STUDIES ON

THE CHEMISTRY OF ARISTOLOCHIA SPECIES
A THESIS

submitted to

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PART I.

THE ETHANOL-SOLUBLE FRACTIONS OF

A. RETICULATA
A. INDICA
A. SERPENTARIA
A. LONGA
Plants of the genus *Aristolochia* have been used medicinally since the 4th. century B.C. and were held in high esteem by the ancient Greek, Roman and Jewish doctors. Extracts are said to have been employed in childbirth, on wounds, ulcers and abscesses, against fevers, asthma, epilepsy and snake-bites, and as bitter tonics and purgatives. Though the plants, and extracts from them, are now no longer in general use, recent work by Shaw showed that *A. elegens* contained an alkaloid of undetermined constitution which caused contraction of the uterus, and an aporphine type base, magnoflorine, has been reported present in *A. debilis* Sieb. and Zucc., and *A. kaempferi* Willd. The pharmacological properties of magnoflorine have not yet been recorded. Extracts obtained from *Aristolochia* species have also been shown to inhibit cultures of *Staphylococcus aureus*, *Micrococcus pyogenes*, *M. citreus* and *B. anthracis* while a product isolated from *A. elegens* was found to possess C-mitotic activity.

These observations indicated that further chemical study of the members of the genus *Aristolochia* was still appropriate. This study was commenced by Stenlake and Williams who described the isolation and detailed examination of the light petroleum-soluble extract of *Aristolochia reticulata* Linn. as part of a wider investigation into the chemical constituents of the plant and
related species. This investigation has now been extended
to include a study of the basic, acidic and other fractions
which can be isolated from *A. reticulata*, *A. indica* Linn.,
*A. serpentaria* Linn. and *A. longa* Linn. by extraction
with ethanol.

The presence of acids and basic material has been
reported in many of the 200 different species of
*Aristolochia*, but unfortunately much confusion of nomen-
clature occurs in the literature. An appraisal of the
relevant publications reveals that "aristolochine" has been
used to describe both acidic and basic
material; "aristolochic acid" describes different acids
and what is obviously the same acid has been named
"aristolochic acid", "isoaristolochic acid", "aristinic acid" and "aristolochine" by different
workers.

Similarly, gross discrepancies are also evident in the
melting points quoted for the same substance, the methyl
ester of aristolochic acid, for example, being variously
reported as melting at 267°C, 250°C, 260-261°C, 280-282°C, 285°C and 286-288°C; again melting
points of 215°C, 275°C, 287-292°C and 281-286°C have been given at different times for
aristolochic acid.

The following discussion of acidic, basic and neutral
substances isolated from the various *Aristolochia* species
represents an attempt to clarify the record.

**Acidic Material.**

In 1820, Chevallier steam distilled the roots of *A. serpentaria* and obtained a yellowish-brown acidic extract which was excessively bitter. The evaporated extract was partially soluble in ethanol giving an amorphous, yellow, bitter and heterogeneous product to which Chevallier ascribed the activity of the root.

Later, Brandes extracted the roots of *A. grandiflora* Gomes (*A. cymbifera* Mart.) with hot ethanol and isolated a golden-yellow crystalline substance from the ether-soluble portion of the extract. The crystals displayed the properties of a plant acid, being acid to litmus, soluble in ammonia, and re-precipitated from the latter by the addition of hydrochloric acid.

Dark orange warty crystals, which were soluble in ethanol and had a persistantly bitter taste were also obtained by Wittstein from the bark of *A. antihystericae* by cold extraction with ether, but were not identified as being acidic.

Still later in 1849, Winkler isolated an amorphous golden-yellow bitter principle from the tops of *A. clematitis*. The product, obtained by extraction with very dilute ammonia and acidifying, was chemically impure, and for this reason was not investigated further, but, according to the author, was identified with the bitter from *A. serpentaria*. 
Two years after this, Frickhinger extracted the powdered roots of *A. clematitis* with ether and obtained on concentration "amber-yellow completely transparent, gleaming clino-rhombic prisms" which he named aristolochia yellow. This was soluble in alkaline solutions and re-precipitated on the addition of acid, and is therefore identifiable with the acidic substance obtained by Brøndes.

The study of *A. clematitis* was continued by Walz who isolated an amorphous golden-yellow bitter substance, aristolochia bitter, as its lead salt from an ammoniacal extract of the plant. The presence or absence of nitrogen in the compound was not recorded and it was given the empirical formula C$_9$H$_{10}$O$_6$, based on the carbon and hydrogen analyses only. Walz also obtained, on steam distillation of the dried plant, a volatile acid which formed crystalline barium, sodium and lead salts. He termed this acid, aristolochic acid, and observed that the lead salt decomposed on heating to give one mol. of formic acid and a half mol. of acetic acid.

Dymok and Warden also isolated a yellow, bitter, semi-crystalline solid by extraction of the roots and stems of *A. indica* with warm ethanol. The product was acidic, dissolving in alkali to give a deep orange solution, from which it was re-precipitated as a yellow amorphous solid on acidification.

These earlier references simply report the presence of
a yellow bitter which, in most cases, is shown to be acidic. The first detailed report on the chemistry of the acidic material appeared in 1892 when Pohl described the isolation of aristolochine (German, Aristoloquin), from the seeds of *A. clematitis* as well as from the roots of *A. longa* and *A. rotunda*. The powdered, defatted seeds or roots were extracted with warm ethanol. Concentration, followed by acidification gave a yellow-brown precipitate which on extraction with ether or ethanol produced aristolochine as yellow microcrystals or orange needles, m.p. 215°C to charring at 220°C. It was soluble in most organic solvents but only slightly soluble in water or benzene, and dissolved in alkali to give a pale brown solution from which it was re-precipitated by acids. The crystals, which analysed to C$_{32}$H$_{22}$N$_2$O$_{13}$, gave a dark green colour with concentrated sulphuric acid. Aristolochine was therefore a nitrogen-containing compound.

Reduction with zinc dust in glacial acetic acid gave a product which was no longer bitter nor physiologically active and which was readily soluble in benzene but only slightly soluble in alkali. A solution of reduced aristolochine in ethanol was fluorescent. Pohl quoted the following analyses for the reduced compound, C 68.95, 69.92%; H 4·36, 4·57%; N 4·66, 4·67%, but did not ascribe a formula to it, merely stating that considerable reduction had taken place. A typical analysis given for aristolochine was
Although he obtained a good crystalline barium salt of aristolochine, Pohl was of the opinion that it could hardly contain a -COOH group since its salts were decomposed by carbon dioxide and also since it completely lost its weak acidic characteristics on reduction. For these reasons, he considered the name aristolochine more apt than aristolochic acid.

Hesse continued the chemical study, extracting the powdered roots of Aristolochia Argentina with ether, which was then saturated with ammonia gas to precipitate the ammonium salts of the acids. The red precipitate which formed consisted of a mixture of the ammonium salts of aristinic, aristidinic and aristolic acids. Recrystallisation from glacial acetic acid gave pure aristinic acid, the other two acids remaining in solution. The material in the mother liquors was dissolved in potassium hydroxide solution; addition of more potassium hydroxide precipitated first crystalline potassium aristinate and then crude amorphous potassium aristidinate. Recrystallisation of the latter from glacial acetic acid gave aristidinic acid. The third acid, which was soluble in the potassium hydroxide solution, was obtained as a flocculent yellow precipitate by addition of hydrochloric acid. This was dissolved in calcium hydroxide solution, again acidified, and then extracted into ether which on evaporation gave crystalline aristolic acid.
Aristinic acid was further purified by formation of its potassium salt. The acid formed greenish-yellow leaflets and needles from glacial acetic acid, m.p. 275°C (decomp.), had a nauseatingly bitter taste and was sparingly soluble in most organic solvents but easily soluble in alcalis. The following analyses were obtained: C 60.16, 60.57%; H 3.68, 3.51%; N 3.46, 3.41, 3.85%. C_{18}H_{13}O_{7}N requires C 60.84%; H 3.66%; N 3.94%. Analyses of the potassium, sodium, ammonium, barium, calcium, copper, lead and silver salts of aristinic acid closely agreed with that of the parent acid.

A Zeisel determination on aristinic acid indicated a methoxyl content of 1.5%. Hesse rather surprisingly suggested that this was probably due to contamination though no impurities were apparent under the microscope, and great care was taken to confirm the purity of the starting material.

Reduction of aristinic acid with zinc dust in glacial acetic acid gave a yellow amorphous material which was not investigated further. The methyl ester was prepared from the silver salt of aristinic acid and methyl iodide and crystallised as yellow needles, m.p. 250°C (approx.), containing C 61.03%; H 3.83%. A methyl ester of the formula C_{18}H_{12}NO_{7}. CH_{3} requires C 61.78%; H 4.06%. The methoxyl content was found to be 11.2% and the difference between this figure and the theoretical for one methoxyl group (8.40%)
was attributed to the same impurity which had supposedly been found in aristinic acid.

Aristidinic acid had the same empirical formula as aristinic acid and differed from it only in melting point (260°C, approx.) and methoxyl content (6.26%) which was lower than the theoretical for one such group (8.73%). Hesse was of the opinion that this difference was due to contamination with aristinic acid.

Aristolic acid formed orange-red needles which melted at 260-270°C (after darkening at 220°C), and had an empirical formula of \( \text{C}_{15}\text{H}_{11}\text{O}_{7} \) or \( \text{C}_{15}\text{H}_{13}\text{O}_{7} \).

The author correlated his work with that of Pohl suggesting that Pohl's aristolochine should be termed aristolochic acid since the analytical figures obtained by Pohl (see page 6) also fitted the formula \( \text{C}_{17}\text{H}_{11}\text{O}_{7} \). Hesse found that all four acids gave the same dark green colour with concentrated sulphuric acid and in his opinion were chemically related, aristidinic acid being methyl aristolochate and aristinic acid being homologous with aristolochic acid.

According to Hesse, the bitter materials isolated by Chevallier and Walz were impure specimens of aristolochic acid but Frickhinger's aristolochia yellow was different because of the depth of colour of the crystals. He suggested that aristinic acid was present in \textbf{A. indica} and maintained that \textbf{A. longa} did not contain any of the acids.
found in *A. Argentina*, despite the fact that Pohl had isolated his aristolochine from this source.

Some twenty-seven years were to elapse before interest (17) in these acids was awakened by Castille with the isolation in 1922 of aristolochic acid from *A. sipho* l'Hérit. The acid had the empirical formula $C_{17}H_{11}O_7N$, was monobasic and appeared identical with Pohl's aristolochine. Its methyl derivative, m.p. 260-261°C (decomp.) proved difficult to saponify and analysed nearer to the dimethyl compound $C_{19}H_{15}O_7N$, than to the monomethyl one, $C_{18}H_{13}O_7N$.

Reduction with zinc dust in glacial acetic acid produced a compound apparently identical with that obtained by Pohl on reduction of aristolochine, corresponding to an empirical formula $C_{17}H_{13}O_4N$.

Fusion of aristolochic acid with solid potassium hydroxide at 250°C yielded ammonia and a residue showing the properties of an anthraquinone as well as a phenolic substance which could be precipitated by bromine. Castille therefore concluded that aristolochic acid was a monobasic acid possessing an anthraquinone nucleus in combination with a tertiary nitrogen atom.

Later, Krishnaswany, Manjunath and Venkato Rao extracted *A. indica* roots with hot ethanol and obtained an intensley bitter yellow crystalline acid, $C_{17}H_{11}O_7N$, m.p. 275°C, in 0.0133% yield after concentration and extraction with ether. This acid had properties very similar to those of Pohl's
aristolochine and Castille's aristolochic acid but as Pohl's acid melted at 215°C, the Indian authors supposed that their compound was an isomer and named it isoaristolochic acid. It contained no methoxyl or methylenedioxy groups but was shown to possess one active hydrogen by the Zerewitinoff method. Refluxing with acetic anhydride for one hour caused no change, starting material being recovered. Other attempts at acetylation also proved abortive. It did not react with the usual reagents for carbonyl compounds, gave no methiodide and was not attacked by boiling 50% aqueous potassium hydroxide. An attempt to prepare a benzoate, however, was successful and a small quantity of a yellow microcrystalline powder, which proved difficult to purify, was isolated. It had a melting point 170-171°C and analysed to C_{24}H_{15}O_3N, in agreement with the formula for the acid.

Methylation of isoaristolochic acid with dimethyl sulphate gave a tasteless derivative, C_{18}H_{13}O_7N, m.p. 267°C (decomp.) which was unaffected when boiled with ethanolic potassium hydroxide for four hours. The authors concluded that the product was an ether and therefore that isoaristolochic acid did not contain a carboxyl group. This agreed with Pohl's statement that aristolochine also contained no -COOH group.

Oxidation of isoaristolochic acid with hydrogen peroxide in dilute potassium hydroxide gave a dibasic acid,
C_{14}H_{11}O_5N \,(C00H)_2\, \text{m.p. } 164.5^\circ C\, \text{which lost one mol. of water when kept at 120^\circ C for 3 hours.}

Rosenmund and Reichstein, who gave an excellent survey of the literature on Aristolochia species, isolated crude aristolochic acid from the root stock of \textit{A. sipho} in approximately 0.3\% yield by extracting the defatted roots and rhizomes with boiling ethanol. The concentrated solution was precipitated with dilute hydrochloric acid and shaken out with ether which was, in turn, extracted with potassium bicarbonate solution. The canary-yellow powder obtained on acidification of this last solution was purified through its sodium salt and recrystallised from dioxan or glacial acetic acid as intense yellow needles, C_{17}H_{11}O_7N\,\text{m.p. } 274-278^\circ C\,\text{(decomp.), confirming Castille's formula and that for aristolochic acid quoted by Hesse, and in agreement with the results obtained by Krishnaswamy et al. for their isoaristolochic acid.}

A Zeisel determination indicated a methoxyl content of 1.3\% as found by Hesse (see page 7). Contrary to Hesse, Rosenmund and Reichstein were of the opinion that this could not be ascribed to impurity but was possibly due to partial cleavage of an N- methyl group since a methyl-imine determination indicated exactly one -N.CH_3 group.

Reaction with diazomethane gave a methyl ester, C_{18}H_{13}O_7N \,\text{m.p. } 280-282^\circ C\,\text{(decomp.) which was saponified only with difficulty and this with considerable decomposition.}
Pohl and Krishnaswamy et al. had concluded from similar observations that a carboxyl group was not present and confirmatory evidence was sought by Rosenmund and Reichstein. It was found that aristolochic acid gave no ferric chloride reaction, which suggested that enolic groups were unlikely to be present, and finally irrefutable evidence for the presence of a carboxyl group was obtained when aristolochic acid was decarboxylated by warming with copper powder in quinoline, to yield an orange-yellow neutral substance, $\text{C}_{16}\text{H}_{11}\text{O}_{5}\text{N}$, m.p. 206-212°C.

Oxidation experiments with alkaline permanganate and chromic acid yielded no characterisable product. Reductive acetylation of aristolochic acid gave a fluorescent substance similar to that obtained by Pohl and Castille. Rosenmund and Reichstein also reduced the methyl ester, employing two different methods. Hydrogenation with platinum oxide in glacial acetic acid gave an unstable bright yellow substance which was soluble in dilute sodium hydroxide and which proved difficult to purify. The best sample melted at 312-315°C with sublimation, and analysed to $\text{C}_{18}\text{H}_{13}\text{O}_{4}\text{N},\frac{1}{2}\text{H}_{2}\text{O}$. Acetylation of this product yielded a crystalline acetate, thought to be a diacetate, $\text{C}_{22}\text{H}_{15}\text{O}_{6}\text{N}$ or $\text{C}_{22}\text{H}_{17}\text{O}_{6}\text{N}$, m.p. 306-308°C. Reductive acetylation of the methyl ester with acetic anhydride, pyridine and zinc dust gave the same acetate.

Reduction of decarboxylated aristolochic acid with plat-
inum oxide in glacial acetic acid produced an almost colourless, strongly fluorescent but unstable product which rapidly became coloured during isolation. Reductive acetylation of the same compound also failed to yield a crystalline product.

Neither aristolochic acid, its methyl ester nor its decarboxylated derivative contained a carbonyl or hydroxyl group. The authors could not draw definite conclusions from their results but postulated the presence of a quinonoid group which could be converted into an unstable phenol. On the basis of their work however, the following partial structure (I) is applicable:

![Structure](image)

This structure can be criticised on the basis that, unless the nitrogen atom is part of an aromatic system, as such it would be strongly zwitterionic and therefore unlikely to be readily extractable from aqueous solution.

Rosenmund and Reichstein went on to review critically the earlier literature and suggested that Frickhinger's aristolochia yellow, Pohl's aristolochine, Hesse's aristinic acid, Castille's aristolochic acid and the isoaristolochic
acid of Krishnaswamy, Manjunath and Venkato Rao were one and the same substance which in their opinion should be called aristolochic acid. They pointed out that Hesse's analyses for aristinic acid (see page 7) also fitted the formula $C_{17}H_{11}O_{7}N$ and that the analyses of some of the salts prepared by him were in better agreement with the lower formula. The melting point of Hesse's compound and their own are identical and the analysis that the former quoted for the methyl ester of aristinic acid (see page 7) was in better agreement with $C_{18}H_{13}O_{7}N$ than the next higher homologue as Hesse had suggested, though melting point discrepancies in the case of the methyl ester still remained. Hesse had reported it as about 250°C whereas Rosenmund and Reichstein found a melting point of 280-282°C (decomp.) for an analytically pure sample. This difference, they suggested, was due to an impurity in Hesse's sample because they themselves obtained m.p. 260-262°C for an impure sample. Castille's melting point for the methyl ester also was 260-261°C with decomposition. (28)

In 1954, Green, Eugster and Karrer isolated aristolochia-cymbifera-acid, $C_{20}H_{32}O_2$, m.p. 107°C, but this was obtained by light petroleum extraction and was obviously not related to any of the previous bitter materials.

It was at this point that the present investigation was commenced but while work was in progress, Pailer and
co-workers published a series of papers and elucidated the structures of both aristolochic acid and a second acid, aristolochic acid - II. (19, 21)

Pailer, Belohlav and Simonitsch extracted the powdered and defatted rhizomes and roots of A. clematitis Linn. with ethanol. The acidic portion of the concentrated extract was obtained by potassium bicarbonate extraction which gave a mixture of acids in which aristolochic acid was the main constituent. It was purified by crystallisation from dimethylformamide-ethanol as orange-red threads, \( \text{C}_{17}\text{H}_{11}\text{O}_{7}\text{N} \), m.p. 287-292°C, 281-286°C (decomp.) depending on rate of heating, the melting points being determined on a microblock.

Treatment with diazomethane gave a methyl ester, \( \text{C}_{18}\text{H}_{13}\text{O}_{7}\text{N} \), m.p. 285°C, 281°C and decarboxylation with copper powder in quinoline gave the expected compound, \( \text{C}_{16}\text{H}_{11}\text{O}_{5}\text{N} \), m.p. 216°C, 212°C. The original acid and the above two derivatives were therefore identical with those obtained by Rosenmund and Reichstein.

Pailer and his co-workers then went on to establish the presence of one methoxyl, one methylenedioxy and one nitro group in aristolochic acid which had as its nucleus the phenanthrene molecule.

Aristolochic acid, by the customary method, gave a methoxyl content of 1.5% (theoretical 9.09%) but by a suitable modification of methoxyl determination in which consideration...
was given to the difficult solubility of the compound, it was established that, in fact, one methoxyl group was present. This was confirmed by a similar methoxyl determination on the methyl ester which was shown to possess two such groupings.

Zinc dust distillation of aristolochic acid yielded phenanthrene, confirmed by its ultraviolet spectrum and melting point, and suggested the partial structure (II) for the acid.

\[
\begin{align*}
\text{Zinc dust distillation of aristolochic acid yielded phenanthrene, confirmed by its ultraviolet spectrum and melting point, and suggested the partial structure (II) for the acid.}
\end{align*}
\]

\[
\begin{align*}
&-\text{COOH} \\
&-\text{O CH}_3 \\
&(\text{CH}_7\text{O}_4\text{N})
\end{align*}
\]

\[(\text{II})\]

Catalytic hydrogenation of the acid or the methyl ester resulted in a hydrogen uptake of three mols, and gave a neutral compound, \( \text{C}_{17}\text{H}_{11}\text{O}_4\text{N} \), m.p. 317-319°C. The authors concluded from this that more than one group was concerned in the reduction, and in the case of the reduction of the methyl ester, methanol was a product of the reaction.

Hydrogenation of the decarboxylated acid also showed an uptake of three mols. of hydrogen and yielded a sensitive basic substance, \( \text{C}_{16}\text{H}_{13}\text{O}_3\text{N} \), m.p. 172-173°C (19), 170°C (21),
which could be acetylated. The same acetate was obtained by reductive acetylation of the decarboxylated compound. The original base after diazotisation and boiling gave a nitrogen-free intensely red substance. These facts indicated the presence of a nitro-group which on reduction gave the lactam of the corresponding amino-acid, and ultraviolet and infrared spectra confirmed the presence of such a group.

The presence of a methylenedioxy group was demonstrated in aristolochic acid, its ester and the decarboxylated acid by heating them with phosphoric acid when formaldehyde (29) was liberated, thus permitting extension of the partial structure to that shown (III).

![Structure III](image)

The complete structure of aristolochic acid was established as given (IV) by oxidation of the decarboxylated acid (V) with hydrogen peroxide in tetrahydrofuran. The dibasicity of the resultant diphenic acid (VI), C_{16}H_{12}O_7, m.p. 246°C (19), 243°C (21), was confirmed by formation of
the dimethyl ester, $\text{C}_{18}\text{H}_{16}\text{O}_7$, m.p. 114°C, which was shown to possess three methoxyl groups. Furthermore, the methylenedioxy group and all the carbon atoms of the starting material were still retained, a fact which could only be explained if the nitro group was in the 9 or 10 position in the molecule. From the observation that aristolochic acid and its methyl ester (VII) gave a lactam (VIII) on reduction, it followed that the carboxyl group must be attached to a carbon atom adjacent to C(9) or C(10).

The positions of the methoxyl and methylenedioxy groups were established by treatment of the diphenic acid with concentrated hydrochloric acid under pressure in the presence of resorcinol to bind the formaldehyde released during the reaction. The acid severed the ether linkages and, with decarboxylation, formed a dihydroxylactone (IX), $\text{C}_{13}\text{H}_8\text{O}_4$, m.p. 204°C, the structure of which was established by potassium permanganate oxidation of the corresponding dimethyl ether (X) to o-methoxy-phthalic acid (XI), characterised as its anhydride.

Confirmatory evidence for the lactone structure (IX) was obtained by synthesis. 1,5,6-Trimethoxyphenanthrene-10-carboxylic acid was oxidised in two stages to a diphenic, dibasic acid (XII), $\text{C}_{17}\text{H}_{16}\text{O}_7$, which on treatment with concentrated hydrochloric acid, gave the required lactone.

Pailer, Belohav and Simonitsch therefore concluded that aristolochic acid is 3,4-methylenedioxy-8-methoxy-10-nitro-
phenanthrene-l-carboxylic acid. It therefore follows that the sensitive basic substance obtained on catalytic hydrogenation of decarboxylated aristolochic acid is as shown (XIII).

A second acid of similar structure to aristolochic acid was found along with aristolochic acid in the sodium bicarbonate-soluble portion of an ethanolic extract of *A. clematidis*.

This was at first called nor-aristolochic acid but in a later publication was re-named aristolochic acid -II. It had an empirical formula $C_{16}H_{9}O_{6}N$ and was reported as melting at $209^\circ C$ though the authors stated that it was not completely pure. Separation of aristolochic acid -II from aristolochic acid (IV) proved difficult. An attempted fractional crystallisation using the ammonium salts was not satisfactory and pure fractions could not be obtained from the potassium salts using conventional methods such as solvent precipitation, counter current distribution and chromatography. Eventually a separation was achieved by esterifying the acid mixture and chromatographing on alumina. Aristolochic acid -II methyl ester, m.p. $274^\circ C$, came through first followed by the methyl ester of aristolochic acid, m.p. $287-288^\circ C$. The former, $C_{17}H_{11}O_{6}N$, contained one methoxyl, one methylenedioxy and one nitro group. The ultraviolet spectrum had a maximum at $251 m\mu$, typical of a phenanthrene derivative, which suggested that aristo-
Aristolochic acid-II was a methylenedioxy nitrophenanthrene carboxylic acid.

Aristolochic acid -II m.p. 269-271°C (decomp.) was obtained from its methyl ester in extremely poor yield (6mg. from 104 mg.) so all degradative work was carried out on the methyl ester and the readily obtained decarboxylated acid -II which could be separated with ease by chromatographic means from a mixture of the two decarboxylated acids.

Structural investigation was accomplished using methods similar to those employed in the elucidation of aristolochic acid(IV). For the same reasons as before, it was deduced that the nitro group occupied position 10 and the carboxyl group position 1 in a phenanthrene nucleus. The problem was therefore reduced to finding the points of attachment of the methylenedioxy group.

Aristolochic acid -II methyl ester (XIV) was heated in a sealed tube with resorcin and hydrochloric acid and the resulting dihydroxy-compound (XV) oxidised, without purification, with alkaline potassium permanganate. Phthalic anhydride (XVI) was identified as a breakdown product, proving that the methylenedioxy group was attached to the same ring as the carboxyl group, otherwise hemimellitic acid (XVII) would have been produced.

The structure of aristolochic acid -II was therefore 2,3- or 3,4-methylenedioxy-10-nitrophenanthrene-1-carboxylic
To distinguish between these two possibilities, decarboxylated aristolochic acid -II (XVIII) was reduced and then, with some difficulty, acetylated under hydrogen. The resulting 10-diacetylamino- 3,4-methylenedioxyphenanthrene (XIX) was identical with synthetic material prepared in an unambiguous manner.

Further proof was obtained by oxidation of decarboxylated aristolochic acid -II (XVIII) to a methylenedioxydiphenyldiacetic acid, C₁₅H₁₀O₆ (XX) which was not identical with synthetic 4,5-methylenedioxydiphenyl-2,2'-dicarboxylic acid and must therefore have been the corresponding 5,6-methylene-
dioxy isomer. Treatment of this with hydrochloric acid under pressure gave a compound identical with synthetic 3,4-benz-8-hydroxycoumarin (XXI).
Aristolochic acid -II (XXII) was therefore 3,4-methyleneedioxy-10-nitrophenanthrene-1-carboxylic acid and only differed from aristolochic acid (IV) in not possessing a methoxyl group.

Since the appearance of the publications by Pailer, Belohlav and Simonitsch and Pailer and Schleppnik, other workers have reported the presence of aristolochic acid (IV) in *A. kaempferi* and *A. debilis*.

**Basic Material**

A review of the investigations into the so-called basic constituents of *Aristolochia* species reveals that there is confusion both in names and identity of the substances isolated as bases, and there can be little doubt that much of the material referred to as basic in earlier references was,
in fact, acidic. In some cases, also, only the isolation of the bitter substance is reported, no attempt being made to classify it as basic or acidic.  

Thus, Chevallier treated a steam distillate of the roots of *A. serpentaria* with lead acetate. The resultant precipitate was immediately bitter and irritant to the throat due to adsorbed material which could be extracted with ethanol. This gave a water-soluble product which no longer was precipitated on the addition of lead acetate. Feneulle confirmed Chevallier's work and isolated the same yellow bitter substance from the filtrate obtained on removal of the precipitate with lead acetate. Neither author claimed that the above bitter was basic, but when Ferguson isolated aristolochine from *A. reticulata* he was of the opinion that all three substances were identical. Winkler, on the other hand, claimed that his bitter, which possessed acidic properties, was also the same as the one isolated by Chevallier and Feneulle. Ferguson obtained aristolochine from *A. reticulata* as light yellow bitter crystals using conventional means which established its basic character. Thus, the concentrated ethanolic extract was dissolved in ether and extracted with dilute hydrochloric acid, the aqueous solution basified, and the alkaloid removed by extraction with ether. Various colour tests were recorded for the base, which evolved ammonia on heating with soda lime, but neither yield nor analysis was reported.
The basic material which Hesse isolated from *A. Argentina* was also designated aristolochine though it gave different colour reactions from Ferguson's base. Hesse used two methods, either treating the powdered roots with sodium carbonate and extracting with ether, or alternatively extracting directly with hot ethanol. In this second method the concentrated extract was made alkaline and shaken out with ether from which dilute acids removed the alkaloid. The free base and its salts were amorphous. Neither analyses nor further investigation was possible since aristolochine was mixed with another substance which could not be removed from the very small quantities of base generally obtained. Hesse also reported aristolochine as a probable constituent of *A. indica*, but that no alkaloids whatsoever were present in *A. longa*.

Although Butte had previously reported the absence of basic material in *A. cymbifera*, Peckolt obtained a dull white flaky crystalline base, cassuvin, from this source by extracting the root bark with tartaric acid in ethanol. The concentrated extract was basified and the base removed into chloroform from which it was obtained as odourless and tasteless crystals with a nauseating after-taste.

In agreement with Hesse's opinion, Dymok and Warden isolated a little basic material from *A. indica* on extract-
ing an acidified concentrated ethanolic extract with ether. The yellow non-crystalline varnish obtained gave positive tests with common alkaloidal reagents. And, from the same source, Krishnaswamy, Manjunath and Venkato Rao reported the isolation of a crystalline alkaloid, again called aristolochine. The study of this base was continued by the first two authors. The crushed roots of *A. indica* were extracted with ethanol which, on concentration, was treated with dilute hydrochloric acid. This acid extract was basified with ammonia and repeatedly extracted with chloroform. The base was removed from the chloroform solution into dilute hydrochloric acid which, on slow concentration, deposited a microcrystalline hydrochloride, \(\text{C}_{17}\text{H}_{19}\text{O}_3\text{N}\cdot\text{HCl}\), m.p. 268°C (decomp.). Recrystallisation from methanol gave the base, \(\text{C}_{17}\text{H}_{19}\text{O}_3\text{N}\), m.p. 215°C, \([\alpha]_D^{25} -268.5^\circ\), in 0.04% yield, whereas recrystallisation from toluene or benzene gave crystalline addition products, m.p. 159°C (decomp.) and 163°C (decomp.) respectively. The base formed a picrate which melted at 222°C with decomposition, and which failed to recrystallise. A crystalline picrolonate, m.p. 232°C (decomp.) was also obtained.

Some of the properties ascribed to aristolochine are rather puzzling. It was sparingly soluble in most common organic solvents though it dissolved readily in alkalis from which a saturated solution of carbon dioxide re-precipitated it. This suggests that a phenolic or other weakly acidic
group is present in the molecule and yet the Indian workers failed to obtain a colour with ferric chloride. They also reported the absence of carbonyl and methylenedioxy groups but one methoxyl group, one N-dimethyl group and one reactive hydrogen atom, determined by the Zerewitinoff method, were found to be present.

The report of the work done on the alkaloid from \textit{A. indica} was published in 1937 but no further work on it has appeared since that date.

More recently, in 1947, Shaw commenced a general survey on alkaloids from Australian flora and simply reported that \textit{A. elegens} contained an alkaloid which caused contraction of the uterus. No further work on this species has since been reported.

In 1957, the presence of an aporphine-type quaternary base, magnoflorine, was reported in \textit{A. kaempferi} Willd.

\begin{center}
\begin{tikzpicture}
\node at (0,0) {CH$_3$O} ;
\node at (1.5,1.5) {N} ;
\node at (1.5,0.5) {CH$_3$} ;
\node at (0.5,0) {HO} ;
\node at (0.5,1) {HO} ;
\node at (-1,0) {CH$_3$O} ;
\node at (1.7,1) {$X^-$} ;
\end{tikzpicture}
\end{center}

\textbf{(XXII)}
and *A. debilis* Sieb. et Zucc. The base was isolated as a reineckate and on regeneration was converted into the styphnate, \( \text{C}_{20}\text{H}_{24}\text{O}_4\text{N} \). \( \text{C}_6\text{H}_2\text{O}_8\text{N}_3 \), m.p. 230°C. Magnoflorine is identical with corytuberine methyl iodide (XXIII). It has, very recently, also been found present in *A. clematidis*.

### Neutral Material

By far the greater proportion of neutral material in *Aristolochia* species is removed during the initial defatting with light petroleum. Krishnaswamy, Manjunath and Venkata Rao, however, isolated a small quantity of a phytosterolin from the non-volatile portion of a hot ethanolic extract of the root of *A. indica*. On concentration of the ethanol, the material precipitated in very poor yield (0.00053%) and required repeated recrystallisations from large volumes of ethanol to raise the melting point to 285 - 290°C. It gave the colour reactions characteristic of phytosterols and an acetyl derivative, m.p. 162 - 163°C was readily obtained. A phytosterol, m.p. 146°C, was produced on hydrolysing with an amyl alcoholic solution of hydrochloric acid; so also was a sugar capable of reducing Fehling's solution, which indicated that the original substance was a glycoside.

A similar substance was obtained by Kind and Celentano from *A. serpentaria*. In this case, an ether extract of the drug deposited a solid material m.p. 263°C which was only
slightly soluble in chloroform and boiling ethanol. Acetylation with acetic anhydride in pyridine gave an acetate identified as β-sitosteryl-β-D-glucoside tetraacetate, which on saponification gave the original glycoside in a pure state. Hydrolysis of this glycoside produced β-sitosterol, characterised as the benzoate. Identification of the glycoside as β-sitosteryl-β-D glucoside was confirmed by synthesis of the tetraacetate.

Green, Eugster and Karrer\(^{(28)}\) have also isolated allantoin from an ethanolic extract of *A. cymbifera* Mart.\(^{(15)}\) Allantoin has also been identified in the water soluble portion of an ethanolic extract obtained from *A. indica*.\(^{(10)}\)
DISCUSSION
OF
EXPERIMENTAL
WORK
The object of the investigation was the isolation and identification of the chemical constituents which could be obtained from *A. reticulata, A. indica, A. serpentaria* and *A. longa* by extraction with ethanol, after preliminary removal of petrol-soluble compounds. Such an investigation was necessary if the contradictory findings summarised in the previous section were to be resolved. Emphasis has therefore been placed on the study of the acidic and basic materials which were isolated from these four species of *Aristolochia*, for it is in the reports on similar materials obtained from other species of *Aristolochia* that most confusion exists.

To avoid unnecessary repetition, only the work done on *A. reticulata* is reported in detail though comprehensive reports for the other three species are given when the methods used were significantly different.
PRELIMINARY EXTRACTIONS

A. reticulata

Four batches of authenticated A. reticulata, which had previously been defatted with light petroleum, were extracted separately by cold percolation with ethanol. Concentration of the extracts under reduced pressure gave almost black thick oils, still containing some solvent, from one of which a neutral solid, m.p. 260 - 270°C, separated on standing. This was reserved for future examination.

Each oil was dissolved in ether and extracted with dilute hydrochloric acid to remove basic and water-soluble material. Subsequent extraction of the ethereal solution with 2% aqueous potassium hydrogen carbonate followed by acidification of the aqueous layer gave a yellowish-brown precipitate of crude acids. The ethereal solution was further extracted with 5% aqueous sodium carbonate and finally with 5% aqueous sodium hydroxide before being evaporated to give a large yield of neutral oil.

Acidification of the aqueous sodium carbonate solution gave a negligible quantity of resinous material which was discarded. Similar treatment of the aqueous sodium hydroxide solution, followed by extraction with ether gave three layers - an ethereal layer which yielded an almost black resinous material on which no further work was done; a middle oily layer, probably an emulsion, which was reserved but not further in-
vestigated; an aqueous layer which was discarded.

The appended scheme (page 35) summarises this preliminary extraction.

Extraction of the marc from one of the batches of A. reticulata with hot ethanol produced, on concentration, a very dark oil which deposited needle crystals, m.p. 223 - 232°C, not identical with the material obtained from the cold extract. After removal of these crystals, the oil was treated as before.

A. indica

The powdered, defatted roots and rhizomes of an authenticated sample of A. indica were extracted with ethanol by cold percolation and the extract treated exactly as in the process used for A. reticulata, though in this case no neutral solid separated on the initial concentration of the ethanolic solution.

A. serpentaria

Two authentic batches consisting of roots and rhizomes of A. serpentaria were examined and sorted by hand to remove contaminants including pieces of A. reticulata which closely resemble A. serpentaria, appreciable amounts of the roots and rhizomes of Hydrastis canadensis, small quantities of Podophyllum peltatum and Polygala senega, as well as some aerial parts of A. serpentaria.
Crude Drug (A. reticulata)

Concentrated Ethnolic Extract

NEUTRAL SOLID MATERIAL
m.p. 260-270° C
m.p. 287-295° C (from A. serpentaria)

Dissolved in ether and extracted with dilute HCl

Ether Layer
+ 2% KHC03

Ether layer
+ 5% Na2CO3

Ether layer
+ 5% NaOH

Evaporate

NEUTRAL OIL

RESIN ACIDS

Negligible residue (Discarded)

CRUDE ACIDS

Marc

Aqueous Layer
containing BASIC and WATER-SOLUBLE MATERIAL

Aqueous Layer

+ dilute HCl or dilute CH3COOH
The roots and rhizomes were powdered and defatted, and then percolated with cold ethanol. Concentration of the percolate gave a thick black oil which, on cooling for four days at 0°C, deposited a brown crystalline solid, m.p. 287 - 295°C, which was reserved for further examination. The thick oily filtrate was separated into fractions by the method described under *A. reticulata*.

*A. longa*, Linn.

*Index Kewensis* lists four different species of *Aristo-lochium* each referred to as *A. longa*. Of these, three are identical with other species, the fourth one, *A. longa*, Linn., being the official plant of this name. The authenticity of a sample of *A. longa*, Linn. was confirmed by Dr. Metcalfe of the Royal Botanic Gardens, Kew.

The powdered roots and rhizomes were macerated and extracted with light petroleum until the percolate was almost colourless. The light-brown oil obtained on evaporation (0.96%) deposited solid globules, m.p. 45°C, which on recrystallisation from methanol were shown to be a mixture of two substances melting at 52 - 56°C and 87 - 93°C. Owing to the very small yield of these substances, they were not investigated further. The marc from the above extraction was macerated and then percolated with cold ethanol to give a deep orange extract. During concentration a yellow crystalline solid was slowly deposited and repeatedly separated by
filtration before final evaporation of the solvent was possible. The oily product was subjected to the same treatment as the corresponding oil from *A. reticulata*. No neutral solid separated out in this case.
A. reticulata

The crude acids were dissolved in either hot glacial acetic acid or hot dioxan to give solutions which deposited crystals on cooling. Further batches of crystalline material, numbering ten in all, were obtained by successive concentration and crystallisation of the mother liquors. The residual mother liquors which were almost black yielded only resinous acidic material which was not studied further.

The appearance, tube melting point and $R_F$ value (determined on Whatman No. 1 paper using 4:1 ethanol - 5% formic acid as solvent) of each fraction were compared. (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Appearance</th>
<th>m.p. °C</th>
<th>$R_F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Orange-yellow needles</td>
<td>269 - 270</td>
<td>0.92</td>
</tr>
<tr>
<td>2</td>
<td>Brown-yellow plates, orange-yellow when powdered</td>
<td>272 - 273</td>
<td>0.92</td>
</tr>
<tr>
<td>3</td>
<td>Pale brown-yellow needles</td>
<td>272 - 275</td>
<td>0.91</td>
</tr>
<tr>
<td>4</td>
<td>Pale brown-yellow needles</td>
<td>271 - 273</td>
<td>0.915</td>
</tr>
<tr>
<td>5</td>
<td>Yellow needles</td>
<td>275 - 277</td>
<td>0.93</td>
</tr>
<tr>
<td>6</td>
<td>Orange plates</td>
<td>273</td>
<td>0.94</td>
</tr>
<tr>
<td>7</td>
<td>Brown-yellow plates</td>
<td>268</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Orange-red needles</td>
<td>266</td>
<td>0.78</td>
</tr>
<tr>
<td>9</td>
<td>Orange-brown needles</td>
<td>268</td>
<td>0.79</td>
</tr>
<tr>
<td>10</td>
<td>Orange needles</td>
<td>270</td>
<td>-</td>
</tr>
</tbody>
</table>
Fractions 1-6 and fractions 8-10 were bulked and treated as two crude acidic products, A and B respectively.

**Product A.**

Recrystallisation of this product from glacial acetic acid or dioxan gave yellow, non-fluorescent needles, C_{17}H_{11}O_{7}N, m.p. 275 - 277°C (decomp., tube) which formed a deep orange solution in aqueous sodium hydroxide. The original material could be recovered as an amorphous solid from this solution on the addition of acid. The crystals gave a green colour with concentrated sulphuric acid in agreement with the descriptions of aristolochic acid from A. sipho (18) and A. Argentina (14).

Aristolochic acid from A. clematitis, also C_{17}H_{11}O_{7}N, (21) has been reported as forming orange-red threads from N,N-dimethylformamide—ethanol, m.p. 281 - 286°C (decomp., microblock). Pure product A also readily crystallised from this mixed solvent as orange-red needles, m.p. 275 - 277°C (decomp., tube). Both forms of product A melt at 284 - 285°C (decomp.) on a microblock.

Product A contains one methoxyl group as determined by a modified Zeisel method in which distillation was allowed to continue for six hours. The insolubility of the substance requires extra time for distillation and no doubt explains the low figures obtained by Hesse and Rosenmund and Reichstein (14) (18).
for the methoxyl content.

The ultraviolet absorption spectrum of product A was recorded (Figure 1) and showed peaks at 223\(\mu\) (log\(\varepsilon\) 4.49), 250\(\mu\) (log\(\varepsilon\) 4.47), 318\(\mu\) (log\(\varepsilon\) 4.11) and 390\(\mu\) (log\(\varepsilon\) 3.85) which are in excellent agreement with those recorded for methyl aristolochate from \textit{A. sipho} and almost identical with those obtained by Pailer and his co-workers for aristolochic acid from \textit{A. clematidis}.

Decarboxylation of product A with copper powder in quinoline gave the neutral substance, \(C_{16}H_{11}O_5\), as orange needles, m.p. 213\({}^\circ\)C (microblock), identical with that obtained by Rosenmund and Reichstein who reported a melting point of 206 - 212\({}^\circ\)C. The ultraviolet absorption spectrum of decarboxylated product A agrees with that recorded by Pailer, Belohlav and Simonitsch for 1-methoxy-5,6-methylenedioxy-9-nitrophenanthrene (V).

Hydrogenation of product A was attempted first by catalytic methods using both 20\% palladium on charcoal and platinum oxide as catalysts and employing either dioxan or glacial acetic acid as solvent. Difficulties arose due to the poor solubility of product A in these solvents, or indeed any solvent, so that only small quantities could be handled at a time. The catalytic reductions, which produced a fluorescent product, indicated a hydrogen uptake of three mols. but were invariably incomplete even after prolonged reaction. A selection of such reductions is reported in Table 2.
Figure 1

Ultraviolet absorption of aristolochic acid (IV)
(product A)

log $E$

WAVELENGTH ($m\mu$)
ion with zinc and acetic acid, on the other hand, was reasonably fast and comparatively large quantities of product A could be used in the reaction. The neutral compound isolated, $C_{17}H_{11}O_{4}N$, has previously been identified as 9-amino-1-methoxy-5,6-methylenedioxy-8-phenanthroic lactam (VIII) (21).

Table 2.

<table>
<thead>
<tr>
<th>Redn.</th>
<th>Weight of Product A (mg.)</th>
<th>Solvent</th>
<th>Conc.</th>
<th>Catalyst</th>
<th>Time</th>
<th>Uptake of Hydrogen (mols.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>104.0</td>
<td>Glacial acetic</td>
<td>0.104% PtO$_2$</td>
<td>160min.</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>41.5</td>
<td>Dioxan</td>
<td>0.415% 20%Pd on charcoal</td>
<td>30min.</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>50.0</td>
<td>Glacial acetic</td>
<td>0.05%  PtO$_2$</td>
<td>60min.</td>
<td>2.94</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>68.0</td>
<td>Glacial acetic</td>
<td>0.068% PtO$_2$</td>
<td>5hrs.</td>
<td>2.85</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>320.0</td>
<td>Dioxan</td>
<td>0.40%  20%Pd on charcoal</td>
<td>4hrs.</td>
<td>2.15</td>
<td></td>
</tr>
</tbody>
</table>

It is undoubtedly identical with the neutral yellow substance (17) erroneously formulated as $C_{17}H_{13}O_{4}N$ obtained by Castille on reduction of aristolochic acid from A. sipho by zinc and acetic acid. It is also identical with the reduction product, said to be $C_{18}H_{13}O_{4}N \cdot \frac{1}{2}H_{2}O$, obtained by Rosenmund and Reichstein (18) as shown by comparison of the respective acetates whose melting points and ultraviolet spectra are in excellent agreement. Furthermore, the analyses of the so-called "diacetate" obtained by Rosenmund and Reichstein agree more
closely with that for the lactam monoacetate:

(18)

Found: C 67.68, 67.88, 67.97%

H 3.89, 3.95, 4.24%

N 4.72, -, 4.60%

Proposed: C_{22}H_{17}O_{6}N requires C 67.51, H 4.38, N 3.58%

Diacetates: C_{22}H_{15}O_{6}N requires C 67.86, H 3.89, N 3.61%

Monoacetate: C_{19}H_{13}O_{5}N requires C 68.06, H 3.91, N 4.18%

The preceding evidence establishes that product A is identical with aristolochic acid obtainable from various species of Aristolochia.

Product B.

Recrystallisation of crude product B from glacial acetic acid or ethanol gave a mixture of yellow and red crystals, easily detected with a hand lens. Paper chromatography, using the same ethanol-formic acid system as before, gave two compact spots, the first one yellow and non-fluorescent with an \( R_F \) value of 0.91, and the second one, a yellow spot which fluoresced blue-green in ultraviolet light, with an \( R_F \) value of 0.77. Product B was therefore a mixture of aristolochic acid and another compound.

Attempts to separate the two components using an acid-washed alumina column failed due to the very low solubility of both substances in all suitable solvents. A silica gel column,
buffered to pH 7.2, also proved useless for the same reason. Sublimation at high temperature and under reduced pressure resulted in decomposition. A partial separation was eventually effected by a purely physical method which depended upon the higher density of the red crystals compared with the yellow ones. The mixture of crystals was suspended by shaking in ethanol and the supernatent liquid containing most of the yellow crystals quickly decanted leaving a fairly pure sample of product B. This was finally purified by repeated crystallisations from ethanol.

Pure product B was obtained as red needles, m.p. 286.5°C (microblock) without decomposition. Elementary analysis gave the formula as C_{19}H_{15}O_{6}N and a Zeisel determination in which distillation was allowed to continue for 4 or 5 hours (due to the insolubility of the material) showed the presence of three methoxyl groups in the molecule. Product B was therefore not identical with Hesse's aristolic acid and, as it also differed from aristolochic acid-II obtained from A. clematidis, it was designated aristo-red.

The carbon-oxygen ratio of aristo-red confirmed that it was not simply a dimethoxy-derivative of aristolochic acid though the analysis and method of extraction indicated a structural similarity between both molecules.

Aristo-red showed an intense fluorescence in ultraviolet light both in the solid state and in solution. It was readily acetylated with acetic anhydride to give a pale-orange
crystalline acetate, m.p. 276 - 278°C (microblock), which also
erred fluorescent properties, but attempted reduction with
zinc and glacial acetic acid caused immediate decomposition
and no product could be isolated. In these respects, aristo-
red resembled the lactam (Vlll) rather than aristolochic
acid (IV).

The absence of nitro- and carboxyl groups in aristo-red
was shown by the study of infrared absorption curves. Thus,
a C-N0\textsubscript{2} group shows two strong bands in the ranges 1500-
1560cm.\textsuperscript{-1} and 1300-1360cm.\textsuperscript{-1} (40). These two bands are pres-
ent in aristolochic acid at 1525 and 1343cm.\textsuperscript{-1} and in 1-methoxy-
5,6-methylenedioxy-9-nitrophenanthrene (decarboxylated aristo-
lochic acid) at 1515 and 1343cm.\textsuperscript{-1} respectively. In both
these regions, however, the infrared spectra of aristo-red and
its acetate show no such bands.

The absence of a carboxyl group in aristo-red and its
acetate is easily demonstrated. In infrared spectra of org-
amic acids, there are five bands to be considered in detecting
a carboxyl group. Three of these bands simply provide con-
firmatory evidence but the bands in the range 2500-2700cm.\textsuperscript{-1}
and near 1700cm.\textsuperscript{-1} are most highly characteristic. The range
2500-2700cm.\textsuperscript{-1} is one where bands due to other groups seldom
occur and, though the absolute intensity of the band is not
very great, it provides the surest way of detecting a carboxyl
(41) group. Absorption in this region, which is due to the -OH
stretching vibrations, consists of one to three bands. A
study of the six relevant infrared spectra (Figures 2 - 7) shows that aristolochic acid alone gives broad absorption in this region with two peaks at 2590 and 2640 cm\(^{-1}\) whereas decarboxylated aristolochic acid, the lactam, the lactam acetate, aristo-red and its acetate do not absorb and therefore do not possess a carboxyl group.

Strong confirmatory evidence that aristo-red is closely related to aristolochic acid lactam (Vlll) was obtained from further comparisons of the infra-red and ultraviolet absorption spectra of aristo-red and its acetate with those of the lactam (Vlll) and its acetate. Comparison of the complete infrared spectra of aristo-red (Figure 5) and the lactam (Figure 4) reveals remarkable similarities with each band in the one having a corresponding band in the other. The same similarity is found to be present on comparing aristo-red acetate (Figure 7) with the lactam acetate (Figure 6). A broad band in the region 3000 - 3300 cm\(^{-1}\) (the lactam -NH- region) is present in aristo-red and the lactam from aristolochic acid (Figure 4) but is absent from the spectra of the acetates (Figures 6, 7), and in the 1650 - 1700 cm\(^{-1}\) region (the lactam carbonyl region), aristo-red shows a broad band and peak at 1704 cm\(^{-1}\), and a peak at 1652 cm\(^{-1}\) which move to 1728 cm\(^{-1}\) and 1702 cm\(^{-1}\) respectively on acetylation. Similarly the lactam (Vlll) shows broad absorption in this region with a peak at 1691 cm\(^{-1}\), and a second peak at 1655 cm\(^{-1}\) which move to 1724 cm\(^{-1}\) and 1702 cm\(^{-1}\) respectively on acetylation. The two
bands in the region of 1702 and 1724 cm$^{-1}$, shown by both acetates, are typical of NN-diacylarylamines.(42,43,44)

![NN-Diacylarylamines](image1)

![Lactam Acetates](image2)

The ultraviolet absorption spectra of the four compounds (Figure 8) show four distinct regions of absorption in contrast with aristolochic acid (Figure 1) which has only three. Furthermore, the absorptions of all four compounds are very similar in wavelength and relative intensities (Figure 8) indicating their similarity. Alkali solubility of aristo-red is therefore not due to a carboxyl group but is explicable in terms of the lactam grouping (18) which also explains the formation of a monoacetate.

These observations suggest that aristo-red can be formulated as the dimethoxy-derivative of the lactam of aristolochic acid (XXIV).
Figure 2

DECARBOXYLATED ARISTOLOCHIC ACID

Medium: KBr disc.
Conc: 0.32% w/w.

Figure 3

ARISTOLOCHIC ACID

Medium: KBr disc.
Conc: 0.28% w/w.
Figure 4

**LACTAM.**

*(REDUCED ARISTOCLINIC ACID)*

**MEDIUM:** KBr disc

**CONC:** 0.35% w/w

Figure 5

**ARISTO-RED**

**MEDIUM:** KBr disc.

**CONC** 0.35% w/w.
Figure 8

Ultraviolet absorption of

aristo-red (xxiv)
aristo-red acetate
the lactam acetate
the lactam (viii)

log ε

WAVELENGTH (mμ)
Due to the very small quantity of aristo-red isolated, the positions occupied by the remaining two methoxyl groups on the phenanthrene nucleus could not be determined. An attempt to increase the yield of aristo-red by acetylating a crude mixture of aristolochic acid (which does not acetylate) and aristo-red failed. This was probably due to decomposition of aristolochic acid and the resultant impossibility of re-crystallising the orange product eventually obtained. Sublimation also caused decomposition.

The acidic material obtained on hot extraction of _A. reticulata_ was also found to consist of aristolochic acid and aristo-red.

**_A. indica_**

In this case, a much cleaner crude acid fraction was obtained which, on recrystallisation from glacial acetic acid or dioxan gave a good yield of only one acidic product identified as aristolochic acid by its analysis, melting point, $R_f$ value, ultraviolet absorption spectrum and colour with concentrated sulphuric acid. Reduction with zinc and acetic acid yielded the lactam (VIII).

**_A. serpentaria_**

The crude acid fraction from this source was investigated using the same techniques as were employed for the corresponding fraction from _A. reticulata_. The same physical and
chemical methods of identification confirmed that the acid mixture consisted of both aristolochic acid and aristo-red.

**A. longa**

The precipitate which separated during concentration of the ethanol extract was identified by the usual methods as aristolochic acid. Careful fractional crystallisations from glacial acetic acid and paper chromatographic techniques established that the only acidic product present was aristolochic acid. The isolation of aristolochic acid from *A. longa* (11) clears up the controversial reports of both its presence (14) and absence in this source.

**General Conclusion**

From the present work and on reference to the relevant literature, it appears that aristolochic acid, in varying yields, is a product characteristic of all the *Aristolochia* species so far examined, whereas aristo-red has been found to be present only in the North American varieties (Table 3). It is of interest to compare this with the findings of Steele, (45) Stenlake and Williams who concluded that aristolactone (XXV), also, was a constituent characteristic of *A. reticulata* and *A. serpentaria* only.

The author is of the opinion that aristinic acid and (14) aristidinic acid, both isolated by Hesse from *A. Argentina*,
are one and the same substance. Their empirical formulae are identical and their respective properties differ only in melting point and methoxyl content. The slight difference in the melting points can be readily attributed to the presence of impurity and also to the fact that they melt with decomposition. The difference in methoxyl content is almost certainly due to practical difficulties in its determination unless a modified Zeisel method is used.

Aristolic acid, $\text{C}_{15}\text{H}_{11-13}\text{O}_{0.7}\text{N}$, m.p. 260 - 270°C, from the same source clearly differs from aristolochic acid.
### Table 3.

<table>
<thead>
<tr>
<th>Source</th>
<th>Aristo-locbic Acid(%)</th>
<th>Secondary Acidic Constituents</th>
<th>Aristolactone (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Argentina, Griseb.</td>
<td>x</td>
<td>aristolic acid</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>A. bracteata, Retz.</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>46</td>
</tr>
<tr>
<td>A. clematitis, Linn.</td>
<td>x</td>
<td>aristolochic acid -II</td>
<td>-</td>
<td>19,20,21</td>
</tr>
<tr>
<td>A. debilis, Sieb. et Zucc.</td>
<td>x</td>
<td>-</td>
<td>-</td>
<td>31</td>
</tr>
<tr>
<td>A. indica, Linn.</td>
<td>0.013</td>
<td>-</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>0.07</td>
<td>0</td>
<td>0</td>
<td>45,pres,wrk.</td>
</tr>
<tr>
<td>A. kaempferi, Willd.</td>
<td>x</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>A. longa, Linn.</td>
<td>0.20</td>
<td>0</td>
<td>0</td>
<td>45,pres,wrk.</td>
</tr>
<tr>
<td>A. reticulata, Linn.</td>
<td>0.022</td>
<td>aristo-red</td>
<td>0.158</td>
<td>45,pres,wrk.</td>
</tr>
<tr>
<td>A. serpentaria, Linn.</td>
<td>0.046</td>
<td>aristo-red</td>
<td>0.091</td>
<td>45,pres,wrk.</td>
</tr>
<tr>
<td></td>
<td>0.92</td>
<td>-</td>
<td>-</td>
<td>47</td>
</tr>
<tr>
<td>A. sipho, l'Hérit</td>
<td>0.30</td>
<td>-</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td>A. maxima, Jacq.</td>
<td>x</td>
<td>-</td>
<td>-</td>
<td>47</td>
</tr>
<tr>
<td>A. pandurata, Jacq.</td>
<td>x</td>
<td>-</td>
<td>-</td>
<td>47</td>
</tr>
</tbody>
</table>

**x** Present in unstated amount  

- No specific search recorded  

0 Substance absent
Recently, Cavallito and Bailey isolated a crystalline substance, $C_{16}H_{11}O_7N$, from *Asarum canadense var. reflexum* (fam. *Aristolochiaceae*) which they termed substance B. Apart from a discrepancy in elementary analysis, the properties of substance B are those of aristolochic acid, (Table 4), the method of extraction in both cases being essentially identical.

### Table 4

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Substance B</th>
<th>Aristolochic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Appearance</strong></td>
<td>Yellow needles</td>
<td>Yellow needles</td>
</tr>
<tr>
<td><strong>m.p.</strong></td>
<td>Darkens between 230-260°C without melting</td>
<td>Darkens similarly, then melts at 275-277°C (decomp.)</td>
</tr>
<tr>
<td><strong>Analysis</strong></td>
<td>Found: C 58.2, H 3.5, N 4.55%</td>
<td>Required: C 59.8, H 3.2, N 4.1%</td>
</tr>
<tr>
<td><strong>Ultraviolet Absorption Spectrum</strong></td>
<td>$\lambda_{\text{max.}}$ (Based on $C_{17}H_{11}O_7N$)</td>
<td>$\lambda_{\text{max.}}$ (Based on $C_{17}H_{11}O_7N$)</td>
</tr>
<tr>
<td></td>
<td>$\varepsilon$</td>
<td>$\varepsilon$</td>
</tr>
<tr>
<td>250</td>
<td>29,325</td>
<td>223</td>
</tr>
<tr>
<td>318</td>
<td>12,820</td>
<td>250</td>
</tr>
<tr>
<td>390</td>
<td>6,615</td>
<td>318</td>
</tr>
<tr>
<td></td>
<td></td>
<td>390</td>
</tr>
</tbody>
</table>
EXAMINATION OF BASIC AND WATER-SOLUBLE MATERIAL

This was obtained from the original concentrated ethanolic extract by dissolving the latter in ether and extracting with dilute hydrochloric acid (see page 35). The treatment was the same for each of the four species of Aristolochia investigated and is shown diagrammatically in the appended scheme (page 53).

Yellow Neutral Material

This was obtained only from *A. reticulata* as shown in the appended scheme. An alternative method of isolation was to set aside the aqueous acidic solution at room temperature for a few days when a greenish-yellow oil precipitated. Recrystallisation of the precipitate from dioxan gave a yellow solid, m.p. 318 - 322°C (decomp., microblock) which was further purified by sublimation in vacuo at 300 - 310°C to give orange prism crystals m.p. 324°C (microblock), 318 - 320°C (sealed tube), both with decomposition and much sublimation.

The compound analysed to C_{15}H_{7}O_5.OCH_3. Zinc dust distillation produced an unidentifiable yellow oily distillate which possessed a distinct phenolic odour. Addition of ferric chloride in ethanol to an ethanolic solution of the substance gave a very dark greenish-brown colour which became purplish-brown on dilution, also indicative of phenolic properties. The presence of three phenolic hydroxyl groups was confirmed.
Aqueous acidic solution of Basic and Water-Soluble Material (see page 35)

Basify (dilute ammonia) and extract with ether.

Ether layer
+ dilute HCl

Ether layer  Aqueous layer

Basify (dilute ammonia) and extract with 1. ether, 2. chloroform.

Ether layer  Chloroform layer  Aqueous layers

YELLOW NEUTRAL MATERIAL  ETHER-SOLUBLE BASES  CHLOROFORM-SOLUBLE BASES  WATER-SOLUBLE BASES

acidify to congo red + ammonium reineckate
by the preparation of a white crystalline triacetate, $C_{20}H_{16}O_9$, m.p. 214.5 - 215°C, which fluoresced brilliant green in ultraviolet light and no longer gave a colour with ferric chloride. An attempt to determine the equivalent weight of this triacetate by saponification resulted in its decomposition and failure to isolate the original material.

Methylation of the neutral material with diazomethane did not take place until a drop of distilled water was included as a catalyst and gave a dimethyl ether, $C_{16}H_{14}O_6$, which melted at 160 - 161°C (microblock), 159 - 160°C (tube). It followed, therefore, that this ether still contained a phenolic group whose presence was confirmed by the formation of a red-brown colour with ethanolic ferric chloride solution.

On the basis of the evidence so far presented, this neutral compound possessed one methoxyl and three hydroxyl groups, two of which could be methylated. It must therefore be a trihydroxy-methoxy-derivative of a parent compound $C_{13}H_8O_2$, such as xanthone (XXVI).

![XXVI]

The isolation of such a product by the route described could then be explained in terms of a parent water-soluble xanthone glycoside which slowly hydrolysed in acid solution to give the
Many facts appeared to confirm that the yellow neutral material was a xanthone: colour, high melting point and ability to sublime are all characteristic physical properties of such compounds. Further, in concentrated sulphuric acid or in strong caustic soda solution, it gave a bright yellow solution which fluoresced brilliantly in ultraviolet light. The lower melting points of the acetate and the methyl ether compared with that of the parent compound, is also similar to that observed in other xanthones (50) and the non-reactivity of one of the hydroxyl groups to diazomethane could be explained in terms of chelation, and is in agreement with the neutral material being a 1, 3, 5-trihydroxy-3-methoxyxanthone (51) similar to swertianol (XXVII).

\[
\begin{align*}
\text{OCH}_3 \\
\text{OH} \\
\text{OH}
\end{align*}
\]

(XXVII)

Due to the small quantity of material isolated, other evidence was essential to prove the structure and with this in mind, a study of the ultraviolet absorption curves of many xanthones was made in order to establish a possible structure. The ultraviolet absorption of the yellow neutral material and its methyl ether were compared with the spectra of 32 other
xanthones and, as far as the positions of the maxima and mini-
ma were concerned, agreement was excellent. A serious dis-
crepancy, however, did occur for without exception, the $\log \varepsilon$ of
the maximum in the 350m$\mu$ region in the 32 xanthone spectra
studied was much less than the $\log \varepsilon$ of the peak near 255m$\mu$.
This is not so in the absorption curves of the yellow neutral
material or its methyl ether and for comparison relevent
figures for some of the xanthones investigated are given in
Table 5. A more detailed study of comparative and subtractive
spectra is described in Appendix I.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Max. m$\mu$</th>
<th>log $\varepsilon$</th>
<th>Compound</th>
<th>Max. m$\mu$</th>
<th>Log $\varepsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,7-trihydroxyxanthone</td>
<td>398</td>
<td>3.57</td>
<td>1,3,5,6-tetramethoxyxanthone</td>
<td>305</td>
<td>4.30</td>
</tr>
<tr>
<td></td>
<td>239</td>
<td>4.40</td>
<td></td>
<td>245</td>
<td>4.66</td>
</tr>
<tr>
<td>1,4,7-trihydroxyxanthone</td>
<td>410</td>
<td>3.67</td>
<td>1,3-dihydroxyxanthone</td>
<td>340</td>
<td>3.79</td>
</tr>
<tr>
<td></td>
<td>237</td>
<td>4.40</td>
<td></td>
<td>252</td>
<td>4.48</td>
</tr>
<tr>
<td>Aspergillone</td>
<td>(52)</td>
<td></td>
<td></td>
<td>(53)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>364</td>
<td>3.12</td>
<td>4-hydroxyxanthone</td>
<td>353</td>
<td>3.03</td>
</tr>
<tr>
<td></td>
<td>242</td>
<td>3.94</td>
<td></td>
<td>250</td>
<td>3.93</td>
</tr>
<tr>
<td>Yellow Neutral Material</td>
<td></td>
<td></td>
<td>Methyl ether</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Calculated as C$<em>{14}$H$</em>{10}$O$_6$)</td>
<td>371</td>
<td>4.28</td>
<td>(Calculated as C$<em>{16}$H$</em>{14}$O$_6$)</td>
<td>353</td>
<td>4.22</td>
</tr>
<tr>
<td></td>
<td>255</td>
<td>4.27</td>
<td></td>
<td>254</td>
<td>4.26</td>
</tr>
</tbody>
</table>
In the light of this observation, re-appraisal of the evidence for the xanthone structure led to the observation that the analysis of the yellow neutral material, apart from the methoxyl result, was in better agreement with \( C_{16}H_{12}O_7 \) than with \( C_{14}H_{10}O_6 \) (Table 6). A tetrahydroxymonomethoxy-flavone structure \( \text{(XXVIII)} \) therefore, became a distinct possibility as flavones possess many properties similar to those of xanthones.

\[
\begin{align*}
\text{(XXVIII)} & \quad \begin{array}{c}
\text{2} \quad \text{3} \\
\text{1} \quad \text{4} \\
\text{7} \quad \text{6} \\
\text{5} \quad \text{4'} \\
\text{2'} \\
\end{array} \\
\quad - \quad \quad (\text{OH})_4 \\
\quad - \quad \quad \text{OCH}_3 \\
\end{align*}
\]

Further support was given by the analytical figures for the acetate and methyl ether which are in agreement with the formation of a tetraacetate, \( C_{22}H_{18}O_{10} \), and a trimethyl ether, \( C_{19}H_{18}O_7 \), by the reactions previously discussed (Table 6). The failure of all four hydroxyl groups to methylate can equally well be explained in terms of a 5-hydroxy-flavone structure.

A study of the ultraviolet absorption spectra of flavones confirmed that the yellow neutral material was, in fact, a member of this group of colouring materials. It has been found that in most cases there are two pronounced peaks at about 250\(\mu\)m and 350\(\mu\)m with a less pronounced peak or inflection near
### Table 6

<table>
<thead>
<tr>
<th>Compound</th>
<th>% C</th>
<th>% H</th>
<th>% O</th>
<th>% OCH₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow Neutral Material</td>
<td>60.8</td>
<td>3.69</td>
<td>35.9</td>
<td>11.54</td>
</tr>
<tr>
<td>C_{14}H_{10}O_{6} requires</td>
<td>61.3</td>
<td>3.68</td>
<td>35.0</td>
<td>11.32</td>
</tr>
<tr>
<td>C_{16}H_{12}O_{7} requires</td>
<td>60.8</td>
<td>3.32</td>
<td>35.45</td>
<td>9.82</td>
</tr>
<tr>
<td>Acetate</td>
<td>60.2</td>
<td>4.50</td>
<td>-</td>
<td>6.65</td>
</tr>
<tr>
<td>C_{20}H_{16}O_{9} requires</td>
<td>60.0</td>
<td>4.01</td>
<td>-</td>
<td>7.75</td>
</tr>
<tr>
<td>C_{22}H_{18}O_{10} requires</td>
<td>59.7</td>
<td>4.10</td>
<td>-</td>
<td>7.00</td>
</tr>
<tr>
<td>Methyl ether</td>
<td>63.5</td>
<td>5.37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C_{16}H_{14}O_{6} requires</td>
<td>63.6</td>
<td>4.67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C_{19}H_{18}O_{7} requires</td>
<td>63.7</td>
<td>5.07</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

270mµ. This description accurately describes the absorption curves of the yellow neutral material and of its methyl ether. In addition, the two spectra show a low intensity inflection near 300mµ which is also shown by quercetin derivatives. More important, in such derivatives the molecular extinction coefficient of the peak near 250mµ is invariably similar to that of the maximum near 350mµ (54, 55). Direct comparison of the ultraviolet spectrum of the yellow neutral material with that of quercetin showed that they were almost identical (Table 7) and confirmation that the yellow neutral material
was a monomethyl ether was obtained by comparing the properties of the neutral material trimethyl ether and 5-hydroxy-3,7,3',4'-tetramethoxyflavone (quercetin-3,7,3',4'-tetramethyl ether). Both are pale yellow needles, m.p. 159 - 160°C, and give the same colour with ferric chloride. Furthermore, apart from a small but consistent difference in log values, due presumably to experimental error in weighing the small quantity (<1 mg.) required for the spectral determination, the ultraviolet absorption spectra are superimposable (Figure 9). It follows then that the original flavone was one of four compounds - rhamnetin (XXIX), isorhamnetin (XXX), quercetin-3-methyl ether (XXXI) and quercetin-4'-methyl ether (XXXII) - all of which are known compounds. The observed constants of the flavone and its tetraacetate however, did not agree with those available for these four compounds (Table 8). The fifth isomer,
quercetin-5-methyl ether was excluded as a possible structure since diazomethane would yield a pentamethoxy- and not a tetramethoxy-quercetin. Table 8 shows that there is a considerable variation of recorded melting points for the same substance due to the fact that they are accompanied by decomposition. This makes them unreliable for characterisation purposes, but none-the-less it would seem reasonable to exclude both quercetin-4'-methyl ether and quercetin-3-methyl ether as probable structures on this basis. Observations by Jurd and Horowitz also exclude these two isomers. They
Ultraviolet absorption of

Figure 9

5-hydroxy-3,7,3',4'-tetramethoxyflavone

(trimethyl ether of flavone from A. reticulata)
<table>
<thead>
<tr>
<th>Substance</th>
<th>m.p. (°C)</th>
<th>Ref.</th>
<th>( \lambda_{\text{max}} )</th>
<th>Ref.</th>
<th>( \log \varepsilon )</th>
<th>Ref.</th>
<th>Tetraacetate m.p. (°C)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin-7-methyl ether (rhamnetin)</td>
<td>294-296</td>
<td>56</td>
<td>256</td>
<td>55</td>
<td>4.40</td>
<td>55</td>
<td>186-188</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>292-293</td>
<td>57</td>
<td>371</td>
<td>55</td>
<td>4.41</td>
<td>55</td>
<td>186-187</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>&gt;300</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>190-192</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>290-294</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>183-185</td>
<td>59</td>
</tr>
<tr>
<td>Quercetin-3'-methyl ether (isorhamnetin)</td>
<td>296</td>
<td>60</td>
<td>255</td>
<td>61</td>
<td>--</td>
<td>61</td>
<td>198-199</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>305</td>
<td>62</td>
<td>365-380</td>
<td>61</td>
<td>(flat)</td>
<td></td>
<td>205-207</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>307</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>205</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>295</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>198-200</td>
<td>64</td>
</tr>
<tr>
<td>Quercetin-4'-methyl ether</td>
<td>240</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>202</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>259-260</td>
<td>66</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>203-204</td>
<td>66</td>
</tr>
<tr>
<td>Quercetin-5-methyl ether</td>
<td></td>
<td></td>
<td>254</td>
<td>55</td>
<td>4.30</td>
<td>55</td>
<td>369</td>
<td>4.25</td>
</tr>
<tr>
<td>Quercetin-3-methyl ether</td>
<td>272-273</td>
<td>67</td>
<td>258</td>
<td>55</td>
<td>4.31</td>
<td>55</td>
<td>360</td>
<td>4.31</td>
</tr>
<tr>
<td>Quercetin-x-methyl ether (present work)</td>
<td>318-322</td>
<td></td>
<td>255</td>
<td>55</td>
<td>4.32</td>
<td>214-215</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(microblock)</td>
<td></td>
<td>370</td>
<td>372</td>
<td>4.34</td>
<td>(microblock).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
showed that 3,4'-dihydroxyflavones were unstable in ethanolic sodium ethoxide and were able to follow this instability spectrophotometrically. The yellow neutral material is unstable under the same conditions (Figure 10) confirming that the 3 and 4' positions in the flavone nucleus are occupied by hydroxyl groups. These observations reduced the number of possibilities to two so that the yellow neutral material was therefore either rhamnetin (XXIX) or isorhamnetin (XXX).

The ultraviolet absorption spectrum of the neutral material in ethanolic sodium ethoxide differed from that recorded for rhamnetin but unfortunately the absorption spectrum of isorhamnetin under the same conditions was not available for comparison. However, the spectrum in ethanol was unaffected by boric acid - sodium acetate indicating that vicinal dihydroxy groups were not present in the molecule in support of its identity with isorhamnetin. This also seemed the most reasonable conclusion on grounds of melting points (Table 8) and ultraviolet absorption spectra, for although the spectra of rhamnetin and isorhamnetin are very similar, the former has a very broad minimum in the 300m\(\mu\) region whereas the latter has a sharp minimum near 290m\(\mu\).

Confirmation of the identity of the flavone as isorhamnetin required an authentic sample of this substance which has been reported to be present in Red Squill but an attempt to isolate the pigment from this source using the published method was not successful. Small samples of synthetic isorhamnetin
and its tetraacetate were eventually obtained from Professor G. Tappi (61) and compared with those obtained from *A. reticulata*. The microblock melting point of isorhamnetin was 318 - 320°C (with sublimation and decomposition after inserting at 310°C) and its ultraviolet absorption spectrum and that of the flavone were identical (Figure 10). Isorhamnetin tetraacetate was a bulky white crystalline product which fluoresced brilliant green in ultraviolet light and which melted at 210 - 211°C (microblock). Its ultraviolet absorption spectrum had maxima at 240m\(\mu\) (\(\epsilon 21,750\)) and 310m\(\mu\) (\(\epsilon 16,700\)) in good agreement with the spectrum of the flavone tetraacetate which had peaks at 239m\(\mu\) (\(\epsilon 20,650\)) and 310m\(\mu\) (\(\epsilon 16,050\)). This evidence therefore confirmed the identity of the flavone from *A. reticulata* as isorhamnetin.
Figure 10
Figure 10.

Ultraviolet absorption of

Isorhamnetin

Flavone from A. reticulata

Flavone from A. reticulata in ethanolic sodium ethoxide
Ether-soluble and chloroform-soluble bases

**A. reticulata**

Evaporation of the ether and chloroform extracts from *A. reticulata* (scheme, page 53) produced negligible quantities of a dark-brown oily base which gave weakly positive reactions with alkaloidal reagents, and was not examined further. No chloroform- or ether-soluble basic material was isolated from the extract which had been obtained by treating the marc with hot ethanol (page 35).

**A. indica**

From this species, greenish-yellow needle crystals (0.0007%) were obtained on evaporating the dark red ether layer (scheme, page 53) to small bulk. Removal of the solvent from the filtrate gave only traces of an almost black non-alkaloidal product which was therefore rejected. The crystals, \( C_{25}H_{23}O_{10}N \), m.p. 339 - 342 °C (decomp., microblock) were soluble in concentrated sulphuric acid giving an orange solution, and fluoresced both in the solid state and in solution. This formula indicated that the base was not identical with that isolated by the Indian workers \((15,35)\) but further work was not possible due to the extremely small quantity isolated. No trace of the base, \( C_{17}H_{19}O_{3}N \), reported to be present in 0.05% yield in *A. indica*, was found and for this reason two further extractions of *A. indica* roots and rhizomes were
undertaken. Once more, negative results were obtained and a possible explanation of this contradiction of the Indian workers' findings will be considered later. Chloroform-soluble bases were not present in the samples of *A. indica* investigated.

**A. serpentaria**

Two samples of *A. serpentaria* were examined separately. Ether-soluble bases were obtained from the first batch of drug (scheme, page 53) as a dark red oil which partially crystallised on standing. It gave positive tests with the usual alkaloidal reagents and was partially soluble in benzene; the benzene-insoluble portion which was free of alkaloids was discarded. Chromatography of the benzene-soluble portion on alumina yielded two yellow fractions, the first being non-fluorescent and the second fluorescing blue-green, under ultraviolet light.

The non-fluorescent base was recrystallised from ether, benzene or chloroform/ethanol to the same melting point of 182°C (decomp., microblock), 178 - 179°C (decomp., tube) and was insoluble in water or dilute hydrochloric acid though a solution in concentrated hydrochloric acid gave a very strong positive reaction with Mayer's reagent. It was slightly soluble in ethanol and very soluble in chloroform and benzene. These characters, particularly the melting point distinguish the base from berberine, the melting point of which varies
with solvent of crystallisation \( [144^\circ C \text{ from ether } (70), 160^\circ C \text{ from water } (71), 145^\circ C \text{ from chloroform } (71)] \). Elementary analysis indicated a formula \( C_{18}H_{15}O_{10}N \) and the ultraviolet absorption spectrum with maxima at 281.5\( \mu m \) (€12,030) and 353\( \mu m \) (€13,365) was indicative of a berberine-type structure. No further work was done on this base because of the very small quantities obtained, but the physical constants indicate that it is not berberine, canadine or hydrastine, the three alkaloids present in the main adulterant of this sample, \( H. \text{canadensis} \).

The fluorescent base was obtained from methanol as almost colourless prisms and was identified as hydrastine by its melting point, elementary analysis, ultraviolet absorption spectrum, solubilities and colour tests. However, it yielded a picrate, m.p. 149\( ^\circ C \) (decomp., tube) which was not in agreement with the reported values of 184\( ^\circ C \) (73) and 190\( ^\circ C \) (74). An authentic sample of hydrastine, obtained from liquid Extract of Hydrastis B.P.C. 1949, yielded a picrate which also melted at 149\( ^\circ C \).

An ethereal solution of the basic material from the second batch of \( A. \text{serpentina} \) was obtained as before and extracted with dilute sulphuric acid. The aqueous layer, on standing, deposited orange crystals (0.025\%\), m.p. 290\( ^\circ C \) (decomp., tube), identified as berberine sulphate by analysis and ultraviolet absorption spectrum. The filtrate on making alkaline, gave an ether-soluble base in 0.027\% yield, after chromatography on alumina. The base was identified by its melting point,
132°C (from methanol), 145°C (from aqueous methanol), as hyd­
rostine which is variously reported as melting at 132°, 135°, (74)
145°C.

No trace of the other base, C_{18}H_{15}O_{10}N, could be found so it is not known whether this base is present in *A. serpentaria* or is derived from some further contaminant. Apart from the doubtful presence of this alkaloid, *A. serpentaria* has therefore been found to contain no ether- or chloroform­
soluble basic material.

*A. longa*

Removal of the ether or chloroform (scheme, page 53) pro­
duced a non-basic dark-brown oil (0.015%), in agreement with (14)
the findings of Hesse who reported the absence of alkaloids in this species.

**General Conclusion**

The work on *A. serpentaria* demonstrates the ease with which adulterants can still escape a careful screening and suggests that earlier reports of the presence of small quantities of basic material in various species of *Aristolochia* might well have been due to adulteration. This may also explain the very small quantity of alkaloid found in *A. indica*. It is probable therefore that no ether- or chloroform-soluble bases are produced by any of the four species of *Aristolochia* examined, though the time of collection of samples might possibly have
an influence on the alkaloidal content and before final conclusions can be made, fresh samples collected at various seasons would have to be examined systematically for alkaloids.
Water-soluble bases

A. reticulata

The crude reineckate of the water-soluble basic material (scheme, page 53) was obtained as a dark-brown solid which was dissolved in dry acetone and filtered from a large amount of amorphous non-alkaloidal impurity. Chromatography of the solution on a column of alumina and elution with acetone gave a compact red zone showing only one fraction to be present. The eluate was concentrated to small bulk and diluted with water to precipitate a pink crystalline reineckate, m.p. 200°C (decomp., tube), \( \text{C}_{17}\text{H}_{20}\text{O}_{3}\text{N} \left[ \text{Cr(SCN)}_4(\text{NH}_3)_2 \right] \cdot 3\text{H}_2\text{O} \). This pure reineckate was decomposed by treating a solution in acetone successively with silver sulphate and barium chloride. Removal of silver reineckate and barium sulphate by filtration and evaporation of the filtrate and washings in vacuo yielded the base chloride as a very hygroscopic, partially crystalline solid. Repeated solution of the product in water removed small quantities of green chromium salts, the presence of which along with the hygroscopic properties of the compound, probably explains the poor analytical figures obtained for the base chloride, \( \text{C}_{17}\text{H}_{20}\text{O}_{3}\text{NCl} \). It was optically active, \([\alpha]_D^\circ +50.83^\circ\), and its ultraviolet absorption spectrum in ethanol showed peaks at 228nm (\( E_{1\text{cm.}}^{1\%} 367 \)) and 286nm (\( E_{1\text{cm.}}^{1\%} 122 \)).

A picrate, aurichloride, and platinichloride of the base were all obtained by conventional methods. The picrate was a
gummy yellow precipitate but on recrystallisation from ethanol a very small yield of crystals, m.p. 178 - 179.5°C was obtained, which was insufficient for analysis. The aurichloride and platinichloride were dark in colour and obviously impure. Attempts to recrystallise them resulted in decomposition, while an attempt to prepare a base iodide was unsuccessful.

The molecular formula of this base corresponds to that of the base reported in *A. indica* but the specific rotation of the base chloride, the melting point of the picrate and the method of isolation all confirm that the two substances are not identical. Shortage of material prevented further investigation of this substance.

*A. indica, A. serpentaria and A. longa*

Extracts from each of these Aristolochias yielded crude reineckates as very dark amorphous solids which were almost totally insoluble in dry acetone. Chromatography on alumina in each case produced negligible quantities of pure reineckate.
Neutral Solids

Concentration of the cold ethanol extract from one batch of *A. reticulata* to a thick black oil and cooling at 0°C gave a small yield (0.01%) of crystalline \( \beta \)-sitosteryl-\( \beta \)-D-glucoside, confirming the observation by Williams that \( \beta \)-sitosterol is present in the plant. The identity of the glucoside was confirmed by conversion to the known tetra-acetate and by hydrolysis with ethanolic hydrochloric acid to \( \beta \)-sitosterol and a reducing sugar, tentatively identified as glucose. \( \beta \)-Sitosteryl-\( \beta \)-D-glucoside was obtained similarly from *A. serpentina* in 0.044% yield in agreement with the findings of Kind and Celentano.

The presence of allantoin has been reported in *A. indica* and *A. cymbifera* but it has not been found in other species. A hot ethanolic extract of *A. reticulata*, however, deposited pale yellow needles (in 0.04% yield) of this substance on concentration, which were identified by comparison with an authentic sample.

These products formed only a small proportion of the total neutral fractions which were viscous oils. Similar oily neutral fractions were obtained from *A. longa*, *A. indica* and *A. serpentina* and the source and yields of these fractions are shown in Table 9. Only one neutral oil, that from *A. reticulata* was further examined, as described below.
Neutral Oil from A. reticulata

The neutral fraction B (Table 9) was fractionated by chromatography in benzene and ethanol on alumina and subsequently by steam distillation to give fractions shown in Table 10 (page 111). Attempts to prepare crystalline derivatives from neutral fractions A, C and D all failed. They were separated into petrol-soluble and petrol-insoluble fractions which were also submitted to steam distillation (Table 11, page 112). Preliminary investigations of fractions I, III, IV, V, VI, VIII, and X failed to yield crystalline or other identifiable material, and these fractions were not investigated further. Fraction VII deposited hexagonal crystals on standing. The oil possessed the odour of borneol but because of the small quantity obtained, this indication of free borneol in the plant could not be confirmed.

Fraction II was a pale yellow, very bright, slightly laevorotatory oil, \([\alpha]_\text{D}^{20} -1.11^\circ\) (in chloroform), \(d_\text{20} 0.963\). It was distilled under reduced pressure and separated into three volatile fractions and a residue which was not further investigated (Table 12). Fraction IX was similarly treated (Table 13).
<table>
<thead>
<tr>
<th>Source</th>
<th>Weight of Powdered Drug</th>
<th>Volume of Percolate</th>
<th>Weight of Neutral oil</th>
<th>%Yield of Neutral oil</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. reticulata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>12.5Kg</td>
<td>&gt; 20 litres</td>
<td>47.0g</td>
<td>0.38</td>
<td>Mobile, reddish-Brown</td>
</tr>
<tr>
<td>Sample 3</td>
<td>12.3Kg</td>
<td>&gt; 20 litres</td>
<td>85.2g</td>
<td>0.665</td>
<td>- do -</td>
</tr>
<tr>
<td>Sample 4</td>
<td>9.5Kg</td>
<td>&gt; 15 litres</td>
<td>51.4g</td>
<td>0.54</td>
<td>- do -</td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. indica</td>
<td>3.0Kg</td>
<td>10 litres</td>
<td>4.86g</td>
<td>0.162</td>
<td>Greenish-red, fairly viscous $\eta_s$ 1.5116</td>
</tr>
<tr>
<td>A. serpentaria</td>
<td>4.2Kg</td>
<td>8 litres</td>
<td>0.77g</td>
<td>0.002</td>
<td>Viscous, reddish-brown</td>
</tr>
<tr>
<td>A. longa</td>
<td>3.01Kg</td>
<td>10 litres</td>
<td>3.75g</td>
<td>0.125</td>
<td>Viscous, dark-red</td>
</tr>
</tbody>
</table>

¥ Percolation continued until eluate was very pale yellow in colour.
### Table 12

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Distillation Temperature °C (0.5mm Hg)</th>
<th>Bath Temp. °C</th>
<th>$n_d^{20}$</th>
<th>Weight (g)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIA</td>
<td>76 - 88</td>
<td>114 - 125</td>
<td>1.4806</td>
<td>3.133</td>
<td>Colourless oil which deposited crystals.</td>
</tr>
<tr>
<td>IIB</td>
<td>88 - 110</td>
<td>120 - 160</td>
<td>1.4888</td>
<td>1.843</td>
<td>- do -</td>
</tr>
<tr>
<td>IIC</td>
<td>102 - 126</td>
<td>156 - 188</td>
<td>1.4980</td>
<td>1.054</td>
<td>Yellow viscous Oil.</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
<td></td>
<td></td>
<td>3.95</td>
<td></td>
</tr>
</tbody>
</table>

### Table 13

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Distillation Temperature °C (0.5mm Hg)</th>
<th>Bath Temp. °C</th>
<th>$n_d^{20}$</th>
<th>Weight (g)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>IXA</td>
<td>74 - 85</td>
<td>120 - 132</td>
<td>1.4785</td>
<td>3.211</td>
<td>Colourless oil $[\alpha]_D^{25} -32.86$, $d_20^{20} 0.973$.</td>
</tr>
<tr>
<td>IXB</td>
<td>85 - 102</td>
<td>132 - 153</td>
<td>1.4881</td>
<td>2.810</td>
<td>Colourless oil $[\alpha]_D^{25} -12.73$, $d_20^{20} 0.942$.</td>
</tr>
<tr>
<td>IXC</td>
<td>102 - 108</td>
<td>153 - 164</td>
<td>1.4980</td>
<td>1.103</td>
<td>Pale yellow oil</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
<td></td>
<td></td>
<td>4.041</td>
<td></td>
</tr>
</tbody>
</table>
These results compare very favourably with those obtained by Williams (Ref. 7, page 67) for his fractions C, D and E which were found to be mixtures of bornyl esters and reticulene and it was reasonable to suppose that the six fractions (Tables 12 and 13) also consisted of such a mixture which had not been fully extracted during percolation with light petroleum.

The crystals from fractions II A and IIB were filtered off, recrystallised from ice-cold light petroleum and identified as borneol by odour, melting point and mixed melting point with authentic borneol, and by preparation of the p-nitrobenzoate. These findings established that free borneol is present in the plant (see also fraction VII). The oily filtrates obtained after removal of the crystals were bulked with fractions IIC, IXA, IXB, IXC and termed fraction XI.

Fraction XI.

Saponification of the oil with ethanolic potassium hydroxide indicated the presence of 19.25% bornyl esters, calculated as formate, assuming the remainder consisted of inert material. Neutral material was extracted with light petroleum after saponification as a deep yellow oil containing borneol which crystallised out. The aqueous liquors remaining after extraction of the borneol-containing oil were acidified and further extracted with light petroleum to give a water-insoluble acid corresponding to that obtained by Williams (7). It was reserved
for future study. Chromatography of the borneol-containing oil yielded four fractions (Table 14).

Table 14

<table>
<thead>
<tr>
<th>Volume of Eluate (ml.)</th>
<th>Eluant</th>
<th>Wt. of Residue (g)</th>
<th>Identification</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Light Petroleum</td>
<td>3.575</td>
<td>XIA</td>
<td>Dextro-rotatory colourless oil, $n_d^i 1.4940$</td>
</tr>
<tr>
<td>120</td>
<td>Light Petroleum</td>
<td>0.420</td>
<td>XIB</td>
<td>Laevo-rotatory colourless oil, $n_d^i 1.5022$</td>
</tr>
<tr>
<td>80</td>
<td>Light Petroleum</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>360</td>
<td>Light Pet. + 5% ethanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>Light Pet. + 5% ethanol</td>
<td>0.327</td>
<td>XIC</td>
<td>Sweet smelling laevo-rotatory oil, $n_d^i 1.4863$ which contained no crystalline material</td>
</tr>
<tr>
<td>150</td>
<td>Light Pet. + 5% ethanol</td>
<td>2.371</td>
<td>XID</td>
<td>Borneol contaminated with sweet smelling oil</td>
</tr>
</tbody>
</table>

Fraction XIA

The oil possessed the odour of reticulene and, with the exception of specific rotation, the physical constants are in agreement with those for reticulene. Redistillation failed to alter the specific rotation (Table 15) but this discrepancy
and the minor differences in the respective infrared absorption spectra can be readily explained by the presence of some volatile impurity.

<table>
<thead>
<tr>
<th></th>
<th>(7)</th>
<th>Fraction XIA.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reticulene</td>
<td>1st. Distn.</td>
</tr>
<tr>
<td>$E_{1cm}^{1%}$</td>
<td>148</td>
<td>136</td>
</tr>
<tr>
<td>$n_{D}$</td>
<td>1.4955</td>
<td>1.4956</td>
</tr>
<tr>
<td>$d_{16}^{16}$</td>
<td>0.913</td>
<td>0.919</td>
</tr>
<tr>
<td>$[\alpha]_{D}^{15}$</td>
<td>+1.6</td>
<td>+7.0</td>
</tr>
<tr>
<td>$[R_{L}]_{D}$</td>
<td>65.2</td>
<td>64.7</td>
</tr>
</tbody>
</table>

**Fraction XIB**

This oil also had an odour of reticulene but because it had a negative rotation, it was kept as a separate fraction, the small quantity of which made further study impossible. In a recent publication, Sorm and his co-workers were able to isolate two isomeric aromadendrenes from *Eucalyptus globulus*, which they named aromadendrene ($[\alpha]_{D}^{10} +24.5^\circ$) and allo-aromadendrene ($[\alpha]_{D}^{10} -21.6^\circ$). Apart from the values for specific rotation, they had similar constants, and an explanation based on isomerism is conceivable in this case.
**Fraction XIC**

Between this fraction and the previous one, 440ml. of eluate were collected which yielded nothing on evaporation proving that fraction XIC was a separate one and not a mixture of reticulene and borneol as suggested by Williams. Also, this fraction did not become yellow on standing as did reticulene, and no p-nitrobenzoate could be prepared, indicating the absence of borneol.

**Fraction XID.** On recrystallisation from light petroleum gave pure borneol.

The ability to separate fraction XI into four components was confirmed by later work on reticulene (see Part II, page 136) and the isolation of the laevorotatory fraction XIB, which was not noted by Williams, could partly explain the difference in $[\alpha]_D$ values for reticulene and fraction XIA. It was therefore apparent that the volatile portion of the neutral oil from *A. reticulata* consisted of light petroleum-soluble fractions which had not been fully extracted with that solvent. The light petroleum-insoluble portion of the neutral oil has been shown to be chemically unreactive and possibly consists of mixtures of hydrocarbons and esters of long-chain alcohols.
Although Krishnaswamy and his co-workers were able to isolate a basic substance $C_{17}H_{19}O_3N$ from *A. indica*, this was not confirmed in the present work. On the other hand much higher yields of aristolochic acid have been obtained than were reported by the Indian workers (Table 3). It is possible therefore that the yields of acids and bases may be complementary and that the discrepancies which have been noted can be explained in terms of seasonal variations. This would imply some biogenetic relationship between acid and base in *A. indica*, a hypothesis which is supported by the identical carbon to nitrogen ratios of the base $C_{17}H_{19}O_3N$ and aristolochic acid $C_{17}H_{11}O_7N$. Further evidence for this possible acid/base relationship is drawn from two sources, (a) the isolation of a base, $C_{17}H_{19}O_3N$, as its reineckate, $C_{17}H_{20}O_3N \cdot [Cr(SCN)_4(NH_3)_2] \cdot 3H_2O$, from *A. reticulata*, (b) the presence of magnoflorine (XXII), $C_{20}H_{24}O_4N$, in *A. kaempferi*, *A. debilis* and *A. clematitis*, a base whose structure bears a formal resemblance to that of aristolochic acid lactam (VII).}

A proposed scheme for the preparation of a basic substance, $C_{17}H_{19}O_3N$, from aristolochic acid (IV) is shown below (page 81). The final product (XXVII) was considered to be of interest
not only because its empirical formula is identical with that of aristolochine, but also with morphine (XXXIII) with which, together with apomorphine (XXXIV), there are structural similarities.

Attempts to prepare the base (XXVII) by the proposed method have so far failed due to the abnormal properties of the primary
Quinoline
Methylation

Due-
Catalytic reduction

C
H$_2$

OCH$_3$

N
CH$_3$

OCH$_3$

H$_2$

H$_2$

H$_2$

H$_2$

H$_2$

N
CH$_3$

OCH$_3$

N
CH$_3$

OCH$_3$

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N
CH$_3$

OCH$_3$

N
CH$_3$
amine (Xlll) which is surprisingly non-reactive.

Decarboxylated aristolochic acid (V) was readily obtained by the method previously reported but reduction of this compound to the primary amine proved difficult. This accords (21) with the findings of Pailer, Belohlav and Simonitsch who obtained it by catalytic reduction as a labile compound, m.p. 170 °C, which required careful isolation. Catalytic hydrogenation with platinum in dry benzene nearly always resulted in incomplete reduction (the weight of catalyst may be critical) but a pale yellow crystalline solid, m.p. 125 - 126.5 °C (microblock), which fluoresced blue-green in the solid state and in solution could be isolated. Recrystallisation of the solid from aqueous methanol or ethanol gave a non-fluorescent pale-brown solid, C_{16}H_{13}O_{3}N, m.p. 168 - 169.5 °C, in agreement (21) with the findings of the Austrian workers.

Due to the poor yields obtained by this reduction, other methods of preparing the primary amine were attempted. Reduction of decarboxylated aristolochic acid (V) with zinc in glacial acetic acid gave a colourless highly fluorescent solution which gradually became red on cooling. The solution was filtered from zinc and zinc acetate and carefully basified with sodium hydroxide solution when a dark-brown precipitate separated which could not be recrystallised from the various solvents used. A further reduction was attempted by refluxing with 5% palladium on charcoal and hydrazine hydrate in ethanol (78). This produced grey crystals, m.p. 166 - 168 °C
which on recrystallisation from aqueous methanol were identical with the pale-brown product obtained on catalytic reduction. The yield of product in this case was even less than in the catalytic method which seems the method of choice.

The base (Xlll) gave a deep-red colour with concentrated sulphuric acid, was insoluble in hot dilute hydrochloric acid and only slightly soluble in warm concentrated hydrochloric acid. Because of its non-reactivity with diazomethane (reported later), together with these unusual solubilities, it appeared doubtful if, in fact, the substance was a primary amine. It was finally proved to be so by preparation of a hydrochloride, m.p. 169°C (decomp., microblock) which analysed rather poorly to $C_{16}H_{13}O_3N$. HCl, and by a comparison of the ultraviolet absorption spectra in both neutral and acid solution (Figure 11). The spectrum in neutral solution was phenanthrenoid in type and in acid solution a hypsochromic shift of the maxima, especially the one at 330m$\mu$, together with the introduction of fine structure above 340m$\mu$ is in agreement with that displayed by 9-aminophenanthrene in neutral and acid solution (79).

Attempts to methylate the base (Xlll) by treating with diazomethane in dry and moist ether, and with methyl iodide in benzene were unsuccessful. Reaction with methyl iodide under reflux and by heating in a sealed tube gave what was probably the N-methyl hydriodide in poor yield.
Figure II

Ultraviolet absorption of 1-methoxy-5,6-methylenedioxy-9-aminophenanthrene (XIII)

---

in ethanol
---

in ethanol - 2N hydrochloric acid (equal parts).

---

log ε

---

WAVELENGTH (mμ)
Due to insufficient quantities of starting material and poor yields of products, this aspect of the problem had to be prematurely broken off at this point, but the results are sufficiently interesting to permit further study, though on a larger scale, when fresh supplies of the crude drug become available.
MICROBIOLOGICAL TESTING OF ARISTOLOCHIC ACID

A 1:1000 aqueous solution (pH 6.96) of sodium aristo-lochate (prepared by dissolving aristolochic acid in water containing an exact equivalent of sodium hydroxide) was tested by the trough-plate method against various bacteria, moulds and yeasts by Dr. E. O. MORRIS of the Royal College of Science and Technology, Glasgow. Complete inhibition was not observed in any case but moderate to marked restriction was apparent in the case of three moulds. Table 16 shows the results after 2 days incubation.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Observation on Organism Growth</th>
<th>Culture Medium and Incubation Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BACTERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Gram negative.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>No apparent restriction</td>
<td>Nutrient agar, 37°</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>No apparent restriction</td>
<td>Nutrient agar, 37°</td>
</tr>
<tr>
<td>E. coli</td>
<td>No apparent restriction</td>
<td>Nutrient agar, 37°</td>
</tr>
<tr>
<td>Salmonella gallinarum</td>
<td>No apparent restriction</td>
<td>Nutrient agar, 37°</td>
</tr>
<tr>
<td>Shigella paradysepteriae</td>
<td>No apparent restriction</td>
<td>Nutrient agar, 37°</td>
</tr>
<tr>
<td>Acetobacter sp.</td>
<td>Very slight restriction</td>
<td>Malt-wort agar, 25°</td>
</tr>
<tr>
<td>ii. Gram positive.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Slight restriction</td>
<td>Nutrient agar, 37°</td>
</tr>
<tr>
<td>Staphylococcus albus</td>
<td>Slight restriction</td>
<td>Nutrient agar, 37°</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Slight restriction</td>
<td>Nutrient agar, 37°</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>Moderate restriction</td>
<td>Nutrient agar, 25°</td>
</tr>
<tr>
<td>Micrococcus citreus</td>
<td>Slight restriction</td>
<td>Nutrient agar, 25°</td>
</tr>
<tr>
<td><strong>YEASTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>Very slight restriction</td>
<td>Malt-wort agar, 25°</td>
</tr>
<tr>
<td>Pichia membranaeafaciens</td>
<td>Very slight restriction</td>
<td>Malt-wort agar, 25°</td>
</tr>
<tr>
<td>Candida utilis</td>
<td>Very slight restriction</td>
<td>Malt-wort agar, 25°</td>
</tr>
<tr>
<td>Nadsonia fulvescens</td>
<td>Very slight restriction</td>
<td>Malt-wort agar, 25°</td>
</tr>
<tr>
<td><strong>MOULDS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td>Very slight restriction</td>
<td>Czapek-Dox (3% glucose), 25°</td>
</tr>
<tr>
<td>Penicillium notatum</td>
<td>Moderate restriction of new transfers.</td>
<td></td>
</tr>
<tr>
<td>Mucor sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizopus nigricans</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


EXPERIMENTAL
EXPERIMENTAL

M.p.s are uncorrected. Rotations were determined in absolute ethanol (unless otherwise stated) in a 1 dcm. tube. Ultraviolet absorption spectra were determined in absolute ethanol on a Hilger Uvispek photoelectric spectrophotometer. \( R_F \) values were determined on Whatman No. 1 paper with 4 : 1 ethanol -5% formic acid as solvent. The author is indebted to Mr. W. McCorkindale, Dr. A. C. Syme and Mr. W. Gardiner for the microanalyses, to Mr. S. G. E. Stevens and Mr. A. J. Cross of Smith, Kline and French Laboratories Limited for the infrared spectra, and to the Pharmacognosy staff for the identification and removal of adulterants from the raw drugs. The light petroleum used boiled over the range 40 - 60°C.
THE ETHANOL-SOLUBLE FRACTION OF *A. RETICULATA* LINN.
Preparation and extraction of the crude drug

Four samples of drug (total weight 34.8 Kg.) were obtained at intervals of several months and each satisfied the pharmaco-cognostical description of A. reticulata. The dried roots and rhizomes of each separate sample were reduced to a fine powder (approx. 60-80 mesh), defatted with light petroleum and dried. The powder, in batches of 6 Kg., was macerated overnight under ethanol in a large copper percolator, then percolated at room temperature with ethanol until the eluant was almost colourless (7-14 days). Concentration of the percolate under reduced pressure gave an almost black thick oil which still contained some solvent.
ISOLATION OF $\beta$-SITOSTERYL-$\beta$-D-GLUCOSIDE

The thick oil obtained from one 6Kg. batch was concentrated further and set aside for 3-4 days when $\beta$-sitosteryl-$\beta$-D-glucoside (0.6g.) separated as a pale brown powder, m.p. 260-270°C (decomp.). Repeated crystallisation from large volumes of ethanol (charcoal) gave a colourless product, m.p. 295-296°C (decomp., microblock), $\lambda_{210\text{nm}}$ ($\epsilon_{1530}$, end absorption). Kind and Celentano gave m.p. 295-297°C.

Found: C 72.3; H 10.5%.
Calculated for C$_{35}$H$_{60}$O$_6$: C 72.9; H 10.5%.

ISOLATION AND TREATMENT OF CRUDE ACIDIC FRACTIONS

The thick oil was dissolved in ether and the almost black solution extracted with dilute hydrochloric acid (treatment of this acid extract is reported on page 92). The ethereal solution was then extracted successively with 2% aqueous potassium hydrogen carbonate, 5% aqueous sodium carbonate and 5% aqueous sodium hydroxide.

Treatment of potassium hydrogen carbonate solution

Acidification with either dilute hydrochloric acid or acetic acid gave a crude mixture of acids (30g.) as an amorphous powder which varied in colour from yellow to reddish-brown.
Isolation of aristolochic acid

Recrystallisation of the crude acid fraction from either dioxan or glacial acetic acid gave aristolochic acid, m.p. 275-277°C (decomp., tube), 284-285°C (decomp., microblock) in various crystalline forms but mainly as yellow microcrystals. Further recrystallisation from \( \text{NN-dimethylformamide} \) — ethanol (1:6) failed to raise the melting points but gave aristolochic acid as orange-yellow needles, (total yield 7.2g.), \( R_F \) 0.91-0.94, \( \lambda_{\text{max}} \) 223(630,000), 250(629,400), 318(613,100), 390m\( \mu \) (67,300). Rosenmund and Reichstein gave m.p. 274-278°C (decomp.) and Pailer, Belohlav and Simonitsch gave m.p. 281-286°C (decomp., microblock).

Found: 
C 60.2, 59.6 ; H 3.3, 3.2 ; N 4.1 ;
OCH\(_3\) 9.15%

Calculated for \( C_{17}H_{11}O_7N \): C 59.8 ; H 3.2 ; N 4.1 ;
OCH\(_3\) 9.1%

Isolation of aristo-red

Repeated concentration of the mother-liquors remaining after the isolation of aristolochic acid yielded mixtures of aristo-red and aristolochic acid which could not be separated on recrystallisation from ethanol, acetone, dioxan or glacial acetic acid. Chromatography from ethanol or dioxan on a column of acid-washed alumina (prepared by adding alumina to acetic acid, leaving overnight then washing free from acid and drying at 120°C) was too slow to be of value in separation.
Chromatography on a buffered silica gel column [prepared by mixing a pH 7.2 buffer solution (5ml.) with silica gel (10g.)] using either chloroform or ether saturated with buffer solution as eluant, was useless for the same reason. Separation was achieved eventually by suspending the mixture of aristo-red and aristolochic acid in ethanol and quickly decanting the solvent which contained most of the less dense crystals of aristolochic acid. The crude aristo-red was repeatedly recrystallised from ethanol as red needles (50mg.), m.p. 236.5°C (microblock), R_f 0.77-0.80 (fluorescent spot), λ_max. 253(ε42,400), 265(ε31,500), 294(ε19,350), 300(ε19,100), 305 (ε13,800), 395mυ (ε8,200) with inflections at 335(ε5850) and 352mυ (ε5000).

Found: C 64.6; H 4.2; N 3.8; OCH_3 25.5, 26.1%

C_{16}H_{6}O_3N(OCH_3)_3 requires: C 64.6; H 4.3; N 3.95; OCH_3 26.3%

Further attempt to separate aristo-red from a mixture with aristolochic acid

The mixture (0.400g.) was refluxed for 1 hour with pyridine (3.5ml.) and acetic anhydride (2ml.). On cooling and adding water a black oily solid (decomposed aristolochic acid) separated and was removed. The filtrate was extracted with ether which gave an orange product (64mg.) on evaporation. Attempts to recrystallise this solid from ethanol, ether, ethyl acetate, light petroleum, glacial acetic acid, pyridine, benz-
ene and various mixtures of these solvents all failed. Sublimation at 260°C/0.1mm. caused decomposition.

Treatment of sodium carbonate solution

Addition of either dilute hydrochloric acid or acetic acid to this highly coloured (reddish-brown) solution gave negligible quantities of a red resinous solid.

Treatment of sodium hydroxide solution

This consisted of two layers, a viscous black oil and an almost black aqueous layer. Acidification (10% hydrochloric acid) of the latter gave a reddish-yellow solid which rapidly became oily.
EXAMINATION OF ACID EXTRACT

This was obtained as reported on page 88. The acidic solution, after a few days, was basified (dilute ammonium hydroxide) and extracted with ether which was in turn extracted with dilute hydrochloric acid. This gave 3 fractions: an ether solution, an aqueous acidic solution, and an aqueous basic solution.

**Isolation of isorhamnetin**

The ether solution was washed with water until free from acid, dried (Na₂SO₄) and evaporated to give yellow microcrystals of isorhamnetin (0.54 g.), m.p. 318-322°C (decomp., microblock) (from dioxan), raised to 324°C on repeated sublimation at 300°C/0.5 m.m. \( \lambda_{\text{max}} \) 255 (ε21,150), 307(ε7,950), 370-372 μm (ε22,100). A sample of isorhamnetin obtained from G. Tappi had m.p. 318-320°C (decomp., microblock), \( \lambda_{\text{max}} \) 255(ε21,250), 307(ε8,150), 370-372 μm (ε22,120).

Found: C 60.8; H 3.7; O 35.9; OCH₃ 11.5%.

Calculated for C₁₅H₉O₆·OCH₃:

C 60.8; H 3.8; O 35.45; OCH₃ 9.8%

_Ultraviolet absorption spectrum of isorhamnetin in ethanolic sodium ethoxide_ (55)

This was carried out by the method of Jurd and Horowitz allowing one hour for the reaction. \( \lambda_{\text{max}} \) 335(ε21,200), 250-
Ultraviolet absorption spectrum of isorhamnetin in the presence of boric acid and sodium acetate

This was recorded using the method of Jurd. \( \lambda_{\text{max}} \)

252\( \mu \) (\( \epsilon 10,480 \) flat).

255(\( \epsilon 20,350 \)), 307(\( \epsilon 7,814 \)), 370-372\( \mu \) (\( \epsilon 21,080 \)).

Attempted isolation of water-insoluble bases.

The aqueous acidic solution was extracted successively with ether then chloroform but removal of the organic solvents gave only traces of dark brown oils which had slight positive reactions with alkaloidal reagents.

Isolation of water-soluble base as reineckate

The aqueous basic solution was acidified to congo-red (dilute sulphuric acid) and treated with a saturated solution of ammonium reineckate in excess. The dark-brown crude base reineckate (31.2g) was dissolved in dry acetone and filtered from a large quantity of non-alkaloidal material. The deep red acetone solution was chromatographed from dry acetone on alumina (20" x 1.3"), the single red band eluted in acetone, and the solution evaporated (water-bath, \(<50^\circ C\)) to give a pink crystalline reineckate which on recrystallisation from aqueous acetone had m.p. 200° C (decomp., tube; insert at 195° C).

Found:  
C 37.8; H 4.8; N 14.8; OCH\(_3\) 4.1, 4.0%

\( \text{C}_{16}\text{H}_{17}\text{O}_2\text{N}(\text{OCH}_3)[\text{Cr(SCN)}_4(\text{NH}_3)_2]\cdot3\text{H}_2\text{O} \) requires:

C 38.3; H 4.9; N 14.9; OCH\(_3\) 4.7%
ISOLATION OF NEUTRAL OIL

The ethereal solution, obtained after successive extractions with aqueous solutions of potassium hydrogen carbonate, sodium carbonate and sodium hydroxide (see page 88), was washed free of alkali with water, dried ($\text{Na}_2\text{SO}_4$) and evaporated to give a large yield of neutral oil (Total weight $=183.6\text{g.}$).

A portion ($22\text{Kg.}$) of the marc remaining from the cold percolate was continuously extracted in batches of $6\text{Kg.}$ with hot ethanol ($14-20\text{ l.}$) until the percolate was pale yellow ($3-6\text{ days}$). Concentration of the percolate and cooling gave a bulky resinous precipitate, which was rejected before further concentration of the liquid to a dark viscous oil.

ISOLATION OF ALLANTOIN

The viscous oil obtained from one $6\text{Kg.}$ batch was further concentrated and on cooling allantoin separated as pale-brown crystals. Recrystallisation from $80\%$ aqueous ethanol with charcoaling gave colourless needles ($1.1\text{g.}$) m.p. and mixed m.p. $232^\circ\text{C}$ (decomp., tube).
Found: C 30.8; H 4.1; N 35.2%
Calculated for C₄H₆O₃N₄: C 30.4; H 3.8; N 35.4%

ISOLATION OF CRUDE ACIDS, BASIC MATERIAL AND NEUTRAL OIL

These fractions were obtained by methods similar to those described under the cold ethanol-soluble fraction.

Acidic material

The crude acid fraction (approx. 8g.) was separated by fractional crystallisation from glacial acetic acid into aristolochic acid (1.80g.), R$_f$ 0.91, and aristo-red (108mg.), R$_f$ 0.77, the latter being contaminated with aristolochic acid.

Basic material

The water-insoluble basic fraction was obtained as an almost black oil (0.5g.) which did not give positive tests with the common alkaloidal reagents.

A bulky brown crude reineckate (4.3g.) was obtained. It was almost completely insoluble in dry acetone.

Neutral oil

The neutral oil (40.1g.) was brown and fragrant and similar to that obtained from the extract with cold ethanol.
The powdered drug (500g.) was macerated for 42 hours with 2 l. of a mixture of water (7 parts), acetone (3 parts) then centrifuged to give a dark-red aqueous acetone extract (1.40 L). The latter was extracted with ether (700, 700, 300, 300 ml.) and the ether removed giving a brown oil which was repeatedly extracted with small volumes of ether (5 x 30 ml.). These were bulked, dried (Na₂SO₄) and evaporated, and in this way, a yellow hygroscopic solid (1.11g.) was obtained which started to melt at 52°C but continued to melt over a very wide range. It was not hydrolysed on heating with sulphuric acid (7%, 2 hours) and showed no tendency to sublime when heated to 320°C.
REACTONS OF ARISTOLOCHIC ACID

Decarboxylation

The acid (102mg.) was refluxed for 10mins. with copper powder (120mg.) and quinoline (16ml.). The mixture was cooled and extracted with ether (40ml.), the ethereal solution washed with dilute hydrochloric acid (50, 50, 25, 25ml.), water (2 x 25ml.), 5% aqueous sodium hydrogen carbonate (50, 25, 25ml.) and water (2 x 25ml.), dried (Na$_2$SO$_4$) and evaporated. The brown residue was chromatographed in benzene on alumina (10cm. x 1.2cm.) and the eluate evaporated. The residue crystallised from chloroform-ethanol and sublimed at 200°C/0.1mm., to yield orange needles (61mg.) of 1-methoxy-5,6-methylenedioxy-9-nitrophenanthrene, m.p. 213°C (microblock), $\lambda_{\text{max.}}$ 247.5(£39,930), 286(£12,510), 310(£9970), 395m$\mu$ (£4460). Pailer, Belohlav and Simonitsch gave m.p. 212°C (microblock).

Found: C 64.9; H 4.1; N 4.5%
Calculated for C$_{16}$H$_{11}$O$_5$N : C 64.65 H 3.7; N 4.7%

Reduction

a) Many catalytic reductions were attempted and the following is a typical example. (See also Table 2)

Aristolochic acid (52.0mg.) was hydrogenated in glacial acetic acid (106 ml.) at a platinum oxide catalyst (50.5mg.). Hydrogen uptake was complete after 30mins. with the absorption
of 19.8 ml. (at N.T.P.). Allowing the appropriate volume for reduction of the catalyst, the volume of hydrogen absorbed by the acid was equivalent to 2.94 double bonds. The fluorescent solution was filtered and the glacial acetic acid removed under reduced pressure to give a yellowish-brown crystalline solid. Repeated recrystallisation from glacial acetic acid failed to give a product with a constant melting point. The best sample melted at 280-282°C (decomp., microblock).

b) Aristolochic acid (360 mg.) was refluxed for 45 mins. with zinc powder (1.04 g.) and glacial acetic acid (20 ml.). The fluorescent solution was filtered hot and on cooling deposited the bulk of the crude product (225 mg.). The mother liquors, treated with water (50 ml.), yielded a further precipitate which was dissolved in chloroform, washed repeatedly with water, dried (Na₂SO₄) and evaporated to give a further 40 mg. of crude product. Sublimation at 240-250°C/0.1 mm. gave greenish-yellow crystals of 9-amino-1-methoxy-5,6-methylene-dioxy-8-phenanthroic lactam, m.p. 320°C (microblock, inserted at 315°C), λ max. 222 (ε 22,900), 242 (ε 30,840), 250 (ε 29,740), 260 (ε 36,130), 291 (ε 15,050), 301 (ε 15,450), 327 (ε 9220), 346 (ε 7190), 395 mμ (ε 8470). Pailer, Belohlav and Simonitsch gave m.p. 319°C.

Found: C 69.8; H 4.2; N 4.8%
Calculated for C_{17}H_{11}O_{4}N : C 69.6; H 3.8; N 4.8%
Preparation of lactam acetate:

a) 9-amino-1-methoxy-5,6-methylenedioxy-8-phenanthroic lactam (100mg.) was refluxed for 30mins. with acetic anhydride (0.5ml.) and pyridine (1ml.). The yellow precipitate which separated during the reaction and on cooling (52mg.) sublimed at 250-260°C/0.1mm. and yielded 9-acetamido-1-methoxy-5,6-methylenedioxy-8-phenanthroic lactam as a greenish-yellow solid, which fluoresced under ultraviolet light both in the solid state and in solution. It had m.p. 283-286°C (decomp., sealed tube), 295°C (decomp., microblock), $\lambda_{\text{max.}}$ 227(€36,200), 242(€47,750), 252(€34,700), 288(€15,800), 300(€13,500), 328 (€8630), 344(€6,600), 388(€8,130), 406mu (€8630). Rosenmund and Reichstein gave m.p. 292-296°C (decomp.) for the so-called "diacetate".

Found: C 68.15; H 3.6; N 4.4; OCH$_3$ 8.5%

C$_{19}$H$_{13}$O$_5$N
requires: C 68.1; H 3.8; N 4.2; OCH$_3$ 9.25%

b) Aristolochic acid (42mg.) was refluxed for lhr. with acetic anhydride (2.5ml.), pyridine (1ml.) and zinc powder (102mg.). The hot solution was filtered free of zinc powder and zinc acetate and on cooling deposited crude lactam acetate (14mg.). The mother liquors were evaporated to give a further 29mg. of crude product. Sublimation at 260°C/0.1mm. gave greenish-yellow crystals, m.p. 295°C (decomp., microblock).
REACTIONS OF ARISTO-RED

Acetylation

Aristo-red (15mg.) was refluxed for 30mins. with acetic anhydride (0.25ml.) and pyridine (0.5ml.). The cooled mixture was extracted with ether which slowly deposited pale orange needles of 9-acetamido-1,3,4,6-tetramethoxy-5,6-methylene-dioxy-8-phenanthroic lactam (6mg.). This fluoresced under ultraviolet light both in the solid state and in solution and had m.p. 276-278°C (microblock), $\lambda_{max}$ 240(635,730), 250.5 (638,860), 291(617,960), 302(615,690, shoulder), 330(65,580, inflection), 343(64410), 385(66680), 400mµ (66740).

Found: N 4.2%

C$_{21}$H$_{17}$O$_7$N requires: N 3.55%

Attempted Reduction

Aristo-red (28mg.) was dissolved in glacial acetic acid (3ml.) and refluxed with zinc dust (68mg.). The solution immediately turned dark brown so refluxing was stopped after 15mins. The solution was flooded with water (100ml.) and extracted with chloroform which removed all the colour. The chloroform was dried (Na$_2$SO$_4$) and evaporated to give a small quantity of a reddish-brown material which could not be crystalised from glacial acetic acid. Paper chromatography gave 2 spots, the main one fluorescent with $R_f$ value 0.30 (aristo-red).
REATIONS OF ISORHAMNETIN

Distillation with zinc dust

Isorhamnetin (50mg.) was intimately mixed with zinc dust (Analar grade) (600mg.) placed in a pyrex tube (12" x 0.3") and covered with a further layer of zinc dust (600mg.). Heat was applied (microflame) and gave a yellow oily distillate with a phenolic odour whose ultraviolet absorption spectrum (qualitative) had a maximum at 253 mp.

Preparation of tetraacetate

Isorhamnetin (40mg.) was refluxed for 30 mins. with acetic anhydride (2ml.) and pyridine (2ml.). To the cooled mixture, water was added dropwise to give white needles (72mg.) which fluoresced brilliant green in ultraviolet light and when re-crystallised from ethanol had m.p. 214-215 °C (microblock), λmax. 239(ε 20,650), 310 μ (ε 16,050). A sample of isorhamnetin-3,4',5,7-tetraacetate obtained from G. Tappi had m.p. 210-211 °C (microblock), λmax. 240(ε 21,750), 310 μ (ε 16,700) and displayed similar fluorescent properties.

Found:       C 60.2; H 4.5; OCH₃ 6.65%
Calculated for C₂₂H₁₈O₁₀: C 59.7; H 4.1; OCH₃ 7.0%.

Preparation of quercetin-3,3',4',7-tetramethyl ether

Isorhamnetin (40mg.) was suspended in dry ether (12ml.) and an excess of diazomethane in dry ether added but no re-
action occurred until a drop of water was added as catalyst. After 3 hours, the excess diazomethane and solvent were removed giving long pale-yellow needles (19mg.) of quercetin-3,3',4,7-tetramethyl ether, m.p. 159-160°C (tube), 160-161°C (microblock) when recrystallised from ethanol. $\lambda_{\text{max}}$ 254 (log€ 4.33), 269 (log€ 4.26), 353μ (log€ 4.305). Gomm and Nierenstein gave m.p. 159-160°C. Briggs and Locker gave $\lambda_{\text{max}}$ 254 (log€ 4.37), 269 (log€ 4.29), 352μ (log€ 4.34).

Found: C 63.5; H 5.4% 
Calculated for $C_{19}H_{18}O_7$: C 63.7; H 5.1%

**Attempted hydrolysis of acetate**

The acetate (26.45mg.) was refluxed for 1 hour with 0.1 N sodium hydroxide solution (5ml.) in ethanol (20ml.). A blank determination was also carried out, omitting the acetate. On cooling, both solutions were titrated with 0.1 N hydrochloric acid using phenolphthalein (15 drops) as indicator. The volume of 0.1 N solution (4.12ml.) equivalent to the acetate (26.45mg.) indicated an equivalent weight of 64.2 (assuming utilisation of 7 equivalents of alkali, calculated equivalent weight for isorhamnetin tetraacetate is 63.2). The slightly acidic aqueous solution was extracted with ether and the latter washed with water, dried (Na$_2$SO$_4$) and evaporated to give a colourless oil (22mg.). Recrystallisation from methanol/ether gave colourless crystals, m.p. 45-51°C (microblock), which showed benzenoid absorption in the ultra-
violet (qualitative).

**ISOLATION AND TREATMENT OF WATER-SOLUBLE BASE**

**Decomposition of base reineckate (page 93)**

The reineckate (0.79g) was dissolved in dry acetone (20ml.) and excess solution of silver sulphate added (0.599% 35.0ml.), followed by an equivalent volume of a solution of barium chloride (1.062% BaCl₂·2H₂O, 15.50ml.) when precipitation of silver reineckate had ceased. The combined precipitates of silver reineckate and barium sulphate were filtered off and washed thoroughly with distilled water; the combined filtrate and washings were evaporated to dryness (water-pump). This gave a very hygroscopic partially crystalline solid of doubtful purity (0.216g.) from which inorganic material, especially chromium salts, could not be completely removed. After repeated solution in water, the yellow base had $\left[\kappa^\theta\right]_b +50.83^\circ$ (C 0.6), $\lambda_{max.} 228\left( E_{icm.} 367\right)$, 286µ ( $E_{icm.} 122\right)$. Found: C 61.0; H 9.2; N 5.6%

The expected base chloride, C₁₁H₂₀O₃NCl would require:

C 63.4; H 6.3.; N 4.4%

**Preparation of base picrate**

The base chloride (50mg.) was dissolved in water (2ml.) and to this solution was added an aqueous solution of picric
acid (0.66%, 4ml.). Recrystallisation of the bulky product from ethanol was accompanied by decomposition and gave crystals (4mg.), m.p. 173-179.5°C (decomp., microblock).

**Attempted preparation of base aurichloride**

The base chloride (82mg.) was dissolved in water (10ml.) and filtered from green insoluble material.

A slight excess of solution of auric chloride (2%\% HAuCl₄·3H₂O) was added and a bulky brown precipitate obtained but an attempted reprecipitation from hot water resulted in decomposition and formation of a gold mirror. A further attempt to dry the initial crude precipitate at 60°C/18mm. also caused decomposition.

**Attempted preparation of base platinichloride**

The base chloride (80mg.) was dissolved in water (10ml.) and, once again, green insoluble material had to be removed. Solution of platinum chloride (5%\% H₂PtCl₆·6H₂O) was added dropwise until precipitation of the crude platinichloride ceased. The pale brown precipitate (72mg.) partially decomposed on attempted recrystallisation from hot water and the dark yellow product obtained (10mg.) was not sufficiently pure for analysis.

**Attempted preparation of base iodide**

The base chloride (80mg.) was dissolved in water (4ml.) filtered, and to the filtrate was added a solution of potassium iodide (20%, 2ml.). The supernatant liquid was de-
canted from the oil which formed and the latter dried in vacuo (water-pump). Attempted recrystallisations from ethanol/ether mixtures always produced a yellow oil.

**REACTIONS OF β-SITOSTERYL-β-D-GLUCOSIDE**

β-Sitosteryl-β-D-glucoside tetraacetate

β-Sitosteryl-β-D-glucoside (77mg.) and sodium acetate (56mg.) were gently refluxed in acetic anhydride (2ml.) for 1 hour. The solution was cooled, poured onto crushed ice (1g.) and left for 30mins., then carefully neutralised (10% NaOH). The crude acetate (93mg.) was recrystallised from aqueous ethanol as lustrous plates, m.p. 166.5-167.5°C (microblock), [α]_D^25 -23.91° (c=0.92 in chloroform) (38)

Kind and Celentano gave m.p. 167.5-168.5°C, [α]_D^25 -23.7° and -24.2°.

Found: C 69.1; H 9.2%

Calculated for C_{43}H_{68}O_{10}: C 69.35; H 9.2%

**Hydrolysis to β-sitosterol**

β-Sitosteryl-β-D-glucoside (165mg.) was refluxed for 9 hours in ethanol (15ml.) and concentrated hydrochloric acid (0.3ml.). The solution was concentrated to 4ml. and water (3ml.) added to precipitate β-sitosterol. Recrystallisation from aqueous methanol gave needles, m.p. 139°C (tube), 140.5-141°C (microblock), mixed m.p. 139-140.5°C (tube),
\[ [\alpha]_D^0 = -37.75^\circ \text{ (c=0.5 in chloroform).} \]

Kind and Celentano gave m.p. 140° C, \([\alpha]_D^0 = -37^\circ \text{ and } -38^\circ .\]

**Found:**

C 82.4; H 12.1%  

**Calculated for \(\text{C}_29\text{H}_50\text{O}_.5\text{CH}_3\text{OH}\):**

C 82.2; H 12.2%.

**Attempted preparation of osazone**

The filtrate (10ml.) obtained on removal of \(\beta\)-sitosterol was decolourised by boiling with charcoal. To a portion of the filtrate (9ml.) was added phenylhydrazine hydrochloride (84mg.) and sodium acetate (124mg.) and the mixture boiled (water bath). After four minutes boiling, a distinct cloudiness suddenly developed, in agreement with the sugar being glucose. Attempted recrystallisation of the small precipitate resulted in loss of the material.

**Test for reducing sugar**

To the boiling filtrate (1ml.), boiling Fehling's solution was added dropwise. The latter was decolourised and a brick red precipitate formed.
TREATMENT OF NEUTRAL OIL FROM A. RETICULATA

Neutral Fraction A (Table 9) - Preliminary reactions

Attempted preparation of 2,4-dinitrophenylhydrazone

The oil (0.2g.) was dissolved in ethanol (2ml.) and a solution of 2,4-dinitrophenylhydrazine (0.3g.) in ethanol (10ml.) and sulphuric acid (0.5ml.) added. The red precipitate which formed immediately was filtered off. Attempted recrystallisation from ethanol gave an oily product.

Attempted preparation of semicarbazone.

The oil (0.223g.) was mixed with a solution of semicarbazide hydrochloride (0.2g.) and sodium acetate (0.2g.) in water (2ml.). The mixture was heated and ethanol added to give a clear solution. The solution was gently refluxed for 10mins. and cooled when a negligible quantity of a flocculent product separated. An attempted recrystallisation from acetone resulted in loss of material.

Attempted acetylation.

The oil (0.55g.) was refluxed for 15mins. with acetic anhydride (5ml.), cooled and the solvent removed under reduced pressure. The resultant oil (0.58g.) failed to crystallise. The acetylation was repeated but this time refluxing was continued for 2 hours. Again, no crystalline material was obtained.
Attempted preparation of azulene

The oil (0.1g.) was gently refluxed in the presence of palladium (20%) on charcoal. After one hour a distinctly blue distillate was obtained which was not further investigated.

Neutral Fraction B — (Table 9)

This fraction (85.2g.) was obtained from the third sample of *A. reticulata* (12.8kg.) by the method reported on page 94. It was dissolved in benzene and chromatographed from this solvent on alumina (220g., 33.5cm. x 3.3cm.). The narrow pale yellow band preceding the main band was collected (25ml.). Evaporation gave an aromatic reddish-yellow mobile oil (4.33g., $n^\circ_\text{D} 1.5184$), termed fraction I (Table 10). The main band (1.28 1.) followed closely and on removing the solvent a dark-red mobile oil (62.84g.) was obtained (fraction a, Table 10). The eluate was then altered to include ethanol and fractions b (200:1), c (50:1), d (20:1) and e (1:1) were successively removed with benzene - ethanol mixtures in the ratios given in parentheses. Fractions b, c, d and e were completely immobile dark-red oils and not examined further (Table 10).

Fraction a

The oil (62.84g.) was steam distilled and the distillate collected in two main portions. A pale yellow oil (11.06g.,
\[ n^\circ_{\text{D}} = 1.4924, \quad d^\circ_{\text{D}} = 0.963, \quad \left[ \alpha \right]^\circ_{\text{D}} = -1.11 \text{ in chloroform} \] (fraction II, Table 10) was separated from the first portion (700ml.) of the distillate. An additional 1400ml. of distillate were collected, combined with the previous 700ml. and extracted with ether. The ether was dried (\( \text{Na}_2\text{SO}_4 \)) and evaporated to give an orange aromatic oil (4.00g., \( n^\circ_{\text{D}} = 1.5095 \)) (fraction III, Table 10).

The portion of fraction a which was not volatile in steam was removed from aqueous suspension into chloroform. Removal of the solvent after drying (\( \text{Na}_2\text{SO}_4 \)) gave a dark red oil which was refluxed (1\( \frac{1}{2} \) hours) with 2\( \text{N} \) ethanolic potassium hydroxide (200ml.). The solution was cooled, diluted with water (600ml.) and extracted with ether (total 700ml.), the ether dried (\( \text{Na}_2\text{SO}_4 \)) and evaporated to give a dark-red viscous oil (22.068g.) (fraction IV, Table 10). The very dark aqueous layer was carefully acidified (dilute hydrochloric acid) and again extracted with ether (total 500ml.) which, after drying (\( \text{Na}_2\text{SO}_4 \)), was removed to give an extremely viscous very dark-red oil with a resinous odour (26.16g.) (fraction V, Table 10).

**Neutral Fractions A, C and D**

These fractions were obtained by the method reported on page 94 from different batches of *A. reticulata* (Table 9). Fractions A and C were bulked and shaken with dry light petrol-
eum (7 x 100ml.) which was evaporated to give a reddish-brown mobile oil (42.45g., $\eta_2^{19.5^\circ} 1.5088$). Identical treatment of fractions D gave a similar oil (28.93g., $\eta_2^{19.5^\circ} 1.5084$). The combined oils were steam distilled as before (see fraction a, page 108) until 900ml. of distillate containing volatile oil were collected. This was extracted with ether which was dried (Na$_2$SO$_4$) and evaporated and gave a pale yellow oil (13.41g., $\eta_2^{20^\circ} 1.4907$, $d_2^{20^\circ} 0.935$, $[\alpha]_d^{20^\circ} +14.37$) (fraction IX, Table 11) which contained hexagonal crystals. The portion not volatile in steam (fraction VIII, Table 11) was a viscous reddish-brown oil (52.95g., $\eta_2^{19^\circ} 1.5171$).

The light petroleum-insoluble portion of fractions A and C (39.5g.) was steam distilled. A negligible quantity of volatile material (0.86g., $\eta_2^{19^\circ} 1.4956$) was obtained (fraction VII, Table 11) together with a portion which was not steam-volatile (fraction VI, Table 11). The light petroleum-insoluble portion of Neutral Fraction D (10.50g.) was extremely viscous and very dark-red in colour (fraction X, Table 11).
Table 10

**NEUTRAL FRACTION B**

(85.2g.)  
(chromatography)

<table>
<thead>
<tr>
<th>4.33g</th>
<th>a) 62.84g</th>
<th>b) 6.46g</th>
<th>c) 5.42g</th>
<th>d) 1.75g</th>
<th>e) 0.49g</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n_20^°C 1.5184)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

I

Steam distillation

Volatile portion

<table>
<thead>
<tr>
<th>11.06g</th>
<th>4.00g</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n_4.5^°C 1.4924)</td>
<td>(n_20^°C 1.5095)</td>
</tr>
</tbody>
</table>

II

Non-volatile portion

Saponification

<table>
<thead>
<tr>
<th>4.00g</th>
<th>Alcohols and non-saponifiable material (22.07g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resin Acids (26.16g.)</td>
</tr>
</tbody>
</table>

III

IV

V.
Table 11

NEUTRAL FRACTIONS A and C

(98.4g.)
Light petroleum

Insoluble portion
(39.5g)

Soluble portion
(42.45g, $\eta^{\circ C} 1.5088$)

Steam distillation

Non-volatile portion
(52.95g, $\eta^{\circ C} 1.5171$)

VIII

Non-volatile portion
(36.75g)

VI

NEUTRAL FRACTION D

(40.0g.)
Light petroleum

Soluble portion
(28.93g, $\eta^{\circ C} 1.5084$) (10.50g.)

Insoluble portion

VII

Bulked and steam distilled

Volatile portion
(13.41g, $\eta^{20\circ C} 1.4907$)

IX
EXAMINATION OF FRACTIONS I - X

Fractions I, III, V, VI, VIII, and X were left at room temperature then at 0°C for a prolonged period of time but no crystalline material separated so these fractions were not examined further.

Fraction II was fractionally distilled under reduced pressure (0.5mm. Hg) and separated into three volatile portions IIA, IIB and IIC, and a residue which was not further investigated. Table 12 (page 74) summarises these results.

Fraction IV This fraction was partially soluble in light petroleum so the oil was suspended in that solvent and chromatographed on alumina (115g, 18cm. x 3.5cm.) using various mixtures of light petroleum, benzene and ethanol as eluants. Eight main fractions were obtained (Table 17), all very viscous and red in colour, none of them showing any tendency to crystallise. They were not examined further.

Fraction VII was a pale yellow oil (0.86g.) with an odour resembling that of borneol. On standing, hexagonal crystals were deposited, but due to the small quantity, no further work was done on them.

Fraction IX was separated into three volatile portions IXA, IXB and IXC, and a residue by the method used for fraction II. The residue was discarded. Table 13 (page 74) summarises the results.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume of eluant (ml.)</th>
<th>Eluant</th>
<th>Weight of residue (g.)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>50</td>
<td>light petroleum</td>
<td>1.083</td>
<td>Small yellow band preceding main band.</td>
</tr>
<tr>
<td>3.</td>
<td>100</td>
<td>light petroleum</td>
<td>0.576</td>
<td>Pale yellow band.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>light petroleum + Benzene (1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>light petroleum + Benzene (5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>400</td>
<td>benzene</td>
<td>2.615</td>
<td>Dark red band.</td>
</tr>
<tr>
<td>5.</td>
<td>700</td>
<td>benzene + ethanol (1%-2%)</td>
<td>1.746</td>
<td>Reddish-yellow band.</td>
</tr>
<tr>
<td>6.</td>
<td>400</td>
<td>benzene + ethanol (5%)</td>
<td>0.620</td>
<td>Pale yellow band.</td>
</tr>
<tr>
<td>7.</td>
<td>300</td>
<td>benzene + ethanol (50%)</td>
<td>0.400</td>
<td>Pale yellow band.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>100</td>
<td>ethanol + glacial acetic acid (1%)</td>
<td>0.322</td>
<td>Red band.</td>
</tr>
</tbody>
</table>
Treatment of fractions IIA, IIB, IIC, IXA, IXB, and IXC

Isolation of (-)-borneol.

Fractions IIa and IIB deposited hexagonal plates of (-)-borneol (150mg.) which, after recrystallisation from ice-cold light petroleum, had m.p. 194-200°C (with sublimation), raised to 196-202°C on admixture with a pure sample of borneol (m.p. 204°C).

p-nitrobenzoate

The crystals (100mg.) were dissolved in pyridine (2ml.) and refluxed gently for 25mins. with p-nitrobenzoyl chloride (150mg.). The mixture was cooled and diluted with distilled water (10ml.) and the bulky off-white precipitate collected, washed with N sodium hydroxide solution (2 x 5ml.) water (3 x 2ml.), and dried in vacuo. Recrystallisation from light petroleum gave needles of (-)-bornyl-p-nitrobenzoate (76mg.), m.p. 134-135°C. Huckel and Kaluba gave m.p. 136°C.

The oily filtrates remaining after removal of (-)-borneol were bulked with fractions IIC, IXA, IXB and IXC to give fraction XI.
TREATMENT OF FRACTION XI - NEUTRAL OIL

The oil (12.91g.) was refluxed for 30 mins. with ethanolic potassium hydroxide (0.66N, 50ml.) and neutralised with 0.5N hydrochloric acid. This indicated an equivalent weight of 949, equivalent to 19.25% bornyl formate. The solution was made slightly alkaline and extracted with light petroleum which was washed with water until free of alkali, dried (Na₂SO₄) and evaporated to give a deep yellow semi-crystalline oil (11.47g.). The latter was treated as reported below. The aqueous liquors remaining after removal of the borneol-containing oil were acidified (dilute hydrochloric acid) and again extracted with light petroleum which was washed with water, dried (Na₂SO₄) and evaporated to give a water-insoluble acid (0.874g.) which was not further examined.

Chromatography of semi-crystalline oil

The oil (11.47g.) was chromatographed on alumina (254g., 38cm. x 3cm.) and the eluate collected in 10ml. portions each of which was examined for optical activity. The small fractions were suitably bulked to give four main fractions XIA, XIB, XIC and XID. Table 14, page 76 summarises the results.

Fraction XIA

The oil (3.575g.) was distilled at 0.5mm., 125-130°C
(bath temp.) and gave a colourless product \( [\alpha]_{D}^{25^\circ} + 7.0 (c=4.05), \)
\( d_{10^\circ}^{25^\circ} 0.919, n_{D}^{25^\circ} 1.4956, E_{1cm} \) at 212 mm = 136 which possessed a reticulene-like odour. Redistillations failed to alter appreciably these physical constants (see Table 15, page 77).

**Fraction XIB.**

This oil (0.420 g.), \( n_{D}^{19^\circ} 1.5022, \) also possessed the odour of reticulene but was slightly laevorotatory. It was not examined further.

**Fraction XIC.** was a very bright, colourless, sweet-smelling mobile oil which did not become viscous, turn yellow or deposit crystals on prolonged storage.

**Attempted preparation of p-nitrobenzoate**

The oil (80 mg.) was dissolved in pyridine (2 ml.) and gently refluxed for 30 mins. with p-nitrobenzoyl chloride (100 mg.). After cooling, the addition of water (10 ml.) failed to precipitate any solid material.

**Fraction XID.** was recrystallised from ice-cold light petroleum and gave \((-\))-borneol, m.p. 205°C.
EXPERIMENTS ON ACID/BASE RELATIONSHIP

Preparation of 1-methoxy-5,6-methyleneedioxy-9-aminophenanthrene (XIII).

Method I

Decarboxylated aristolochic acid (1-methoxy-5,6-methyleneedioxy-9-nitrophenanthrene) (0.11g.) was refluxed for 30mins. with zinc powder (1.3g.) and glacial acetic acid (25ml.) On cooling, the colourless solution gradually darkened and gave a red solution which fluoresced blue-green in ultraviolet light. The cold solution was filtered from zinc and zinc acetate, basified (dilute sodium hydroxide) and the resultant brown precipitate (42mg.) removed, washed with water and dried in vacuo. Attempts were made to recrystallise the product from ethanol, methanol, water, light petroleum and mixtures of these solvents without success, the product always being amorphous. In an attempt to characterise the product as its picrate, it was dissolved in ethanol and a saturated solution of picric acid in ethanol added. No precipitate separated.

Method II

Decarboxylated aristolochic acid (51mg.), 5% palladium-charcoal (21mg.) and hydrazine hydrate (0.5ml.) were refluxed for 10mins. in ethanol (20ml.). After filtering, the solution, still distinctly orange, was evaporated under reduced pressure but yielded starting material, m.p. 205-210°C.
The residue was redissolved in ethanol (20ml.), 5% palladium-charcoal (40mg.) and hydrazine hydrate (0.5ml.) added and refluxing continued for 75mins. This time a yellow solution was obtained. It was filtered and concentrated to 5ml. Addition of water gave grey crystals (14mg.), m.p. 166-168°C (microblock).

**Method III a**

Decarboxylated aristolochic acid (0.357g.) was dissolved in dry benzene (100ml.) and hydrogenated at a platinum catalyst (72.8mg.). Uptake of hydrogen appeared complete after 1 hour at 19°C with the absorption of 58.1ml. of hydrogen (after deducting the volume absorbed by the catalyst). Equivalent uptake: 2.2mls. Filtration of the reaction mixture and evaporation of the filtrate in vacuo yielded a mixture of pale orange and yellow crystals m.p. 120-122°C (microblock) which was shaken with ethanol in which the yellow crystals dissolved. The pale-orange crystals turned yellow but remained undissolved in the ethanol. They were filtered off, repeatedly washed with ethanol and dried in vacuo (yield 131mg., m.p. 166-167°C). Recrystallisation from aqueous ethanol gave pale-brown needle crystals, m.p. 168-169.5°C (microblock).

$\lambda_{max.} = 258(\log\epsilon 4.635), 297(\log\epsilon 3.835), 330(\log\epsilon 4.10), 333\mu(\log\epsilon 3.55)$ - in ethanol.

$\lambda_{max.} = 245(\log\epsilon 4.60, \text{shoulder}), 253(\log\epsilon 4.64), 300(\log\epsilon 4.21), 330(\log\epsilon 3.95, \text{shoulder}), 352(\log\epsilon 3.71), 370(\log\epsilon 3.72)$,
395μ (log€ 3.24) - in 2N hydrochloric acid/ethanol (equal parts). Pailer, Belohlav and Simonitsch gave m.p. 170°C.

Found: C 71.9; H 5.15; N 5.35%

Calculated for C_{16}H_{13}O_{3}N : C 71.9; H 4.9; N 5.25%.

**Method III b**

The previous method was repeated by dissolving decarboxylated aristolochic acid (283mg.) in dry benzene (100ml.) and hydrogenating as before, this time a much larger proportion of platinum catalyst (158mg.). Uptake of hydrogen was complete after 1 hour at 19°C with the absorption of 65.2ml. of hydrogen (after deducting the volume absorbed by the catalyst). Equivalent uptake: 2.9mols. Filtration and evaporation of the filtrate in vacuo yielded pale yellow needles (200mg.), m.p. 125-126.5°C (microblock) after recrystallisation from benzene. Further recrystallisation from ethanol gave pale-brown needles, m.p. 168 - 169°C (microblock).

**Hydrochloride** 1-methoxy-5,6-methylenedioxy-9-aminophenanthrene (20mg.) was dissolved in dry benzene (10ml.) and dry hydrochloric acid passed through the solution for 5mins. The orange solution turned green. The solvent was removed and the pale pink product obtained was recrystallised three times from ethanol/ether as faintly pink needles (6mg.) m.p. 169°C (decomp., microblock).

Found: C 62.4; H 5.6%

C_{16}H_{13}O_{3}N.HCl requires: C 63.2; H 4.7%
Methylation of 1 methoxy-5,6-methylenedioxy-9-aminophenanthrene

**Method I**

The base (Xlll) (50mg.) was dissolved in dry benzene (50ml.) and an excess of diazomethane in dry ether added. No evolution of nitrogen occurred. After 15mins., the solvents and excess diazomethane were removed and the product recrystallised from aqueous ethanol as pale brown needles (35mg.) m.p. 166.5-168°C (i.e. starting material).

**Method II**

The base (Xlll) (60mg.) was suspended in dry ether (20ml.) and an excess of diazomethane in dry ether added. No reaction took place until a drop of water was added as catalyst. The reaction was allowed to continue for 21 hours during which time the base completely dissolved. The solution was filtered, evaporated to dryness and the solid obtained recrystallised from aqueous ethanol as pale brown needles, m.p. 167-169°C (microblock), mixed m.p. with starting material 167-170°C (microblock).

**Method III**

The base (Xlll) (50mg.) was dissolved in dry benzene (50ml.) and refluxed for 30mins. with methyl iodide (148mg.). The solvents were removed and the product recrystallised from aqueous ethanol as pale brown crystals, m.p. 167°C (microblock).

**Method IV**
Method IV

The previous method was repeated using methyl iodide (0.72g.) and refluxing for 90mins. Again, starting material was recovered.

Method V

The base (XIII) (50mg.) was refluxed for 20mins. with methyl iodide (5ml.). During the reaction, an off-white crystalline precipitate separated which was removed and washed with methyl iodide to give colourless needles (22mg.), m.p. 205-207°C (decomp., microblock) after darkening at 160-170°C.

Found: C 47.55; H 3.85%

C₁₇H₁₅O₃N.HI requires: C 49.9; H 3.92%

Method VI

The base (XIII) (70mg.) and methyl iodide (2ml.) were placed in a strong glass ampoule and heated at 120°C for 1 hour. The ampoule was cooled and the contents filtered and washed with methyl iodide to give colourless needles, m.p. 200-202°C (decomp., microblock) after preliminary darkening.

Found: C 51.5; H 4.40%

C₁₇H₁₅O₃N.HI requires: C 49.9; H 3.92%
THE ETHANOL-SOLUBLE FRACTIONS OF A. SERPENTARIA LINN.,

A. INDICA LINN. AND A. LONGA LINN.
To avoid unnecessary repetition, only a brief description is given of the practical work done on these three species when it is of a nature similar to that reported in the section on *A. reticulata*. Other experiments are reported in detail.
Two authentic samples of *A. serpentaria* were obtained and examined separately.

**Sample a.**

The first sample of dried root and rhizome (4.34Kg.), from which appreciable quantities of *Hydrastis canadensis* root and rhizome and other adulterants had been removed, was reduced to a No. 60 powder, defatted with light petroleum (b.p. 40-60°C) and percolated in the cold with ethanol until the percolate was pale brown (7 days). The thick black oil obtained on concentration was left at 0°C for 4 days during which time β-sitosteryl-β-D-glucoside (1.88g.) separated as a brown crystalline solid m.p. 287-295°C, which on repeated recrystallisations from ethanol gave off-white microcrystals, m.p. 295-296°C. The identity of the glucoside was confirmed by the preparation of the tetraacetate, m.p. 166°C. [Kind and Celentano gave m.ps. of 295-297°C, 167.5-168.5°C respectively for β-sitosteryl-β-D-glucoside and its tetraacetate]. The oily filtrate was dissolved in ether and the solution extracted with dilute hydrochloric acid (treatment of this acid extract is reported on page 124).

The crude acid fraction (3.64g.) obtained from the ether
solution by the method used for *A. reticulata*, was recrystallised from glacial acetic acid and gave aristolochic acid (2.00g), m.p. 283°C (decomp., microblock), R_f 0.915, identified further by its ultraviolet absorption spectrum (identical with that described under *A. reticulata*) and conversion to 1-methoxy-5,6-methylenedioxy-9-nitrophenanthrene, orange needles, m.p. 212°C (microblock) by the method previously reported (page 97). Concentration of the glacial acetic acid mother liquors gave, on repeated fractional recrystallisations from ethanol, red needles of aristo-red (35mg.), m.p. 286.5-287.5°C (microblock), R_f 0.78 (fluorescent spot in ultraviolet light). The ultraviolet absorption spectrum agreed with that reported under *A. reticulata*.

A neutral oil (25.7g.) was obtained from the ether solution after the removal of acidic material, by the method outlined in the experimental section on *A. reticulata* (page 94).

**Examination of Acid Extract**

This was obtained as reported on the previous page. The acidic solution was basified (dilute ammonium hydroxide) and extracted with ether which on evaporation gave a dark-red partially crystalline oil (500mg.). The benzene-soluble portion was chromatographed on alumina (5" x 0.5") from benzene to give two fractions. The benzene-insoluble portion was non-alkaloidal.

**Fraction 1.** This fraction came through as a compact non-
fluorescent yellow band which on evaporation and recrystallisation from ether or benzene gave pale yellow prismatic crystals (74mg.) of a base, m.p. 178-179°C (decomp., tube), $\lambda_{\text{max.}}$ 281.5 ($\varepsilon$ 12,030), 353$m\mu$ ($\varepsilon$ 13,356).

Found: C 53.6; H 3.75; N 3.6%

$\text{C}_{18}\text{H}_{15}\text{O}_{10}\text{N}$ requires: C 53.4; H 3.7; N 3.5%

**Fraction 2.** Removal of benzene from the fluorescent solution produced pale yellow prism crystals of hydrastine (62mg.) which gave an olive-green colour with ammonium molybdate in concentrated sulphuric acid. After recrystallisation from methanol the base had m.p. 132°C (tube), $\lambda_{\text{max.}}$ 297$m\mu$ ($\varepsilon$$_{\text{icm.}}$ 196). El Ridi, Khalifa and Mamoon gave $\lambda_{\text{max.}}$ 297$m\mu$ ($\varepsilon$$_{\text{icm.}}$ 200), m.p. 132°C.

Found: C 65.6; H 5.5; N 3.7%

Calculated for $\text{C}_{21}\text{H}_{21}\text{O}_{6}\text{N}$: C 65.8; H 5.5; N 3.7%.

**Picrate**

The picrate was prepared by dissolving the base (18mg.) in methanol (1ml.) and adding a saturated solution of picric acid in ethanol (0.5ml.). Recrystallisation from ethanol gave hydrastine picrate (18mg.), m.p. 148-149°C (decomp., tube).

Found: C 53.2; H 4.25%

Calculated for $\text{C}_{21}\text{H}_{21}\text{O}_{6}\text{N}\cdot\text{C}_6\text{H}_2(\text{NO}_2)_3\text{OH}$: C 52.85; H 3.95%.
Sample b

The second sample of defatted root and rhizome (4.20 kg., No. 60 powder), on concentration of the ethanolic extract, gave a thick black oil from which aristolochic acid, aristo-red and the acid extract were obtained as before.

Examination of Acid Extract

The solution was basified (dilute sodium hydroxide) and extracted into ether which was, in turn, shaken out with sulphuric acid (2.5%). On standing, the aqueous layer deposited orange crystals of berberine sulphate (1.037 g.), m.p. 288-290°C (decomp., microblock), after recrystallisation from ethanol/ether. \( \lambda_{\text{max.}} \) in 88% ethanol 267(\( E_{\text{lcm}} \) 648), 351 m\( \mu \) (\( E_{\text{lcm}} \) 609). El Ridi, Khalifa and Mamoon gave \( \lambda_{\text{max.}} \) 270 (\( E_{\text{lcm}} \) 610), 350 m\( \mu \) (\( E_{\text{lcm}} \) 600) for berberine hydrochloride.

Found: C 55.0; H 4.2; N 3.3; S 7.2%
Calculated for \( \text{C}_{20}\text{H}_{17}\text{O}_4\text{N.H}_2\text{SO}_4 \): C 55.4; H 4.4; N 3.2; S 7.4%

The ether layer gave yellow prism crystals on removal of the solvent, and chromatography from benzene on alumina (6" x 0.5") yielded only hydrastine (1.097 g.), m.p. 132°C (tube) (from methanol), 145°C (tube) (from aqueous methanol). Both melting points have been reported for hydrastine. The picrate prepared as before had m.p. 148-149°C (decomp., tube).

Attempted isolation of water-soluble bases.

The acid extracts from both samples of \( \text{A. serpentaria} \),
which had been basified and extracted with ether to remove basic material, were re-acidified to congo red (dilute sulphuric acid). Addition of a saturated aqueous solution of ammonium reineckate gave an amorphous dark brown solid (5.133g.) which was only slightly soluble in dry acetone. Chromatography from dry acetone on alumina (38g., 6.5" x 0.75") gave a negligible quantity of pure reineckate.

PREPARATION OF HYDRASTINE PICRATE

A sample of hydrastine (0.82g.), m.p. 132°C, was obtained from Liquid Extract of Hydrastis B.P.C. 1949 (50ml.) using the official assay method. The picrate was prepared by dissolving the base (0.1g.) in hot methanol (10ml.) and adding a saturated solution of picric acid in ethanol (5ml.). It was recrystallised from ethanol and had m.p. 149°C (decomp., tube).
A. INDICA LINN.

ISOLATION AND TREATMENT OF THE FRACTION SOLUBLE IN COLD ETHANOL.

The dried root (3Kg.), previously defatted with light petroleum, was extracted with ethanol by cold percolation. The percolate was concentrated to 200ml., acidified with dilute hydrochloric acid and extracted with ether. Treatment of the acid extract is reported below. The ethereal solution was extracted with 2% aqueous potassium hydrogen carbonate and the latter solution acidified to yield aristolochic acid (2.5g.) as yellow needles after recrystallisation from dioxan, m.p. 284° C (decomp., microblock), $\lambda_{\text{max.}}$ 223($\epsilon_{29,300}$), 250($\epsilon_{32,300}$), 317.5($\epsilon_{12,900}$), 391$\mu$m($\epsilon_{6000}$). Reduction with zinc and glacial acetic acid by the method previously reported (page 98) gave 9-amino-1-methoxy-5,6-methylenedioxy-8-phenanthroic lactam, m.p. and mixed m.p. 318° C (microblock).

Examination of acid extract.

The acidic solution was basified (dilute sodium hydroxide) and extracted with ether which was washed free of alkali, dried (Na$_2$SO$_4$) and concentrated to give yellow needles (20mg.) which fluoresced bright yellow in ultraviolet light and had m.p. 339-342° C (decomp., microblock).

Found: C 60.95; H 4.75; N 2.8%

C$_{25}$H$_{23}$O$_{10}$N requires: C 60.4; H 4.6; N 2.8%
Removal of the solvent from the mother liquors gave a negligible quantity of a brown non-alkaloidal oil.

The aqueous alkaline layer from above was re-acidified to congo red (dilute sulphuric acid). Addition of a saturated aqueous solution of ammonium reineckate produced only a little crude reineckate (210mg.) which gave a negligible quantity of pure material when chromatographed with dry acetone on alumina.

**FURTHER ATTEMPTED ISOLATION OF BASIC MATERIAL**

a) The powdered, defatted roots (250g.) were soaked to a dry cake consistency in ammonia solution (S.G. 0.88) and continuously extracted for 24 hours with hot ethanol. The dark red-brown solution was reduced to low bulk and the basic concentrate extracted with chloroform then ether. The chloroform and ether solutions were washed (distilled water), dried ($\text{Na}_2\text{SO}_4$) and evaporated to dryness. Only traces of a dark-brown oil were obtained in each case.

The aqueous solution was then acidified to congo red (dilute sulphuric acid) and a saturated aqueous solution of ammonium reineckate added. The crude reineckate (0.4g.) was dissolved in dry acetone (in which most was insoluble) and chromatographed on alumina which gave only a negligible yield (4mg.) of pure reineckate. On heating, it darkened at 140°C and decomposed without melting.

b) The powdered, defatted roots (250g.) of a different
portion of this sample of *A. indica* were again treated as above, the extraction time being extended to 48 hours. The result was the same as before.
A. LONGA LINN.

ISOLATION AND TREATMENT OF THE FRACTION SOLUBLE IN COLD ETHANOL

The powdered root and rhizome (3.01Kg., No. 60 powder) was extracted with light petroleum until the percolate was almost colourless (4 litres). Removal of the solvent gave an almost odourless, light-brown oil which slowly deposited solid globules, m.p. 45°C (microblock). Recrystallisation from methanol gave two fractions, a poorly soluble portion, m.p. 91-93°C (microblock), and a methanol-soluble portion obtained as a waxy solid, m.p. 52-56°C (microblock), on slow evaporation of the solvent. These fractions were not examined further.

The dried marc from the light petroleum extract was macerated for 2 days with ethanol then percolated in the cold to give a dark-orange extract (10 litres). During concentration, the yellow crystalline solid which separated out (total weight 7.12g.) was repeatedly filtered off before an almost black thick oil was obtained. The residue was acidified with dilute hydrochloric acid and the crude acids extracted with ether (treatment of the acid extract is reported below). Extraction of the ethereal solution with 2% aqueous potassium hydrogen carbonate followed by acidification of the aqueous layer with dilute hydrochloric acid gave the crude acids. Fractional crystallisation of the bulked acid portions from glacial acetic
acid gave eight fractions as yellow microcrystals (total weight 6.01g.), each with m.p. 282-285°C (decomp., microblock), R_f 0.90-0.94, \( \lambda_{\text{max}} \) 250(\( \epsilon 30,600 \)), 317(\( \epsilon 11,500 \)), 390m\( \mu \) (\( \epsilon 5,700 \)), identical with aristolochic acid. Reduction with zinc and glacial acetic acid by the method previously reported (page 98) gave 9-amino-1-methoxy-5,6-methylenedioxy-8-phenanthroic lactam, m.p. 317°C (microblock).

**Examination of acid extract.**

The solution was basified (dilute sodium hydroxide) and extracted with ether which on evaporation gave only a trace of a brown non-alkaloidal oil. The aqueous layer was acidified to congo red (dilute sulphuric acid) and treated with a saturated aqueous solution of ammonium reineckate. The resultant crude precipitate (4.078g.) was completely insoluble in dry acetone, and was rejected.
PART II.

RETICULENE
HISTORICAL INTRODUCTION
The sesquiterpene reticulene was first isolated by (7,8) Stenlake and Williams from the light petroleum-soluble portion of the roots and rhizomes of A. reticulata. On concentration of the percolate, a dark green oil was obtained from which aristolactone, free acids and carbonyl compounds were removed. The steam-volatile portion of the residual oil was then subjected to distillation and of the many fractions obtained, the four which distilled between 114-136°C (bath temp.) at 18mm. Hg, were separately saponified with ethanolic potassium hydroxide. Extraction of each solution with light petroleum yielded an oily semi-crystalline mass, the oil from which was chromatographed on alumina in light petroleum. Two main fractions were obtained. The first, a colourless oil, was reticulene, C_{15}H_{24}, \([\alpha]_{D}^{15^\circ} +1.6; d_{16^\circ}^{16^\circ} 0.913; n_{D}^{20^\circ} 1.4955; \lambda 212m\mu (\varepsilon 2260, \text{in cyclohexane}).

Quantitative bromination of reticulene indicated two double bonds but catalytic hydrogenation consistently showed a hydrogen uptake equivalent to only one ethylenic bond and gave dihydroreticulene, C_{15}H_{26}, b.p. 130-135°C (bath) /18m.m., 
\([\alpha]_{D}^{7^\circ} +4.0; d_{15^\circ}^{15^\circ} 0.900; n_{D}^{7^\circ} 1.4826; \lambda 208m\mu (\varepsilon 1681, \text{in cyclohexane}).

Waves and Perrottet (85) pointed out that the physical constants of reticulene suggested a bicyclic structure in agreement with the bromination figures but quoted the findings of Naves and Perrottet who observed that bromination of aromadendrene (XXXVIII or XXXIX, R = CH_2) and dihydroaromadendrene
caused the absorption of two and one mol. of bromine respectively due to the opening of the cyclopropane ring. The same authors found that catalytic hydrogenation of aromadendrene gave the dihydro compound without abnormal reaction.

\[ R \]

\( (XXXVlll) \)

\( (XXXlX) \)

Williams therefore postulated that, as reticulene behaved in exactly the same way as aromadendrene, it might also be tricyclic and the remarkable similarity of the reported physical properties of aromadendrene, reticulene and their corresponding reduction products afforded support for this suggestion. Reticulene gave no crystalline derivatives and ozonolysis produced a camphoraceous oil together with formaldehyde, identified as its dimedone derivative. This latter fact established the presence of a vinylidene group in reticulene, confirmed by infrared absorption spectra. The camphoraceous oil gave a small quantity of a crystalline semicarbazone, m.p. 200-201°C, in agreement with one form of apoaromadendrone semicarbazone which melted at 201°C, but a 2:4 dinitrophenyl-hydrazone obtained was amorphous. Dehydrogenation of dihydro-
reticulene with palladium on charcoal gave no identifiable products though heating with selenium for six hours gave traces of an azulene.

Williams concluded that reticulene and aromadendrene were, in all probability, one and the same substance but pointed out that complete confirmation required authentic aromadendrene which he was unable to obtain. Two observations which appeared to contradict this conclusion were explained by him. Firstly, the pale yellow colour which dihydroreticulene gave with tetranitromethane, although indicative of unsaturation, was similar to the colours given by saturated substances containing a cyclopropane ring e.g. cycloartanone, and evidence from infrared spectra suggested that such a ring could be present in reticulene and dihydroreticulene. The former had a small peak at 1007cm.\(^{-1}\) which shifted to 1009cm.\(^{-1}\) on reduction. Secondly, the low intensity ultraviolet end absorption of reticulene (\(\epsilon_{2260}\) at 212\(\mu\)) and dihydroreticulene (\(\epsilon_{1681}\) at 208\(\mu\)) did not compare favourably with that reported by Birch and Lahey for apoaromadendrone (XXXVIII or XXXIX, \(R=0\)) (\(\epsilon_{95}\) at 212\(\mu\)) which would be expected to have similar end absorption characteristics as aromadendrene. Williams suggested that these discrepancies might be attributable to the purity of the spectroscopic solvents used.
DISCUSSION

OF

EXPERIMENTAL WORK
ISOLATION OF RETICULENE

Steele precipitated aristolactone from a concentrated extract of A. reticulate by seeding, and then chromatographed the oily filtrate on cellulose-charcoal to give four main fractions. The second fraction which was strongly dextro-rotatory, yielded more aristolactone and a clear mobile brownish oil with a terpene odour. Steele steam distilled the latter and subjected the oily distillate to fractional distillation under reduced pressure to obtain fractions corresponding to those previously obtained by Williams. The fractions with refractive indices in the range 1.4756 to 1.4978 were saponified and the light petroleum-soluble neutral portion, which consisted mainly of reticulene and borneol, was chromatographed from light petroleum on cellulose-charcoal. In this way, borneol was left on the column and an oily mixture was eluted.

This oil has now been chromatographed on alumina and three distinct fractions obtained (Table 18, page 156), the first and main one being reticulene followed closely by a laevorotatory colourless oil of similar odour. A later fraction consisted of an oil with a sweet odour similar to that of borneol, but unlike borneol, it failed to crystallise and did not react with p-nitrobenzoyl chloride. The findings are in agreement with those obtained in Part I (Table 14).
Williams obtained six main fractions on steam distillation of the volatile oil from *A. reticulata* and isolated reticulene from fractions II and III but did not examine fractions IV and V. These fractions have now been combined and distilled under reduced pressure giving the further small fractions shown in Table 19 (page 158). Saponification of fraction C followed by chromatography of the light petroleum-soluble portion gave reticulene.
Since Williams concluded that reticulene and aromadendrene were probably identical, a sample of aromadendrene has become available for comparison through the kindness of Dr. M. D. Sutherland. Dihydroaromadendrene was prepared by catalytic hydrogenation of this material, for comparison with the dihydro-derivative of reticulene. Williams named the latter dihydroreticulene but for reasons reported later, it has been concluded that the product is more correctly termed dihydroisoreticulene. The physical constants and ultraviolet absorption spectra of the four compounds are recorded in Table 20. They show conclusively that despite the near agreement of density, refractive index and optical rotation, there are significant differences in the ultraviolet absorption spectra. In particular it appears that the end absorption of dihydroisoreticulene is not due to impurities in the solvent as suggested by Williams. Its importance lies in affording confirmatory evidence for the presence of an ethylenic bond in dihydroisoreticulene.

The infrared absorption spectra of reticulene and aromadendrene and of their respective dihydro-compounds show many similarities but are not identical. Vinylidene absorption is present at 888 and 1646 cm\textsuperscript{-1} in the spectrum of reticulene.
<table>
<thead>
<tr>
<th>SUBSTANCE</th>
<th>$[\alpha]_D$</th>
<th>$n_D$</th>
<th>d</th>
<th>$\varepsilon_{209\mu\nu}$</th>
<th>$\varepsilon_{212\mu\nu}$</th>
<th>Ref.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulene</td>
<td>+ 0.96°</td>
<td>1.4970</td>
<td>0.915</td>
<td>3998</td>
<td>3040</td>
<td>Present work</td>
<td></td>
</tr>
<tr>
<td>-do-</td>
<td>+ 1.6°</td>
<td>1.4955</td>
<td>0.913</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Aromadendrene</td>
<td>+ 12.4°</td>
<td>1.4950</td>
<td>0.909</td>
<td>2835</td>
<td>2040</td>
<td>Present work</td>
<td></td>
</tr>
<tr>
<td>-do-</td>
<td>+ 7.54°</td>
<td>1.4953</td>
<td>0.911</td>
<td></td>
<td></td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>-do-</td>
<td>+ 0.8°</td>
<td>1.4990</td>
<td>-</td>
<td></td>
<td></td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Dihydroisoreticulene</td>
<td>-</td>
<td>1.4842</td>
<td>0.902</td>
<td>1970†</td>
<td>1770†</td>
<td>Present work</td>
<td></td>
</tr>
<tr>
<td>-do-</td>
<td>+ 4.0°</td>
<td>1.4826</td>
<td>0.900</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Dihydroaromadendrene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>331</td>
<td>266</td>
<td>Present work</td>
<td></td>
</tr>
<tr>
<td>-do-</td>
<td>-12.14°</td>
<td>1.4850</td>
<td>0.900</td>
<td></td>
<td></td>
<td>85</td>
<td></td>
</tr>
</tbody>
</table>

* The molecular extinction coefficients were determined under controlled conditions and so the values obtained by other workers are not quoted.
- Not determined.
and at 885 and 1640 cm\(^{-1}\) in aromadendrene, both maxima disappearing in each case on reduction.

Comparative infrared study suggests that reticulene and dihydroisoreticulene possess a cyclopropane ring with at least one substituent on the \(-\text{CH}_2-\) group. Cole found that hydrocarbons containing the cyclopropane ring sometimes exhibit bands of medium intensity near 1010 cm\(^{-1}\) though it is impossible to identify cyclopropane rings in this region if the substance possesses oxygen-containing substituents. Reticulene, dihydroisoreticulene, aromadendrene and dihydroaromadendrene have no oxygen-containing substituents yet all possess the following type of absorption in the cyclopropane region (Table 21).

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulene</td>
<td>990</td>
<td>999</td>
<td>1014</td>
</tr>
<tr>
<td>Dihydroisoreticulene</td>
<td>987</td>
<td>998</td>
<td>1013</td>
</tr>
<tr>
<td>Aromadendrene</td>
<td>988</td>
<td>999</td>
<td>1014</td>
</tr>
<tr>
<td>Dihydroaromadendrene</td>
<td>984</td>
<td>995</td>
<td>1011</td>
</tr>
</tbody>
</table>
Furthermore, Rees and Shoppee quote that the cyclopropane ring absorbs in the 334 to 888 and 1020cm. regions. The vinylidene group of reticulene and aromadendrene would mask the cyclopropane absorption in the 880-890cm. region but dihydroisoreticulene has a peak at 884cm. and dihydroaromadendrene shows twin absorption peaks at 881 and 888cm. Both aromadendrene and dihydroaromadendrene have been shown chemically to possess a cyclopropane ring and the similarities in their infrared spectra suggest that reticulene and dihydroisoreticulene do also.

The small peaks at 3060 and 3065cm. in reticulene and aromadendrene respectively deserve comment. Cole has found that if a cyclopropane ring, which possesses an unsubstituted \(-\text{CH}_2\)- group, is present in the molecule then characteristic small peaks are shown in the region 3040-3060cm. In the many examples he quotes, these peaks are almost identical with those shown by reticulene and aromadendrene. Bellamy, on the other hand, records that the \(\text{C}=\text{CH}_2\) group also absorbs in the region 3075-3095cm. and it is to this group that the absorption in reticulene and aromadendrene is due for the corresponding dihydro-compounds show no such absorption. The infrared evidence is therefore consistent with all four compounds possessing a cyclopropane ring in which all carbon atoms have substituents other than hydrogen.

A careful comparison of chemical properties also indicates that although similarities exist, reticulene and aromadendrene
are not identical. Both substances behaved the same on cata-
lytic reduction, each taking up hydrogen equivalent to one 
ethylenic bond. Dihydroisoreticulene, however, differed 
from dihydroaromadendrene in giving a much darker colour with 
tetranitromethane. Further, aromadendrene gave an azulene after 1-2 minutes heating with palladium on charcoal whereas 
reticulene failed to do so even after 30 minutes.
The degree of unsaturation in reticulene.

Whereas catalytic hydrogenation suggested the presence of only one double bond in reticulene, the intensity of end absorption in the ultraviolet and the tetranitromethane colour tests indicated two double bonds. Experiments were therefore undertaken to clarify this discrepancy.

Ozonolysis of dihydroisoreticulene was inconclusive as unchanged material was recovered, but the determination of ethylenic bonds by halogenation and by titration with perbenzoic acid afforded useful information which is shown in tables 22 and 23 respectively. The former method, carried out under carefully controlled conditions and with model compounds for comparison, indicated the presence of two double bonds in reticulene and one in dihydroisoreticulene. The latter method was used to confirm the results obtained by halogenation because halogen substitution can lead to erroneous results.

It must therefore be concluded that reticulene contains two double bonds while dihydroisoreticulene contains one such bond which is resistant to both catalytic reduction and ozonolysis.
<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of double bonds. Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydroaromadendrene</td>
<td>0.06 Present work</td>
<td>1 cyclopropane ring. 0 double bonds.</td>
</tr>
<tr>
<td>Hederagenin</td>
<td>0.83 Present work</td>
<td>trisubstituted double bond.</td>
</tr>
<tr>
<td>Hederagenin methyl ester diacetate.</td>
<td>0.90 87</td>
<td>trisubstituted double bond.</td>
</tr>
<tr>
<td>Cyclo-eucalanyl acetate</td>
<td>0.07 Present work</td>
<td>1 cyclopropane ring. 0 double bonds.</td>
</tr>
<tr>
<td>2,3-dimethyl-2,3 methylene-1,4 naphthaquinone</td>
<td>0.00 Present work</td>
<td>1 cyclopropane ring. 0 double bonds.</td>
</tr>
<tr>
<td>Iso-Xetal from aristolactone</td>
<td>1.98 87</td>
<td>Two double bonds.</td>
</tr>
<tr>
<td>α-angelicalactone</td>
<td>0.90 87</td>
<td>trisubstituted double bond.</td>
</tr>
<tr>
<td>Reticulene</td>
<td>1.97 Present work</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.90 87</td>
<td></td>
</tr>
<tr>
<td>Dihydroisoreticulene</td>
<td>0.70 7</td>
<td></td>
</tr>
</tbody>
</table>

* References are to molecular structure.*
<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of Double Bonds</th>
<th>reaction time (hrs)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6  24  48</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.98  1.10</td>
<td></td>
<td>1 trisubstituted double bond.</td>
</tr>
<tr>
<td>Hederagenin</td>
<td>0.26  0.39</td>
<td></td>
<td>1 trisubstituted double bond.</td>
</tr>
<tr>
<td>Cyclo-eucalanyl acetate</td>
<td>0.03</td>
<td></td>
<td>1 cyclopropane ring.</td>
</tr>
<tr>
<td>Aristolactone</td>
<td>2.28  2.56  2.63</td>
<td></td>
<td>3 double bonds.</td>
</tr>
<tr>
<td>Reticulene</td>
<td>1.48  1.54  1.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dihydrorisetriculene</td>
<td>0.43  0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reticulodione</td>
<td>0.10  0.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
On the basis of the above evidence, reticulene possesses two rings and if it is accepted that one of these is a cyclopropane ring, it follows that the other is either a medium ring or else a small one with a large side chain. In an attempt to obtain small identifiable fractions, the sesquiterpene was oxidised with Beckmann's chromic acid mixture at room temperature for 6 days. A gas was released and the solution developed a camphoraceous odour. The solution was then separated into acidic and neutral material but the latter yielded no identifiable fragments. The acidic material was oily and on admixture with ethanol gave only a small quantity of a fatty solid, m.p. 49-53°C, insufficient for characterisation. Chromatographic separation of the residue from the filtrate yielded crystals (m.p. 170°C with sublimation) and two oils. Paper chromatography showed that the three main acidic fractions were dicarboxylic acids, larger in molecular weight than pimelic acid. The fatty solid, m.p. 49-53°C appeared from its R_f value to be a monocarboxylic acid (Table 24.).
<table>
<thead>
<tr>
<th>Substance</th>
<th>$R_F$ in phenol 80%, formic acid 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystals, m.p. 170°C</td>
<td>0.935</td>
</tr>
<tr>
<td>Oil (1)</td>
<td>0.954</td>
</tr>
<tr>
<td>Oil (2)</td>
<td>0.977</td>
</tr>
<tr>
<td>Crystals, m.p. 49-53°C</td>
<td>0.14</td>
</tr>
<tr>
<td>Pimelic acid</td>
<td>0.905</td>
</tr>
<tr>
<td>Adipic acid</td>
<td>0.856</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.77</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Ozonolysis of reticulene yielded a viscous yellow oil of which only a portion was volatile under reduced pressure. The volatile portion was a very pale yellow oil, \( \text{C}_{13}\text{H}_{20}\text{O}_2 \), of camphoraceous odour. The formula indicates the loss of 2 carbon atoms during ozonolysis with the formation of a diketone, reticulodione. Reticulene therefore contains two vinylidene groups. The ultraviolet absorption spectrum of the diketone had a distinct inflection in the carbonyl region (\( \varepsilon \) at 290m\( \mu \) 5.12) and showed end absorption (\( \varepsilon \) at 218m\( \mu \) 394) which confirmed that no ethylenic bond was present. Further confirmation that reticulodione was fully saturated was obtained by titrating with perbenzoic acid (Table 23). The parent hydrocarbon of reticulodione, \( \text{C}_{13}\text{H}_{24} \), would also be fully saturated. To satisfy such an empirical formula, the diketone must contain a two ring system. Twin peaks are present in the carbonyl region of the infrared absorption curve in agreement with the postulated diketone structure. The infrared absorption spectrum in carbon disulphide confirmed that the diketone retained the cyclopropane ring. Typical bands were present at 986, 996 and 1016cm\(^{-1}\) (compare Table 21).
Both double bonds in reticulene react with ozone whereas the double bond in the dihydro-derivative is resistant to attack. This apparent discrepancy can be explained if one postulates that reduction of one double bond is accompanied by a shift of the remaining double bond into a position in the molecule which is resistant to further reduction and ozonolysis.

Excellent confirmation of this postulate is given by a peak at 814 cm\(^{-1}\) in the infrared absorption curve of dihydro-isoreticulene, characteristic of a trisubstituted double bond. This peak is absent from the absorption curve of reticulene.
Williams attempted to dehydrogenate dihydroisoreticulene with palladium on charcoal but obtained no products possessing typical aromatic absorption in the ultraviolet. This experiment was repeated with similar results but when the palladium on charcoal was replaced by selenium and distillation continued for 4 hours at 280-285°C, a blue distillate was obtained which was purified by phosphoric acid separation. The ultraviolet absorption spectrum in ethanol of the resulting azulene, termed reticazulene, was almost identical with the spectrum obtained by Williams (unpublished work) in a similar experiment (Figure 12). The ultraviolet absorption spectrum was also recorded in 50% sulphuric acid. Again it was typical of an azulene but was not identical with any of the relatively few published spectra.

Steele recorded in tabular form the characteristic wavelengths of maximum absorption of many azulenes and comparison showed that reticazulene was not identical with aristazulene (Table 25). The latter possessed an additional maximum at 306mμ and was violet in colour. Reticazulene is blue and therefore is not a 2-alkylazulene.
Figure 12

Ultraviolet absorption of Reticazulene.

- --- - Williams (unpublished work)
- --- - Present work

E (Observed Value)

Wavelength (m/μ)

240 280 320
Further comparison showed that reticazulene could only be a 1,4,8-trialkylazulene (Table 25).

**Table 25**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wavelength of Maximum Absorption (mμ)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aristazulene</td>
<td>245, 279, 289, 306, 333, 348</td>
<td>87</td>
</tr>
<tr>
<td>1,4,8-trimethylazulene</td>
<td>246, 286, - , - , 335, 348</td>
<td>100</td>
</tr>
<tr>
<td>1,4-dimethyl-8-isopropylazulene</td>
<td>246, 287 , 305, 335, 348 (infl)</td>
<td>100</td>
</tr>
<tr>
<td>Reticazulene</td>
<td>245, 285-290, 305, 335, 348 (infl)</td>
<td>101</td>
</tr>
<tr>
<td>Reticazulene</td>
<td>245 , 284 , 305, 335, 348 (infl)</td>
<td>Present work</td>
</tr>
</tbody>
</table>

*Readings in this region not reproducible.*

The spectra of 1,4,8-trimethylazulene and 1,4-dimethyl-8-isopropylazulene differ slightly. The former has an additional inflection near 232mμ while the latter also possesses an additional inflection but in this case near 305mμ.

Reticazulene has no inflection near 232mμ but one is present at 305mμ. This evidence indicates that reticazulene is a 1,4,8-trialkylazulene, probably 1,4-dimethyl-8-isopropylazulene.
A TENTATIVE STRUCTURE FOR RETICULENE

From the preceding evidence, a tentative structure for reticulene can be proposed (XL). Such a structure conforms to the isoprene rule as shown in XLI and would explain the inability to obtain small identifiable fractions on oxidation. It is closely related structurally to aristolactone (XXV) which could conceivably be its precursor in agreement (102) with the findings of Cekan, Herout and Sorm who isolated both a hydrocarbon and a lactone from Matricaria chamomilla L. and showed that the latter was the precursor of the former.

\[
\begin{align*}
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{CH}_3 & \\
\text{H}_3\text{C} & \\
\end{align*}
\]

(XL)

\[
\begin{align*}
\text{CH}_2 & \\
\text{CH}_3 & \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{O} & \text{CO} & \\
\end{align*}
\]

(XXV)
The proposed structure for reticulene also explains why reticazulene was formed only slowly, as a ring closure is necessary similar to that found in the preparation of aristazulene. This ring closure could occur in two ways, each of which would give a 1,4,8-trialkylazulene (XLII, XLI).

On the basis of structure XL for reticulene and by analogy with aristolectone, dihydroisoreticulene can be represented by either structure XLIV or XLV. The former is more probable for in structure XLV, the double bond is conjugated with the cyclopropane ring and the expected value for the molecular extinction coefficient would be higher than that obtained (ε at 210 m\(\mu\) 1970), in agreement with the findings.
In the umbellulone series of compounds \(103,104\),

\[
\text{(XLIV)} \quad \text{(XLV)} \quad \text{(XLVI)}
\]

In the infrared spectrum of reticulodione, which is therefore XLVI, a carbonyl peak at 1703 cm\(^{-1}\) (in CC\(_4\)) and at 1708 cm\(^{-1}\) (thin film) is consistent with the molecule having a 10-membered ring system \(105,106\) which would explain the lack of ketonic activity \(7\) due either to steric hinderance or to the typical "O-inside" configuration of medium ring ketones \(107\).

It must be emphasised that the proposed structure for reticulene is a tentative one which must be confirmed by chemical evidence when more material becomes available.
EXPERIMENTAL

Apart from the conditions used in the determination of $R_F$ values, the comments preceding the experimental section of Part I apply.

The author is indebted to Dr. W. Lawrie and Dr. R. Stevenson for the sample of cyclo-eucalanyl acetate and to Dr. G. Buchanan for the sample of 2,3-dimethyl-2,3 methylene-1,4-naphthaquinone.
The oily mixture obtained from Steele (see page 136) (15.136g.), which still contained some solvent, was chromato-graphed in two portions on alumina (25cm. x 3.5cm.) from light petroleum followed by light petroleum-ethanol. 10ml. or 20ml. fractions were collected and each was examined for optical activity. In this way the oil was separated into three main fractions. Table 18 shows the result of chromato-graphing one of the portions of the oily mixture. Similar treatment of the second portion gave identical fractions so corresponding fractions were combined.

**Combined fraction A:** Removal of solvent gave reticulene as a colourless oil (8.632g.), \( \eta^{18^\circ} = 1.4970 \).

**Combined fraction B:** Removal of solvent gave a colourless laevorotatory oil (1.857g.), \( \eta^{18^\circ} = 1.4930 \), which possessed the odour of reticulene. It was not examined further.

**Combined fraction C:** Removal of solvent gave a yellow oil (3.764g.) with a sweet odour similar to that of borneol. On prolonged standing both at room temperature then at 0\( ^\circ \)C, the oil failed to crystallise. Distillation at 110-115\( ^\circ \)C (bath temp.)/18mm. Hg, gave a colourless oil (2.40g., \( \eta^{19^\circ} = 1.4737 \)) which still possessed an odour similar to that of borneol but at no time during the distillation did sublimation or crystallisation occur (absence of borneol).
<table>
<thead>
<tr>
<th>Eluant</th>
<th>Fraction</th>
<th>Volume of Eluate (ml)</th>
<th>Optical Rotation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light petroleum</td>
<td>1</td>
<td>20</td>
<td>+0.03</td>
<td>light petroleum</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>+0.11</td>
<td>Fractions 1-7 bulked</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>+0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
<td>+0.11</td>
<td>(Fraction A)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>+0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10</td>
<td>+0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>10</td>
<td>+0.04</td>
<td></td>
</tr>
<tr>
<td>Light petroleum</td>
<td>8</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>10</td>
<td>-0.06</td>
<td>Fractions 9-10 bulked</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>-0.04</td>
<td>(Fraction B)</td>
</tr>
<tr>
<td>Light petroleum</td>
<td>11</td>
<td>10</td>
<td>+0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>20</td>
<td>+0.02</td>
<td>Fractions 11-14</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>20</td>
<td>+0.02</td>
<td>No residue on evaporation</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>20</td>
<td>+0.02</td>
<td></td>
</tr>
<tr>
<td>Light petroleum</td>
<td>15</td>
<td>10</td>
<td>-0.23</td>
<td></td>
</tr>
<tr>
<td>10% ethanol</td>
<td>16</td>
<td>20</td>
<td>-0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>20</td>
<td>-0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>20</td>
<td>-0.37</td>
<td>Fractions 15-27 bulked</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>20</td>
<td>-1.41</td>
<td>(Fraction C)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20</td>
<td>-1.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>20</td>
<td>-0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>10</td>
<td>0</td>
<td>Fractions 28-32</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>10</td>
<td></td>
<td>No residue on evaporation</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Attempted preparation of p-nitrobenzoate:** The oil (145mg.) was dissolved in pyridine (2ml.) and refluxed for 25mins. with p-nitrobenzoyl chloride (100mg.). The solution was cooled and water added dropwise. The oily globules which separated failed to crystallise (absence of borneol).

This fraction was not examined further.

b) The oils obtained from Williams (see page 137) were bulked to give a viscous yellow oil (31.013g.) which was distilled under vacuum (18mm. Hg.) and separated into three volatile fractions (total weight 21.928g.) (Table 19).

Fractions A and B were reserved. Fraction C was refluxed with ethanolic potassium hydroxide (50ml., 0.71N) for 30mins., cooled and neutralised (N hydrochloric acid) using solution of phenolphthalein (5ml.) as indicator. The neutral solution was then made weakly basic (dilute sodium hydroxide) and extracted with light petroleum, and this washed (distilled water), dried (Na₂SO₄) and evaporated to give a pale yellow neutral oil (6.75g.). This oil was chromatographed from light petroleum on alumina (201g., 30cm. x 3cm.) and separated into two fractions.

**Fraction 1** was dextrorotatory and distilled at 130-131°C (bath temp.)/18mm. Hg to give reticulene (2.493g.) as a colourless oil, $\beta^5$ 1.4955.

**Fraction 2** was laevorotatory and distilled at 120-125°C (bath temp.)/18mm. Hg to give a colourless oil (2.73g.),
Table 19

<table>
<thead>
<tr>
<th>°C, b.p.</th>
<th>( \eta_{18^\circ} )</th>
<th>Weight (g)</th>
<th>Identification</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>106-108°</td>
<td>1.4780</td>
<td>7.515</td>
<td>A</td>
<td>bornyl formate</td>
</tr>
<tr>
<td>108-116°</td>
<td>1.4817</td>
<td>7.154</td>
<td>B</td>
<td>mainly bornyl formate</td>
</tr>
<tr>
<td>120-130°</td>
<td>1.4932</td>
<td>7.259</td>
<td>C</td>
<td>mainly reticulene</td>
</tr>
</tbody>
</table>

\( \eta_{18^\circ} \) 1.4952, with an odour resembling that of reticulene.
The reticulene fractions isolated as reported in the previous pages were bulked and distilled under reduced pressure (18 mm. Hg) at 130-135°C (bath temp.) to give a colourless oil, \( \eta_{D}^{15^\circ C} \) 1.4970, \( d_{45^\circ C}^{18^\circ C} \) 0.915, \([\alpha]_{D}^{15^\circ C} +0.964 \) (c=5.29), \([R]_{D}^{15^\circ C} 65.3\), \( \lambda_{209 \mu m} (\varepsilon 3998) \), infrared absorption - peaks at 888, 1646, 3060 cm. (vinylidene); 990, 999, 1014 cm. (cyclopropane). Williams gave \( \eta_{D}^{15^\circ C} 1.4972, d_{45^\circ C}^{15^\circ C} 0.914, [\alpha]_{D}^{15^\circ C} +1.1 \) (c=4.2), \([R]_{D}^{15^\circ C} 65.22\) for reticulene.

**REACTIONS OF RETICULENE**

**Reduction**

Reticulene (1.253 g.) was dissolved in ethanol and hydrogenated at a platinum catalyst (0.219 g.) until hydrogen uptake ceased (2 hours, 0.98 ethylenic double bonds). After filtering, the solvent was removed under pressure to give dihydroisoreticulene (1.235 g., \( \eta_{D}^{15^\circ C} 1.4859 \)). Distillation at 128-135°C (bath temp.)/18 mm. Hg, gave the dihydro-compound as a colourless oil, \( \eta_{D}^{15^\circ C} 1.4842, d_{45^\circ C}^{15^\circ C} 0.902 \), which gave an orange colour with tetranitromethane. \( \lambda_{210 \mu m} (\varepsilon 1970) \), Infrared absorption - 987, 998, 1013 cm. (cyclopropane); 814 cm. (trisubstituted double bond). Williams gave b.p. 130-135°C (bath temp.)/18 mm. Hg, \( d_{45^\circ C}^{15^\circ C} 0.900 \), \( \eta_{D}^{15^\circ C} 1.4826 \) for "dihydroreticulene."
Oxidation

Reticulene (1.026g.) was shaken with Beckmann's chromic acid mixture (30ml.) in a well-stoppered flask and almost immediately, the solution turned dark-brown in colour and developed a camphoraceous odour. The reaction was left for 6 days during which time the flask was occasionally opened to release the gas pressure which had built up. The solution was extracted with ether; the ether layer was separated and washed free of acid (distilled water) then extracted with 5% aqueous sodium hydrogen carbonate. The ether layer was again washed (distilled water), dried (Na$_2$SO$_4$) and evaporated to a yellow oil (0.388g.) which partially crystallised. — Fraction 1.

The aqueous sodium hydrogen carbonate solution was acidified (dilute hydrochloric acid) and extracted with ether. The latter was dried (Na$_2$SO$_4$) and evaporated to give acidic material (0.351g.) as an almost colourless oil. — Fraction 2.

Treatment of Fraction 1: The oil was chromatographed on alumina (12cm. x 1.4cm.) from various eluants. Table 26 summarises the results.

Fractions IA and IB were not investigated further. Fraction IC (70mg.) was dissolved in ethanol (2ml.) and to this solution was added 2ml. of a solution of 2,4-dinitrophenylhydrazine prepared by dissolving 2,4-dinitrophenylhydrazine (0.5g.) in concentrated sulphuric acid (1.5ml.),
Table 26

<table>
<thead>
<tr>
<th>Eluant</th>
<th>Volume of eluate (ml)</th>
<th>Identification</th>
<th>Product on removal of solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light petroleum</td>
<td>26</td>
<td>IA</td>
<td>Yellow viscous oil which did not crystalise (31mg.)</td>
</tr>
<tr>
<td>{Light petroleum 90parts / Benzene 10parts}</td>
<td>12</td>
<td>IB</td>
<td>Camphoraceous oil (trace).</td>
</tr>
<tr>
<td>Benzene</td>
<td>50</td>
<td>IC</td>
<td>Yellow camphoraceous oil (70mg.)</td>
</tr>
<tr>
<td>{Benzene 90parts / Ethanol 10parts}</td>
<td>30</td>
<td>ID</td>
<td>Very viscous red oil (150mg.)</td>
</tr>
</tbody>
</table>

Adding dilute sulphuric acid (2.5ml.) and diluting to 15ml. with ethanol but only a faint cloudiness formed. Heating resulted in resinification.

Fraction ID (150mg.) was dissolved in ethanol (3ml.) and the same solution of 2,4-dinitrophenylhydrazine (4ml.) added. A crystalline dark-orange solid slowly separated, m.p. 63-78°C. Recrystallisation from aqueous ethanol failed to alter this m.p. so no further work was done on this fraction.

Treatment of Fraction 2 The oil was triturated with a few drops of ethanol and a small precipitate (15mg.) separated out. It was removed and recrystallised from ether/ethanol as a fatty solid which melted over a range 49-53°C (microblock), R_f 0.14 (in phenol: water: formic acid, 80: 19: 1). The
solvent was removed from the filtrate and the oil obtained chromatographed on charcoal, cellulose (1:3) (7.5g., 10cm. x 2cm.). The eluate was collected in 5ml. portions. Table 27 shows the results.

<table>
<thead>
<tr>
<th>Eluant</th>
<th>Volume of Eluate (ml)</th>
<th>Identification</th>
<th>Product on removal of solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light petroleum</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light petroleum + 1% benzene</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light petroleum + 10% benzene</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light petroleum + 50% benzene</td>
<td>40</td>
<td>2A</td>
<td>Colourless crystals contaminated with yellow oil.</td>
</tr>
<tr>
<td>Benzene</td>
<td>110</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>2B</td>
<td>Pale yellow acidic oil, ( R_F 0.954 )</td>
</tr>
<tr>
<td>Benzene + 5% ethanol</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2C</td>
<td>Yellow acidic oil (102mg.), ( R_F 0.977 ).</td>
</tr>
</tbody>
</table>

\( R_F \) values determined on Whatman No. 1 paper using phenol: water: formic acid (80:19:1) as solvent.
Fraction 2A. The oil was removed from the crystals by filtering and washing with ice-cold light petroleum after which the crystals had m.p. 170°C (microblock), with sublimation, Rf 0.935, using same system as before (Table 27).

(Contrast succinic acid which has m.p. 184°C with sublimation, Rf 0.66, in same system)

No further work was done on fractions 2A, 2B, 2C.

Ozonolysis.

The oil (2.04g.) was dissolved in dry ethyl acetate (50ml.) and the solution cooled to -3°C. A stream of ozonised oxygen was passed first through this solution and then through a solution of potassium iodide (3g.) and dilute acetic acid (10ml.) in water (40ml.). Ozonolysis was continued until iodine was freely released from the potassium iodide trap. The ethyl acetate solution was allowed to reach room temperature and the ozonide catalytically reduced at a platinum catalyst. After reduction the solvent was removed in vacuo to give a viscous, yellow camphoraceous oil, reticulodione, (1.97g.) Distillation at 170°C (bath temp.)/2.5mm. Hg, slowly caused decomposition but a portion (283mg.) distilled as a viscous almost colourless oil, λ218(ε 394), 240(ε 337, small maximum), 290μ (ε105, inflection), infrared absorption peaks at 1703, 1738cm. (in CCl₄); 1707, 1737cm. (in CS₂); 1707, 1733cm. (thin film) (diketone); 986,996,1016 (in CS₂) (cyclopropane).
Attempted dehydrogenation

Reticulene (75mg.) was mixed with 20% palladium on charcoal (22mg.) and the mixture gently refluxed for 30 mins. in an apparatus similar to that used by Williams in the dehydrogenation of aristolactone. The distillate did not become blue or violet.
**REACTIONS OF DIHYDROISORETICULENE**

**Attempted ozonolysis**

The oil (0.637g.) was dissolved in dry ethyl acetate (30ml.) and the solution cooled to 0°C. A stream of ozonised oxygen was passed first through this solution then through an acidified (dilute acetic acid) solution of potassium iodide (6% w/v). Iodine was immediately released from the latter solution. Ozonolysis, however, was continued for 10 mins. beyond the theoretical time for one double bond and the ethyl acetate solution then reduced at a platinum catalyst. After filtering, the solvent was removed under reduced pressure to give a pale yellow oil (0.575g.), $\gamma^2$ 1.4860, $\lambda 212nm (61300)$, which still gave an orange colour with tetranitromethane (i.e. dihydroisoreticulene).

**Dehydrogenation**

**Method 1** The oil (0.5g.) was mixed with 20% palladium on charcoal (100mg.) and gently refluxed for 4 hours at 270°C as before (page 164). Only a trace of a blue colouration was apparent after this time.

**Method 2** The oil (0.45g.) was mixed with powdered selenium (0.52g.) and gently refluxed at 260-270°C as before (page 164). Almost immediately, the unmistakable odour of hydrogen selenide was apparent but distillation was allowed to continue for 4 hours during which time the distillate became distinctly
blue in colour. The distillate was dissolved in cyclohexane (10ml.) which was then extracted with phosphoric acid (90\% \text{w/w}, 10ml.). The phosphoric acid layer was separated and diluted with water (26ml.) and the azulene, reticazulene, removed by extracting with cyclohexane which was washed (distilled water), dried (\text{Na}_2\text{SO}_4) and diluted with cyclohexane in order to give ultraviolet absorption readings in a suitable range (see table 25). The ultraviolet absorption spectrum was recorded in 50\% sulphuric acid by diluting the blue distillate (see above) (0.1ml.) with an equal volume of ethanol and adding 50\% sulphuric acid until readings in a suitable range were obtained: \lambda_{\text{max.}} 225, 268-270, 372\mu \text{m}.
AROMADENDRENE

The sample was obtained from Dr. Sutherland and possessed the physical constants shown in Table 20. Infra-red absorption - peaks at 885, 1640, 3065 cm\(^{-1}\) (vinylidene); 988, 999, 1014 cm\(^{-1}\) (cyclopropane).

**REACTIONS OF AROMADENDRENE**

**Dehydrogenation**

The oil (68 mg.) was mixed with 20% palladium on charcoal (20 mg.) and gently refluxed under conditions identical with those used in the attempted dehydrogenation of reticulene (page 164). After only 1½ mins. the distillate was distinctly blue in colour.

**Reduction**

The oil (0.55 g.) was dissolved in ethanol and hydrogenated at a platinum oxide catalyst (97 mg.) until hydrogen uptake ceased (30 mins.). The volume of hydrogen utilised (81 ml. at 14 °C) was equivalent to 0.97 ethylenic bonds (after deduction of the appropriate volume for the catalyst). The solution was filtered and the solvent removed to give dihydroaromadendrene as a colourless oil which gave a very pale yellow colour with tetranitromethane, \(\lambda_{209 \text{nm}(6331)}\), infrared absorption - peaks at 984, 995, 1011 cm\(^{-1}\) (cyclopropane).
To ensure reproducible conditions, the cells of the spectrophotometer were repeatedly washed with ethanol for spectrophotometric purposes until a constant blank reading was obtained. All transfers of the solutions under test were then done by pipette and absorption readings for the four substances were accepted only when the final blank reading agreed with the initial one.
DETERMINATION OF IODINE VALUES

All values were determined using a modification of the pyridine bromide method of the British Pharmacopoeia 1958. The determinations were carried out in dry glass-stoppered bottles of approximately 50ml. capacity into which the substance under test (10-50mg.) was weighed and dissolved in carbon tetrachloride (1ml.) Excess pyridine bromide solution (20ml.) was added by pipette, allowing exactly one minute drainage time, then the stoppered bottle was placed in the dark for the required time (10mins.) measured accurately from the moment the initial drop of reagent entered the bottle. Solution of potassium iodide (10%, 10ml.) was added and the liberated iodine titrated to a very pale yellow colour with sodium thiosulphate solution (0.1N), and then to a colourless end-point on adding starch mucilage. A blank was carried out simultaneously with every determination and the number of double bonds calculated according to the formula:

$$\text{No. of double bonds} = \frac{(a - b) x m}{w \times 2000 \times 10}$$

where

- \(a\) = blank titration (ml. of 0.1N thiosulphate)
- \(b\) = test titration (ml. of 0.1N thiosulphate)
- \(m\) = molecular weight of substance under test.
- \(w\) = weight of substance (g.)

Table 22 (page 144) shows the results of a number of determinations.
PERBENZOIC ACID TITRATIONS

Preparation of Reagent

Perbenzoic acid was prepared according to the method in Vogel using one-fifth quantities. The dried chloroform solution of the acid was diluted to 100ml. with chloroform and the exact perbenzoic acid content determined according to the published method using 3ml. of the chloroform solution. Each ml. of the solution contained 42.86mg. of perbenzoic acid.

Determination of unsaturation

The substance under test (20-50mg.) was weighed into a small sample tube (25mm. x 8mm.) and the total transferred to a glass-stoppered bottle (50ml. capacity). Chloroform (3ml.) and solution of perbenzoic acid (2-3ml.) were added and the bottle and contents left at 0°C for the specified time (6-48 hours), then solution of potassium iodide (10%, 10ml.) was added and the liberated iodine titrated with sodium thiosulphate (0.1N) using starch mucilage as indicator. A blank was carried out simultaneously with every determination. The number of double bonds was calculated according to the formula used in determination of iodine values.

Table 23 (page 145) summarises the results.
APPENDIX I.

STUDIES ON THE ULTRAVIOLET ABSORPTION SPECTRA OF XANTHONES
Direct comparison of xanthone spectra was used by Mull (53) and Nord (53) to establish a structure for ravenelin, which was confirmed by synthesis, and also to propose tentative structures for rubrofusarin and nor-rubrofusarin. Similar methods have also been used by Shah, Kulkarni and Dalai (52) and by Lund (110) in the study of xanthone structures. In reaching their conclusions, however, Mull and Nord appear to have ignored similarities in the spectra of 1,6- and 1,8-dihydroxyxanthones which invalidate the conclusion that the spectra of the latter are uniquely distinguishable from those of other hydroxyxanthones. Further doubt was cast on the value of direct comparisons of hydroxyxanthone spectra by Lund, Robertson and Whalley (111) who were unable to establish a correlation between the ultraviolet spectra and the position of hydroxyl groups. However, the known differences in the spectra of hydroxyxanthones and the potential value of ultraviolet spectra when working with small quantities of material led to the present re-appraisal of the method.

An examination of the ultraviolet absorption spectrum of xanthone (XXVI) in ethanol (53,112,113) shows the presence of a shoulder at 232μ (S), and maxima at 238μ (A), 260μ (B),
286μ (C) and 337μ (D), peaks B and D being broad. Diagrammatically, the spectrum has the following composition (Figure 13).

The ultraviolet absorption spectra of thirty-one substituted hydroxyxanthones have been examined and found in almost every case to conform to a similar pattern of five main absorption peaks or four such peaks and a shoulder corresponding to (S), (Table 28).

**Maxima S, A and B.**

The spectra of 2-hydroxy-, 3-hydroxy-, 4-hydroxy-, and 4-methoxy-xanthone all show clearly the same four areas of maximum absorption as xanthone, though at different wavelengths, together with a shoulder in the 230μ region. It is significant that none of these compounds are substituted in the 1-position of the xanthone molecule. Of the remaining
Table 28

<table>
<thead>
<tr>
<th>Substance</th>
<th>S Max.</th>
<th>Min.</th>
<th>A Max.</th>
<th>Min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Xanthone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. 1.OH Xanthone</td>
<td>230(sh)</td>
<td>-</td>
<td>238</td>
<td>251</td>
</tr>
<tr>
<td>3. 1.OCH3 Xanthone</td>
<td>228</td>
<td>237</td>
<td>251</td>
<td>267</td>
</tr>
<tr>
<td>4. 1.OH 3.CH3 Xanthone</td>
<td>234</td>
<td>240</td>
<td>251</td>
<td>269</td>
</tr>
<tr>
<td>5. 3.OH Xanthone</td>
<td>230(sh)</td>
<td>-</td>
<td>235</td>
<td>253</td>
</tr>
<tr>
<td>6. 2.OH Xanthone</td>
<td>225(sh)</td>
<td>-</td>
<td>237</td>
<td>-</td>
</tr>
<tr>
<td>7. 4.OH Xanthone</td>
<td>233(sh)</td>
<td>-</td>
<td>250</td>
<td>273</td>
</tr>
<tr>
<td>8. 4.OCH3 Xanthone</td>
<td>233(sh)</td>
<td>-</td>
<td>246</td>
<td>-</td>
</tr>
<tr>
<td>9. 1.OH 5.CH3 Xanthone</td>
<td>232</td>
<td>240</td>
<td>249</td>
<td>272</td>
</tr>
<tr>
<td>10.1:3(CH)2 Xanthone</td>
<td>233</td>
<td>248</td>
<td>252</td>
<td>268</td>
</tr>
<tr>
<td>11.1:5(OH)2 3.CH3 Xanthone</td>
<td>233</td>
<td>243</td>
<td>250</td>
<td>-</td>
</tr>
<tr>
<td>12.1:6(OH)2 Xanthone</td>
<td>228</td>
<td>241</td>
<td>248</td>
<td>-</td>
</tr>
<tr>
<td>13.1:7(OH)2 3.CH3 Xanthone</td>
<td>236</td>
<td>246</td>
<td>249</td>
<td>-</td>
</tr>
<tr>
<td>14.1:8(OH)2 Xanthone</td>
<td>228</td>
<td>236</td>
<td>251</td>
<td>-</td>
</tr>
<tr>
<td>15.1:2:7(CH)3 Xanthone</td>
<td>-</td>
<td>-</td>
<td>239</td>
<td>254</td>
</tr>
<tr>
<td>16.1:4:7(CH)3 Xanthone</td>
<td>-</td>
<td>-</td>
<td>237</td>
<td>249</td>
</tr>
<tr>
<td>17.1:4(OH)2 7.OCH3 Xanthone</td>
<td>-</td>
<td>-</td>
<td>237</td>
<td>249</td>
</tr>
<tr>
<td>18.1:3:5(CH)3 Xanthone</td>
<td>218</td>
<td>(220)</td>
<td>240</td>
<td>-</td>
</tr>
<tr>
<td>19.1:3:6(CH)3 Xanthone</td>
<td>226</td>
<td>240</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20.1:3:7(CH)3 Xanthone</td>
<td>234</td>
<td>245</td>
<td>257</td>
<td>269</td>
</tr>
<tr>
<td>21.1:4:8(CH)3 3.CH3 Xanthone</td>
<td>233</td>
<td>238</td>
<td>238</td>
<td>259</td>
</tr>
<tr>
<td>22.1:5:6(CH)3 3.CH3 Xanthone</td>
<td>227</td>
<td>240</td>
<td>249</td>
<td>-</td>
</tr>
<tr>
<td>23.1:6:7(CH)3 3.CH3 Xanthone</td>
<td>230</td>
<td>241</td>
<td>246</td>
<td>-</td>
</tr>
<tr>
<td>24.1.OH 6:7(OCH3)2 3.CH3 &quot;</td>
<td>230</td>
<td>233</td>
<td>254</td>
<td>-</td>
</tr>
<tr>
<td>25.1:6:8(OH)3 3.CH3 Xanthone</td>
<td>228</td>
<td>234</td>
<td>250</td>
<td>-</td>
</tr>
<tr>
<td>26. Rubrofusarin.</td>
<td>224</td>
<td>-</td>
<td>250(sh)</td>
<td>-</td>
</tr>
<tr>
<td>27. Nor Rubrofusarin</td>
<td>225</td>
<td>236</td>
<td>247</td>
<td>258</td>
</tr>
<tr>
<td>28. Aspergillone.</td>
<td>-</td>
<td>-</td>
<td>242</td>
<td>261</td>
</tr>
<tr>
<td>29. Decussatin.</td>
<td>240</td>
<td>246</td>
<td>260</td>
<td>278</td>
</tr>
<tr>
<td>30. Swertinin.</td>
<td>236</td>
<td>252</td>
<td>267</td>
<td>282</td>
</tr>
<tr>
<td>31.1.OH 3:5:6(OCH3)3 Xanthone</td>
<td>215(sh)</td>
<td>218</td>
<td>243</td>
<td>265</td>
</tr>
<tr>
<td>32.1:3:5:6(OCH3)4 Xanthone</td>
<td>219</td>
<td>245</td>
<td>260</td>
<td>-</td>
</tr>
</tbody>
</table>

† Flavone from A. reticulata 232(sh) 237 255 -
† Trimethyl ether of above 225(sh) 235 251 265

† Included for comparison only - see page 56.

(sh) = shoulder
<table>
<thead>
<tr>
<th>B Max.</th>
<th>Min.</th>
<th>C Max.</th>
<th>Min.</th>
<th>D Max.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>260</td>
<td>281</td>
<td>286</td>
<td>300</td>
<td>337</td>
<td>53, 112</td>
</tr>
<tr>
<td>280</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>261 - 268</td>
<td>273</td>
<td>306 s</td>
<td></td>
<td>332(3)</td>
<td>53</td>
</tr>
<tr>
<td>280</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>267(3)</td>
<td>273</td>
<td>300</td>
<td></td>
<td>345(3)</td>
<td>52</td>
</tr>
<tr>
<td>265(3)</td>
<td>273</td>
<td>303</td>
<td></td>
<td>355</td>
<td>53</td>
</tr>
<tr>
<td>280</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>270(3), 233(3)</td>
<td>294</td>
<td>307 s</td>
<td></td>
<td>345(3)</td>
<td>52</td>
</tr>
<tr>
<td>264(3)</td>
<td></td>
<td>319</td>
<td></td>
<td>336</td>
<td>52</td>
</tr>
<tr>
<td>281(3)</td>
<td></td>
<td>333</td>
<td></td>
<td>367</td>
<td>52</td>
</tr>
<tr>
<td>273(3)</td>
<td></td>
<td>378</td>
<td></td>
<td>390</td>
<td>53</td>
</tr>
<tr>
<td>264(3)</td>
<td>273</td>
<td>297</td>
<td></td>
<td>341</td>
<td>52</td>
</tr>
<tr>
<td>286(3), 306(3)</td>
<td>276</td>
<td>325 s</td>
<td></td>
<td>400</td>
<td>Present work</td>
</tr>
<tr>
<td>278(3)</td>
<td></td>
<td>349</td>
<td></td>
<td>&gt;400</td>
<td>53</td>
</tr>
<tr>
<td>279</td>
<td></td>
<td>352</td>
<td></td>
<td>399</td>
<td>53, 52</td>
</tr>
<tr>
<td>279</td>
<td></td>
<td>343</td>
<td></td>
<td>364</td>
<td>52</td>
</tr>
<tr>
<td>295(3)</td>
<td></td>
<td>350</td>
<td></td>
<td>375</td>
<td>110</td>
</tr>
<tr>
<td>284</td>
<td>290</td>
<td>315</td>
<td></td>
<td>360</td>
<td>110</td>
</tr>
<tr>
<td>285(3)</td>
<td></td>
<td>305</td>
<td></td>
<td>340(3)</td>
<td>Present work</td>
</tr>
</tbody>
</table>

* Strong peak
- Not present
Blank space denotes spectrum not recorded in this region.
twenty-seven xanthones examined, twenty-four are known to be 1-hydroxy- or 1-methoxy-substituted and the other three (Table 28, xanthones 26, 27, 28) suspected to be of this type. In twenty-one of these xanthones, the shoulder (S) is replaced by a maximum near 230m$\mu$ and correspondingly, a minimum is introduced near 240m$\mu$. This is accompanied in all these cases by a bathochromic shift of maxima A and B. In the other six cases (Table 28, xanthones 15, 16, 17, 18, 31, 32) all are substituted in the 1- position (XXVI) yet no minimum is apparent in the 240m$\mu$ region. Again, this contradicts Mull and Nord and the Indian workers who concluded that 1-hydroxy- or 1-methoxy-xanthones have a characteristic minimum in this region. It is more correct to say that if a minimum is present around 240m$\mu$, the xanthone possesses a 1-hydroxy- or 1-methoxy-substituent but the absence of this minimum does not necessarily imply the absence of such a substituent.

Maximum C

Positions 3 and 6 are equivalent. The Indian workers were of the opinion that the presence of a hydroxyl group in the 3-(or 6-) position of the xanthone nucleus resulted in a hypsochromic shift of maximum D (Figure 13). Compilation of Table 28 immediately showed this to be untrue. Substitution of a hydroxyl, methoxyl or possibly even a methyl group in the 3-(or 6-) position causes maximum C to shift to higher wavelengths, often with a large increase in the value of log $\varepsilon$. 

...
Figure 14 shows this effect. The graph of 3-hydroxyxanthone has been superimposed upon that of xanthone and it is clear that the positions of the minima and maxima correspond except in the region 285-330\(\mu\) (i.e. in the region of maximum C). This bathochromic shift of maximum C may result in either maximum B or D appearing only as shoulders, or in some cases, maximum B disappearing altogether. Figures 15 and 16 illustrate the gradual dominance of maximum C at the expense of maxima B and D. The maximum at C in all the 3- and 6-substituted xanthones examined is a strong peak and in almost all cases occurs near 300\(\mu\). (Table 28.)

1,8-dihydroxyxanthone also shows a large bathochromic shift of maximum C to 333\(\mu\). This same shift is shown by 1,4,8-trihydroxy-3-methylxanthone, 1,6,8-trihydroxy-3-methylxanthone, decussatin and swertinin, where the effect of the 8-substituent is apparently greater than that of the 3- or 6-substituent.

**Maximum D**

With three exceptions, (Table 28, xanthones 5, 19, 22 which are all substituted in the 3-position), substitution in the xanthone molecule results in a bathochromic shift of maximum D which, in most cases, lies between 340-360\(\mu\). Eleven derivatives, however, have this maximum above 365\(\mu\) (Table 28, xanthones 14, 15, 16, 17, 20, 21, 26, 27, 28, 29, 30). Xanthones 14, 21, 29, and 30 are 1,8-substituted so it
would seem that a further characteristic of such substitution is the presence of a maximum above 365\(\mu\). Xanthones 15, 16, 17 and 20 are all 1,7-substituted and from the limited number of examples available it is concluded that a characteristic of such substitution is the presence of a maximum near 400\(\mu\) (xanthones 15, 16, 17) except when a 3- or 6-substituent is present in which case this maximum is at a much lower wavelength (xanthones 13, 20, 23). In these cases, in contrast with 1,8-substitution, the 3- or 6-substituent is apparently the dominating one.

The structures of the remaining xanthones (xanthones 26, 27, 28) are unknown. They do not possess a strong absorption peak near 330\(\mu\) so it would seem probable that these substances are 1,7-substituted and possess no substituents in the 3- or 6-positions. A direct comparison of the ultraviolet absorption spectra of nor-rubrofusarin and 12,7-trihydroxy-xanthone agreed with this conclusion (Figure 17) indicating that nor-rubrofusarin was probably a 12,7-trihydroxy-x-methyl-xanthone. This contradicts the postulate of Mull and Nord.

**Subtractive Spectra**

Deduction of xanthone structures from spectral evidence has always been by direct comparison of spectra but the question of the contribution to the spectrum due to the position of a substituent has not been considered. As
it is possible to measure, in terms of displacement of ultraviolet absorption maxima, the contribution due to the hydroxyl, methoxyl or other conjugating substituents in derivatives (114) of molecules such as naphthalene, quinoline or isoquinoline the possibility of deducing the ultraviolet absorption spectrum of one xanthone from other xanthone spectra was thought to be worth investigating.

A theoretical spectrum for 3-hydroxyxanthone (equivalent to 6-hydroxyxanthone) was calculated from

(a) 1,6-dihydroxyxanthone  
(b) 1-hydroxyxanthone  
(c) xanthone

(a) minus (b) plus (c) was calculated at various wavelengths in terms of $\varepsilon$ and it was immediately clear that the general shape of the curve and the wavelengths of maximum and minimum absorption were in very good agreement with that of the actual curve for 3-hydroxyxanthone though there was a discrepancy in the calculated values of $\log \varepsilon$. (Table 29 and Figure 18).
Table 29

The Ultraviolet Absorption Spectrum of 3-hydroxyxanthone

<table>
<thead>
<tr>
<th></th>
<th>Calculated m(\mu)</th>
<th>Actual m(\mu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>236</td>
<td>235</td>
</tr>
<tr>
<td>Minimum</td>
<td>252</td>
<td>253</td>
</tr>
<tr>
<td>Maximum</td>
<td>261 - 265</td>
<td>261 - 268</td>
</tr>
<tr>
<td>Minimum</td>
<td>278</td>
<td>278</td>
</tr>
<tr>
<td>Maximum</td>
<td>310</td>
<td>306</td>
</tr>
</tbody>
</table>

It was therefore apparent that some additive property could be demonstrated.

1,3,5,6-Tetrahydroxyxanthone

The theoretical curve for 1,3,5,6-tetrahydroxyxanthone was then calculated from 1,3,5-trihydroxyxanthone, 3-hydroxyxanthone (= 6-hydroxyxanthone) and xanthone itself. This calculated curve was compared with those of 1,3,5,6-tetramethoxyxanthone and 1-hydroxy-3,5,6-trimethoxyxanthone and the similarities in the shape of the curve and the wavelengths of minimum and maximum absorption were again striking. (Table 30, Figure 19). Once more, the calculated values of \(\log \epsilon\) were not in agreement with the actual values.
Table 30

The Ultraviolet Absorption Spectra of 1,3,5,6-substituted xanthones

<table>
<thead>
<tr>
<th></th>
<th>1,3,5,6-tetramethoxyxanthone</th>
<th>Calculated curve of 1,3,5,6-tetrahydroxyxanthone</th>
<th>1-hydroxy-3,5,6-trimethoxyxanthone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>mμ 245</td>
<td>mμ 249</td>
<td>mμ 245</td>
</tr>
<tr>
<td>Minimum</td>
<td>mμ 260</td>
<td>mμ 260</td>
<td>mμ 265</td>
</tr>
<tr>
<td>Small maximum or shoulder</td>
<td>mμ 285</td>
<td>mμ 285</td>
<td>mμ 285</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>mμ 290</td>
<td>mμ 290</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>mμ 305</td>
<td>mμ 315</td>
</tr>
</tbody>
</table>

1,3,7-Trihydroxyxanthone

A theoretical curve was calculated for 1,3,7-trihydroxyxanthone but unfortunately a non-ambiguous curve was not possible. 1,3-Dihydroxyxanthone + 2-hydroxyxanthone = xanthone could give either 1,2,3-trihydroxyxanthone or 1,3,7-trihydroxyxanthone though the latter seemed more probable as the contribution due to an isolated 2-hydroxy- (ε 7-hydroxy-) group was being added. This was indeed the case. Although the shape of the calculated curve was not in such close agreement as in the previous two examples (Figure 20), the positions of the calculated minima and maxima were again very similar to those of the actual curve for 1,3,7-trihydroxy-
xanthone (Table 31).

Table 31

Ultraviolet Absorption Spectrum of 1,3,7-trihydroxyxanthone.

<table>
<thead>
<tr>
<th></th>
<th>Actual curve (mμ)</th>
<th>Calculated curve (mμ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>235</td>
<td>235</td>
</tr>
<tr>
<td>Minimum</td>
<td>240</td>
<td>243</td>
</tr>
<tr>
<td>Maximum</td>
<td>252</td>
<td>256</td>
</tr>
<tr>
<td>Minimum</td>
<td>271</td>
<td>271</td>
</tr>
<tr>
<td>Shoulder</td>
<td>280</td>
<td>280</td>
</tr>
<tr>
<td>Maximum</td>
<td>304</td>
<td>308</td>
</tr>
<tr>
<td>Minimum</td>
<td>332</td>
<td>332</td>
</tr>
<tr>
<td>Maximum</td>
<td>&gt;360</td>
<td>368</td>
</tr>
</tbody>
</table>

1,5,6-Trihydroxy-3-methylxanthone

To 1,5-dihydroxy-3-methylxanthone was added the contribution due to a 3-hydroxy-(= 6-hydroxy-) group, this contribution being obtained by subtracting the spectrum of xanthone from that of 3-hydroxyxanthone. The result was the theoretical ultraviolet absorption spectrum of 1,5,6-trihydroxy-3-methylxanthone. The shape of this curve was not in good agreement with the actual spectrum but, once again the
calculated positions of the minima and maxima were reasonably close (Figure 21, Table 32.)

Table 32

**Ultraviolet Absorption Spectrum of 1,5,6-trihydroxy-3-methyl-xanthone**

<table>
<thead>
<tr>
<th>Actual Curve (μ)</th>
<th>Calculated Curve (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>231</td>
</tr>
<tr>
<td>Minimum</td>
<td>240</td>
</tr>
<tr>
<td>Maximum</td>
<td>251</td>
</tr>
<tr>
<td>Minimum</td>
<td>278</td>
</tr>
<tr>
<td>Maximum</td>
<td>306</td>
</tr>
<tr>
<td>Shoulder</td>
<td>320</td>
</tr>
</tbody>
</table>

* Inflection

**Decussatin and Swertinin**

By direct comparisons of ultraviolet absorption spectra, Shah, Kulkarni and Dalal deduced that the structures of decussatin (R'=H, R''=CH₃) and swertinin (R'=R''=H) were as shown (XLIll) with the fourth substituent, a methoxyl group, in ring B but not in position 5. Decussatin and swertinin were therefore 1,2,6,3- or 1,2,7,3-tetrasubstituted xanthones.
On the wrong assumption (Table 28) that the minimum and maximum at 370 and 380 m\(\mu\) respectively in 1,8-dihydroxyxanthone were additional and absent from other hydroxyxanthones, the Indian workers postulated a hypsochromic shift of the other maxima and for this reason concluded that the fourth substituent occupied the 6-position (XLVII). Assuming that the spectra of decussatin and swertinin conform to the general pattern (Figure 13) it is clear that all maxima are at wavelengths higher than those of xanthone. This phenomenon is shown by 1,7- and 1,8-substituted xanthones (see before) so the possibility that the fourth substituent occupies position 7 cannot be excluded. The theoretical curves for 1,2,6,3-tetrahydroxy- and 1,2,7,8-tetrahydroxyxanthone were therefore calculated and compared with the actual curves of decussatin and swertinin. Table 33 shows that the 7-substituted xanthone is a better fit but it is obvious that spectral studies cannot distinguish between the possibilities.
Table 33

Ultraviolet Absorption Spectra of Decussatin and Swertinin

<table>
<thead>
<tr>
<th></th>
<th>Decussatin (μm)</th>
<th>Swertinin (μm)</th>
<th>Calculated curve of 1,2,7,3-tetra-hydroxyxanthone (μm)</th>
<th>Calculated curve of 1,2,6,8-tetra-hydroxyxanthone (μm)</th>
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<td>240</td>
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<td>237</td>
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<td>267</td>
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<td>259</td>
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<tr>
<td>Minimum</td>
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<tr>
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<tr>
<td>Maximum</td>
<td>375</td>
<td>390</td>
<td>&gt; 375</td>
<td>370</td>
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</table>

1,4,8-Trihydroxyxanthone

The ultraviolet absorption spectrum of 1,4,8-trihydroxyxanthone was calculated from 1,8-dihydroxyxanthone, 4-hydroxyxanthone and xanthone. It was compared with the spectrum of Ravenelin (1,4,8-tri hydroxy-3-methylxanthone) but they were dissimilar (Table 34).

1,4,7-Trihydroxyxanthone

The theoretical curve for 1,4,7-tri hydroxyxanthone was also quite unlike the actual curve (Figure 22). The curve was calculated from 1,5,7-tri hydroxyxanthone, 3-hydroxyxanthone
and 4-hydroxyxanthone, however, which could give rise to either a 1,4,7-trihydroxyxanthone or a 1,5,7-trihydroxyxanthone and because of this ambiguity, no stress can be placed on this result.

1,2,7-Trihydroxy-3-methylxanthone.

A portion of the theoretical curve of 1,2,7-trihydroxy-3-methylxanthone was calculated from 1,7-dihydroxy-3-methylxanthone, 2-hydroxyxanthone and xanthone. The curve obtained had a large maximum in the neighbourhood of 300µ, which is absent from 1,2,7-trihydroxyxanthone again illustrating dissimilarity.

From the preceding results it can be seen that some additive property is present in the spectra of xanthenes but
it is quite obvious that other effects, the contributions of which cannot be anticipated, must also be considered. In some cases these factors may be of a minor nature and hence a theoretical spectrum similar to the actual spectrum would be obtained, but in other cases, they appear to contribute largely to the spectrum.

One such factor is the presence of a methyl group. A comparison of the spectra of 1-hydroxyxanthone, 1-hydroxy-3-methylxanthone and 1-hydroxy-5-methylxanthone showed that, apart from differences in log $\varepsilon$ values, these spectra were almost identical and hence it was assumed that the methyl group had little effect on the shape of the spectrum. Such a conclusion might not be applicable in the case of more heavily substituted xanthones. A separate investigation into the effect of the methyl group on the spectra of xanthones is required before further work can be done on the additive effect.

A second factor is the problem of interaction between vicinal and other neighbouring groups. The discrepancies observed between the calculated and observed spectra of 1,4,3-, 1,4,7- and 1,2,7-trihydroxyxanthones may no doubt be explained in this way. To investigate such an effect, the spectra of many more xanthones would have to be examined. The syntheses and ultraviolet absorption study of such xanthones would also be a separate investigation.
EXPERIMENTAL
The author is indebted to Drs. F. E. King and T. J. King for samples of 1,3,5,6-tetramethoxyxanthone and 1-hydroxy-3,5,6-trimethoxyxanthone and for a translation of the paper by Tanase (see reference 50). He also is indebted to Professor T. S. Wheeler for samples of 1,2,7-trihydroxyxanthone, 1,4,7-trihydroxyxanthone and 1,4-dihydroxy-7-methoxyxanthone.
The spectra of xanthones 15, 16, 17, 31 and 32 (Table 28) were determined in absolute ethanol on a Hilger Uvispek photoelectric spectrophotometer. The absorption spectra quoted by Lund were in terms of frequency and were therefore replotted in terms of wavelength as were the spectra given by Mull and Nord. For accurate measurement, the spectra published by the latter authors were projected onto a suitable scale before wavelengths of minimum and maximum absorption were recorded. The accuracy of this method was confirmed by comparing the resultant graph of xanthone with other published spectra (see Table 28).

**Calculation of Theoretical Spectra**

As most published spectra are accurate only to the second decimal place in terms of log€, the resultant theoretical spectra cannot be more accurate. In some cases the calculated value of € is a small negative value but this is of no importance as interest is focussed only on the wavelengths of minimum and maximum absorption and in the general shape of the theoretical graph. In any case, after calculating the values of € at various wavelengths, an arbitrary constant can be added to each before conversion into log €. This has no effect on the wavelengths of minimum and maximum absorption and, provided that the constant is reasonably small, it does not
affect the shape of the graph (see Figure 18).

The theoretical curves were all calculated in identical fashion so, to avoid repetition, a work sheet is recorded for one typical example.
Theoretical ultraviolet absorption curve of 3-hydroxyxanthone

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<th>( \mu m )</th>
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<th>b. ( \epsilon )</th>
<th>c. ( \epsilon )</th>
<th>d. ( \epsilon )</th>
<th>e. ( \epsilon )</th>
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Modified theoretical ultraviolet absorption curve of 3-hydroxyxanthone

This curve was calculated from the previous one by adding an arbitrary constant to the calculated value of $\varepsilon$ so that all new values of $\varepsilon$ became positive.

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Figure 17.

1,2,7-(OH)$_3$-xanthone

Norrubrofusarin

Wavelength (m$\mu$)
Figure 18.
3-OH-xanthone.

- calculated (see p. 189)
- actual

Wavelength (mÅ)
Figure 19.

Log E vs Wavelength (m/µ)

1,3,5,6-(OCH₃)₄-xanthone

1,3,5,6-(OH)₄-xanthone (calculated)

1-OH 3,5,6-(OCH₃)₃-xanthone

1,3,5,6-(OH)₄-xanthone (calculated)
Figure 2a.
1,3,7-(OH)$_3$-xanthone
Figure 21.
Calculated curve of 1,5,6-(OH)3-3-CH3-xanthone
Figure 22

$1,4,7-(\text{OH})_3$-xanthone.
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