STUDIES ON

THE CHEMISTRY OF *ARISTOLOCHIA* SPECIES

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A THESIS

submitted to

The UNIVERSITY of GLASGOW

by

Ronald Thomson Coutts

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Glasgow.

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PART I.

THE ETHANOL-SOLUBLE FRACTIONS OF

_A._ _RETICULATA_

_A._ _INDICA_

_A._ _SERPENTARIA_

_A._ _LONGA_

HISTORICAL INTRODUCTION

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 [1]. Though the plants, and extracts from them, are now no longer in general use, recent work by Shaw [2] 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 [3] 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 [4], Micrococcus pyogenes [5], M. citreus [5] and B. anthracis [5] while a product isolated from A. elegens was found [6] 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 [7,8,9] 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 [10] of Aristolochia, but unfortunately much confusion of nomenclature occurs in the literature. An appraisal of the relevent publications reveals that "aristolochine" has been used to describe both acidic [11] and basic [12,13,14,15] material; "aristolochic acid" describes different acids [11,16] and what is obviously the same acid has been named "aristolochic acid" [17], "isoaristolochic acid" [15], "aristinic acid" [14] and "aristolochine" [11] 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 [15], 250°C [14], 260-261°C [17], 280-282°C [18], 285°C [19] and 286-288°C [20]; again melting points of 215°C [11], 275°C [14,15], 287-292°C [19] and 281-286°C (21) 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 (12) 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 (22) 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 (23) from the bark of *A. antihystericae* by cold extraction with ether, but were not identified as being acidic.

Still later in 1849, Winkler (24) 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[25] 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 Brandes.

The study of A. clematitis was continued by Walz[16,26] 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 $C_9H_{10}O_6$, 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[27] 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[11] described the isolation of aristolochine (German, Aristolochin), 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 $C_{32}H_{22}N_2O_{13}$, 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

C 59.93%; H 3.57%; N 4.23%.

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[14] continued the chemical study, extracting the powdered roots of A. 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 alkalis. The following analyses were obtained: C 60.16, 60.57%; H 3.68, 3.51%; N 3.46, 3.41, 3.85%. $C_{18}H_{13}O_7N$ 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 $C_{15}H_{11}O_7N$ or $C_{15}H_{13}O_7N$.

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 $C_{17}H_{11}O_7N$. 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 _A. indica_ and maintained that _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 in these acids was awakened by Castille [(17)] with the isolation in 1922 of aristolochic acid from *A. sipho* l'Hérit. The acid had the empirical formula $C_{17}H_{11}O_{7}N$, 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 $C_{19}H_{15}O_{7}N$, than to the monomethyl one, $C_{18}H_{13}O_{7}N$.

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 $C_{17}H_{13}O_{4}N$.

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, $C_{17}H_{11}O_{7}N$, 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 $C_{24}H_{15}O_8N$, 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,

$C_{14}H_{11}O_5N\ (COOH)_2$, m.p. 164.5°C , which lost one mol. of water when kept at 120°C for 3 hours.

Rosenmund and Reichstein (18), who gave an excellent survey of the literature on Aristolochia species, isolated crude aristolochic acid from the root stock of _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, $C_{17}H_{11}O_7N$, m.p. 274-278°C (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, $C_{18}H_{13}O_7N$, m.p. 280-282°C (decomp.) which was saponified only with difficulty and this with considerable decomposition.

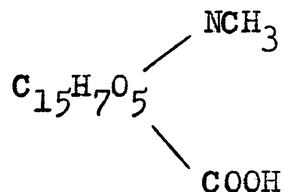
Pohl and Krishnaswamy et al. had concluded from similar observations that a carboxyl group was not present and confirmatory evidence was sought by Rosenmund and Reichstein. It was found that aristolochic acid gave no ferric chloride reaction, which suggested that enolic groups were unlikely to be present, 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, $C_{16}H_{11}O_5N$, 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 $C_{18}H_{13}O_4N.\frac{1}{2}H_2O$. Acetylation of this product yielded a crystalline acetate, thought to be a diacetate, $C_{22}H_{15}O_6N$ or $C_{22}H_{17}O_6N$, 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:



(I)

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 $C_{17}H_{11}O_7N$ 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 $C_{18}H_{13}O_7N$ 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.

In 1954, Green, Eugster and Karrer[(28)] 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.

Pailer, Belohlav and Simonitsch [19,21] 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, $C_{17}H_{11}O_7N$, m.p. 287-292°C [19], 281-286°C [21] (decomp.) depending on rate of heating, the melting points being determined on a microblock.

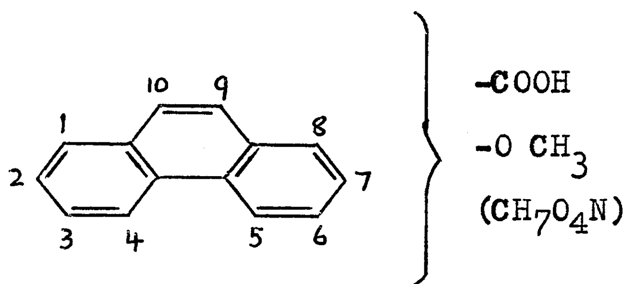
Treatment with diazomethane gave a methyl ester, $C_{18}H_{13}O_7N$, m.p. 285°C [19], 281°C [21] and decarboxylation with copper powder in quinoline gave the expected compound, $C_{16}H_{11}O_5N$, m.p. 216°C [19], 212°C [21]. The original acid and the above two derivatives were therefore identical with 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.



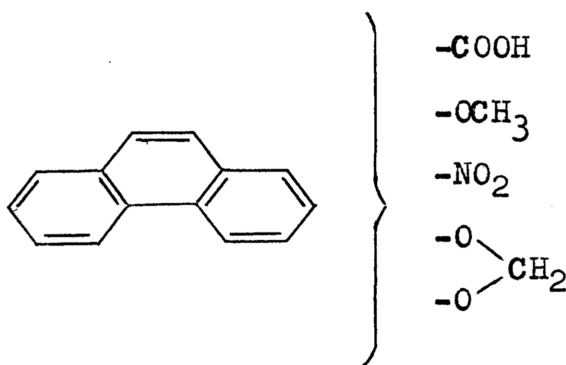
(II)

Catalytic hydrogenation of the acid or the methyl ester resulted in a hydrogen uptake of three mols, and gave a neutral compound, $C_{17}H_{11}O_4N$, 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, $C_{16}H_{13}O_3N$, 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 was liberated [29], thus permitting extension of the partial structure to that shown (III).



(III)

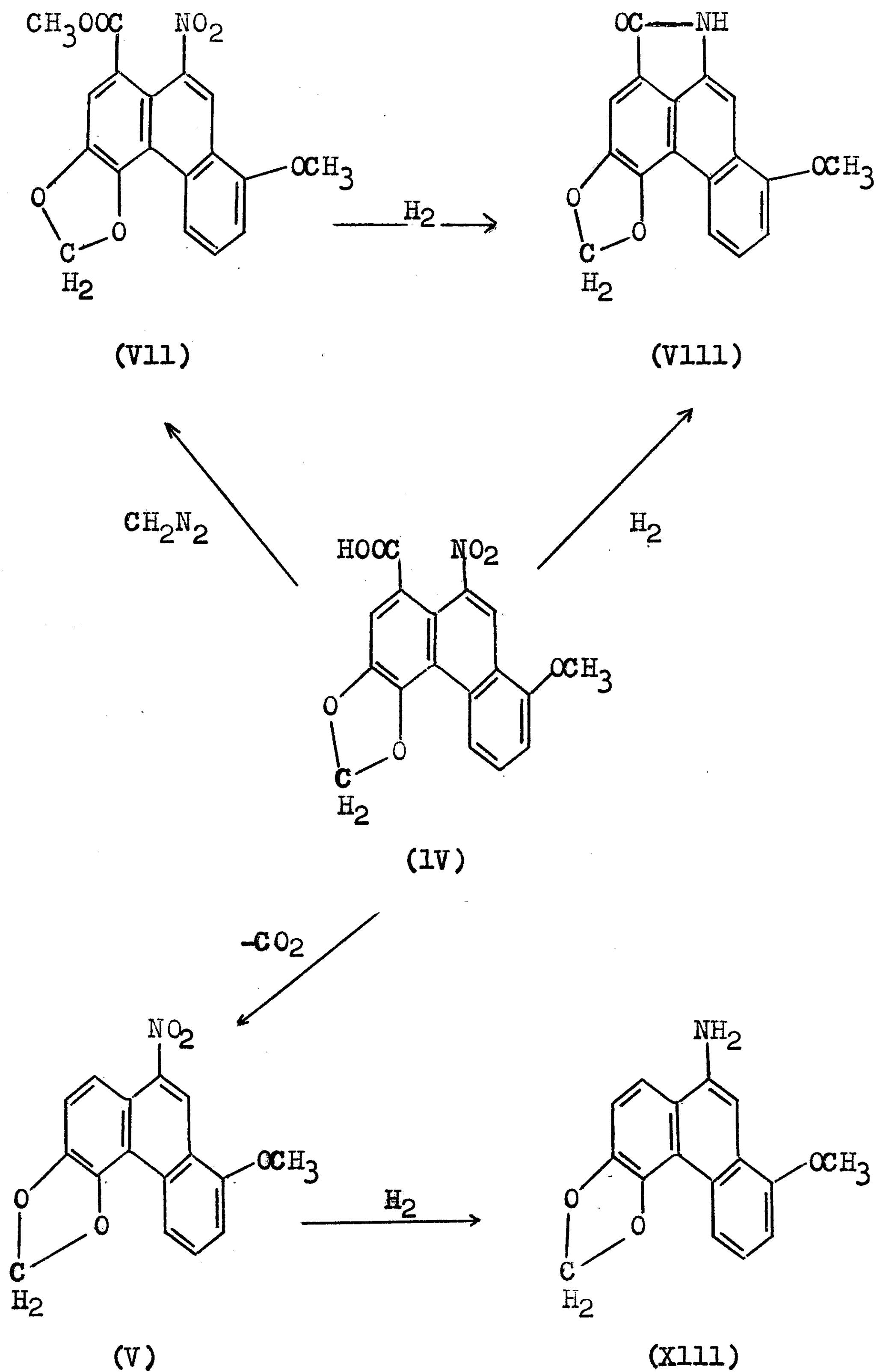
The complete structure of aristolochic acid was established as given (lV) by oxidation of the decarboxylated acid (V) with hydrogen peroxide in tetrahydrofuran. The dibasicity of the resultant diphenic acid (Vl), $C_{16}H_{12}O_7$, m.p. 246°C [19], 243°C [21], was confirmed by formation of

the dimethyl ester, $C_{18}H_{16}O_7$, 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 (V11) gave a lactam (V111) 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 (1X), $C_{13}H_8O_4$, 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 (X1), characterised as its anhydride.

Confirmatory evidence for the lactone structure (1X) was obtained by synthesis. 1,5,6 -Trimethoxyphenanthrene-10-carboxylic acid was oxidised in two stages to a diphenic, dibasic acid (X11), $C_{17}H_{16}O_7$, 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-



CH3OOC
NO2
OCH3
O
O
C
H2
H2
OC
NH
OCH3
O
O
C
H2
(V11)
(V111)
CH2N2
H2
HOOC
NO2
OCH3
O
O
C
H2
(1V)
-CO2
NO2
OCH3
O
O
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H2
H2
NH2
OCH3
O
O
C
H2
(V)
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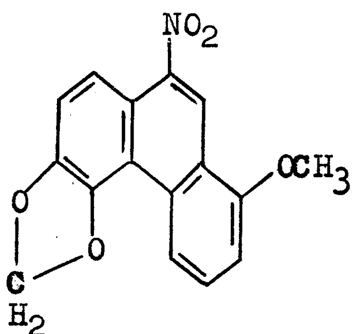
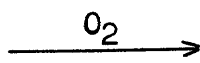
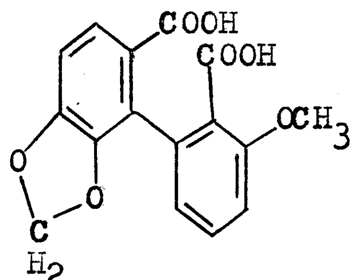
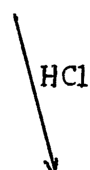
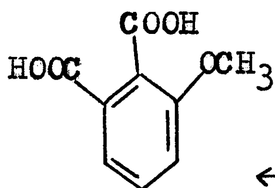
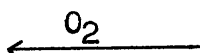


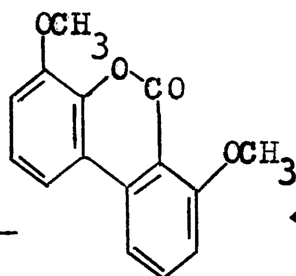


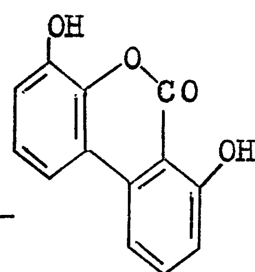


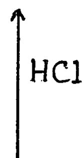


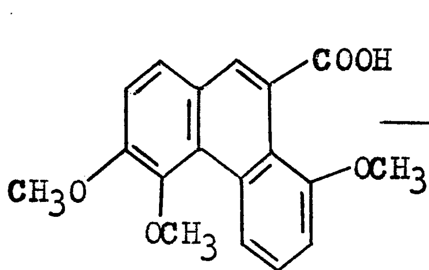


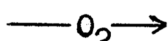


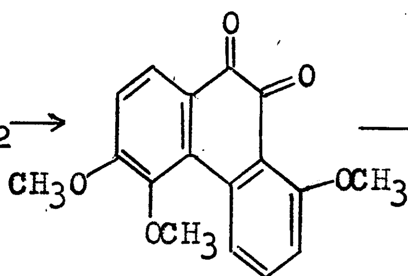


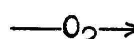


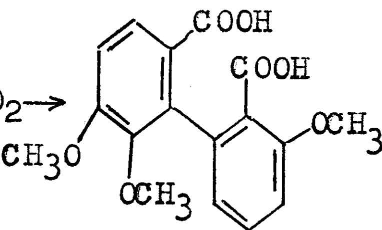












(X11)

phenanthrene-l-carboxylic acid. It therefore follows that the sensitive basic substance obtained on catalytic hydrogenation of decarboxylated aristolochic acid is as shown (X111).

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. clematitis.

This was at first called nor-aristolochic acid[19] but in a later publication was re-named aristolochic acid - II[20]. It had an empirical formula $C_{16}H_9O_6N$ and was reported as melting at 209°C though the authors stated that it was not completely pure. Separation of aristolochic acid -II from aristolochic acid (lV) 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°C, came through first followed by the methyl ester of aristolochic acid, m.p. 287-288°C. The former, $C_{17}H_{11}O_6N$, contained one methoxyl, one methylenedioxy and one nitro group. The ultraviolet spectrum had a maximum at 251mμ, typical of a phenanthrene derivative, which suggested that aristo-

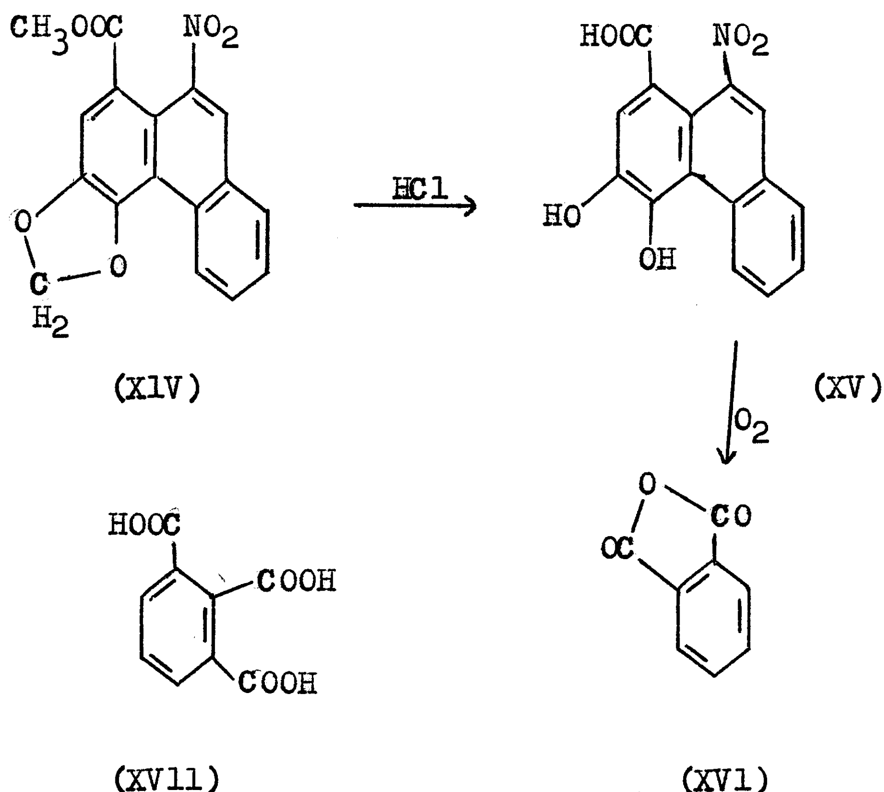
lochic acid -II was a methylenedioxynitrophenanthrenecarboxylic acid.

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(lV). 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 (XlV) 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 (XVl) was identified as a breakdown product, proving that the methylenedioxy group was attached to the same ring as the carboxyl group, otherwise hemimellitic acid (XVll) would have been produced.

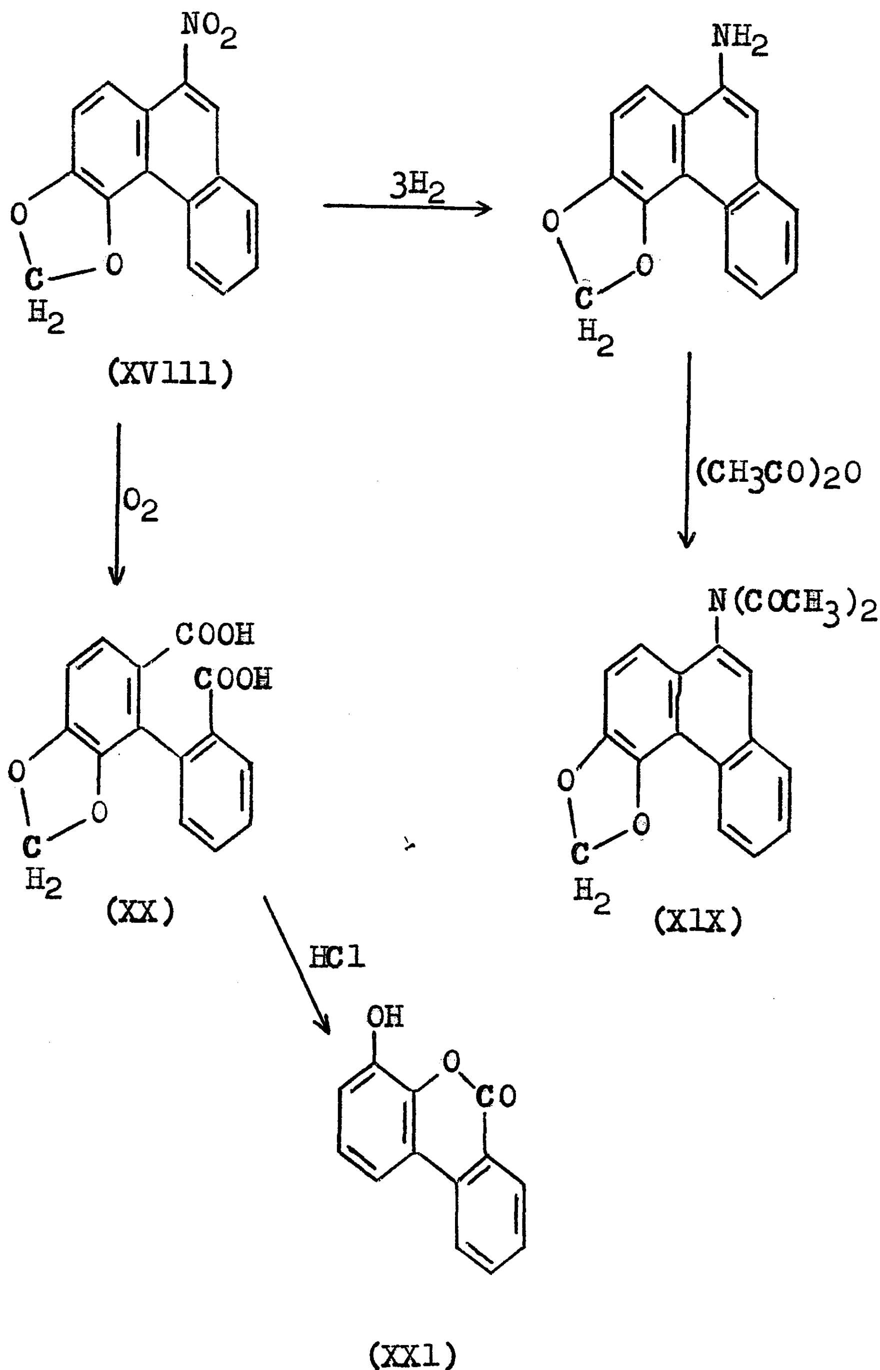
The structure of aristolochic acid -II was therefore 2,3- or 3,4-methylenedioxy-10-nitrophenanthrene-1-carboxylic



acid. To distinguish between these two possibilities, decarboxylated aristolochic acid -II (XV111) was reduced and then, with some difficulty, acetylated under hydrogen. The resulting 10-diacetylamino- 3,4-methylenedioxyphenanthrene (X1X) was identical with synthetic material prepared in an unambiguous manner.[30]

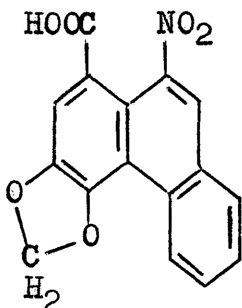
Further proof was obtained by oxidation of decarboxylated aristolochic acid -II (XV111) to a methylenedioxydiphenyldicarboxylic acid, $C_{15}H_{10}O_6$ (XX) which was not identical with synthetic 4,5-methylenedioxydiphenyl-2,2'-dicarboxylic acid and must therefore have been the corresponding 5,6-methylene-

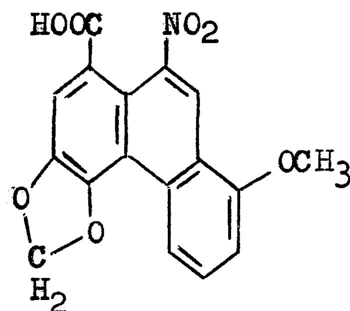
dioxy isomer. Treatment of this with hydrochloric acid under pressure gave a compound identical with synthetic 3,4-benz-8-hydroxycoumarin (XX1).



(XX1)

Aristolochic acid -II (XXll) was therefore 3,4-methylenedioxy-10-nitrophenanthrene-1-carboxylic acid and only differed from aristolochic acid (lV) in not possessing a methoxyl group.





(XXll) (lV)

Since the appearance of the publications by Pailer, Belohlav and Simonitsch and Pailer and Schleppnik, other workers have reported the presence of aristolochic acid (lV) in A. kaempferi[3] and A. debilis[3,31].

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[12] 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[32] 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[13] isolated aristolochine from A. reticulata he was of the opinion that all three substances were identical. Winkler[24], 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[14] 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[33] had previously reported the absence of basic material in *A. cymbifera*, Peckolt[34] 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 aftertaste.

In agreement with Hesse's opinion, Dymok and Warden[27] 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 [15] reported the isolation of a crystalline alkaloid, again called aristolochine. The study of this base was continued by the first two authors [35]. 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, $C_{17}H_{19}O_3N.HCl$, m.p. 268°C (decomp.). Recrystallisation from methanol gave the base, $C_{17}H_{19}O_3N$, m.p. 215°C, $[\alpha]_D^{25}$ -268.5°, 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.

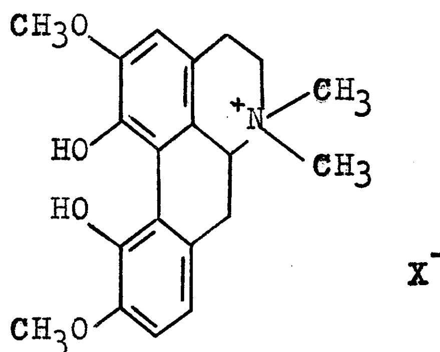
Some of the properties ascribed to aristolochine are rather puzzling. It was sparingly soluble in most common organic solvents though it dissolved readily in alkalis from which a saturated solution of carbon dioxide re-precipitated it. This suggests that a phenolic or other weakly acidic

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 A. indica was published in 1937 but no further work on it has appeared since that date.

More recently, in 1947, Shaw[2] commenced a general survey on alkaloids from Australian flora and simply reported that 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[3] in A. kaempferi Willd.



(XX111)

and A. debilis Sieb. et Zucc. The base was isolated as a reineckate and on regeneration was converted into the styphnate, $C_{20}H_{24}O_4N. C_6H_2O_8N_3$, m.p. 230°C. Magnoflorine is identical with corytuberine methyliodide (XX111)[36]. It has, very recently, also been found present in A. clematitis[37].

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 Venkato Rao[15], 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[38] 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.. Allantoin has also be identified[15] in the water soluble portion of an ethanolic extract obtained from _A. indica_.

D I S C U S S I O N

O F

E X P E R I M E N T A L

W O R K

INTRODUCTION TO THE PRESENT 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.

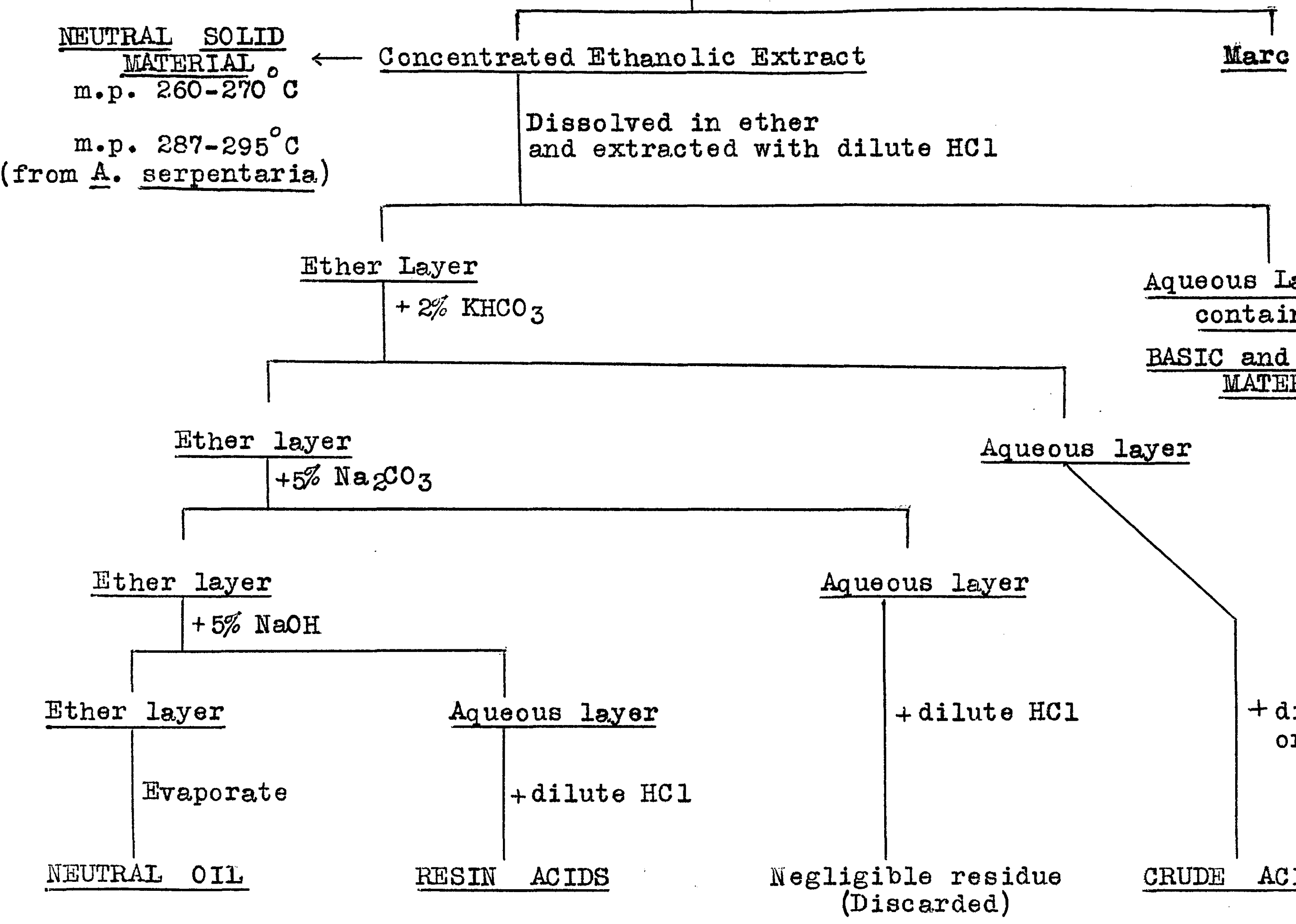
Extraction of the marc from one of the batches of _A. reticulata_ with hot ethanol produced, on concentration , a very dark oil which deposited needle crystals, m.p. 223 - 232°C, not identical with the material obtained from the cold extract. After removal of these crystals, the oil was treated as before.

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)
NEUTRAL SOLID MATERIAL m.p. 260-270°C
m.p. 287-295°C (from A. serpentaria)
Concentrated Ethanolic Extract
Marc
Dissolved in ether and extracted with dilute HCl
Ether Layer
+2% KHCO3
Aqueous Layer containing
BASIC and WATER-SOLUBLE MATERIAL
Ether layer
+5% Na2CO3
Aqueous layer
Ether layer
+5% NaOH
Aqueous layer
Ether layer
Aqueous layer
+dilute HCl
+dilute HCl or dilute CH3COOH
Evaporate
+dilute HCl
NEUTRAL OIL
RESIN ACIDS
Negligible residue (Discarded)
CRUDE ACIDS

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 Aristolochia, each referred to as A. longa[39]. 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.

EXAMINATION OF CRUDE ACID FRACTIONS

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.

Fraction	Appearance	m.p. °C	R_F
1	Orange-yellow needles	269 - 270	0.92
2	Brown-yellow plates, orange-yellow when powdered.	272 - 273	0.92
3	Pale brown-yellow needles	272 - 275	0.91
4	Pale brown-yellow needles	271 - 273	0.915
5	Yellow needles	275 - 277	0.93
6	Orange plates	273	0.94
7	Brown-yellow plates	268	-
8	Orange-red needles	266	0.78
9	Orange-brown needles	268	0.79
10	Orange needles	270	-

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, $C_{17}H_{11}O_7N$, 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 $C_{17}H_{11}O_7N$, has been reported[(21)] as forming orange-red threads from NN-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[(14)] and Rosenmund and Reichstein[(18)]

for the methoxyl content.

The ultraviolet absorption spectrum of product A was recorded (Figure 1) and showed peaks at 223mμ (log ε 4.49), 250mμ (log ε 4.47), 318mμ (log ε 4.11) and 390mμ (log ε 3.85) which are in excellent agreement with those recorded[18] for methyl aristolochate from A. sipho and almost identical with those obtained by Pailer and his co-workers for aristolochic acid from A. clematitis[20].

Decarboxylation of product A with copper powder in quinoline gave the neutral substance, $C_{16}H_{11}O_5N$, as orange needles, m.p. 213°C (microblock), identical with that obtained by Rosenmund and Reichstein[18] who reported a melting point of 206 - 212°C. The ultraviolet absorption spectrum of decarboxylated product A agrees with that recorded by Pailer, Belohlav and Simonitsch[21] 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. Reduct-

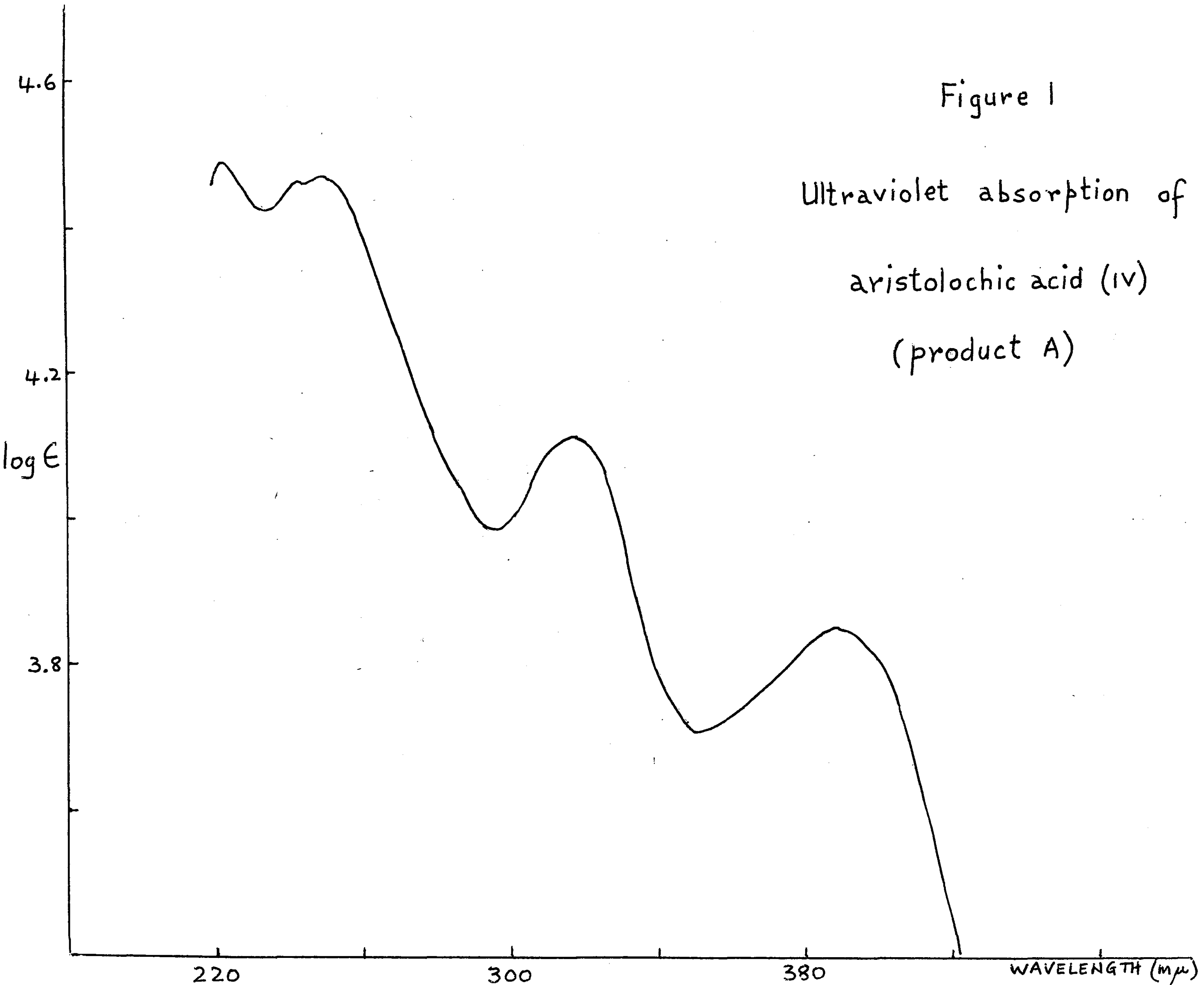


Figure 1
Ultraviolet absorption of
aristolochic acid (IV)
(product A)
4.6
4.2
log ϵ
3.8
220
300
380
WAVELENGTH (mμ)

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, $C_{17}H_{11}O_4N$, has previously been identified as 9-amino-1-methoxy-5,6-methylenedioxy-8-phenanthroic lactam (V111)[21].

Table 2.

Redn.	Weight of Product A (mg.)	Solvent	Conc.	Catalyst	Time	Uptake of Hydrogen (mols.)
1	104.0	glacial acetic acid	0.104%	PtO_2	160min.	2.70
2	41.5	Dioxan	0.415%	20%Pd on charcoal	30min.	3.00
3	50.0	glacial acetic acid	0.05%	PtO_2	60min.	2.94
4	68.0	glacial acetic acid	0.068%	PtO_2	5hrs.	2.85
5	320.0	dioxan	0.40%	20%Pd on charcoal	4hrs.	2.15

It is undoubtedly identical with the neutral yellow substance erroneously formulated as $C_{17}H_{13}O_4N$ obtained by Castille[17] on reduction of aristolochic acid from A. sipho by zinc and acetic acid. It is also identical with the reduction product, said to be $C_{18}H_{13}O_4N.\frac{1}{2}H_2O$, 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:

Found[18] : C 67.68 , 67.88 , 67.97%

H 3.89 , 3.95 , 4.24%

N 4.72 , - , 4.60%

Proposed: Diacetates: $C_{22}H_{17}O_6N$ requires C 67.51,H 4.38,N 3.58%

$C_{22}H_{15}O_6N$ requires C 67.86,H 3.89,N 3.61%

Monoacetate: $C_{19}H_{13}O_5N$ 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 $C_{19}H_{15}O_6N$ 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[14] and, as it also differed from aristolochic acid -II obtained from <u>A. clematitis</u>[20], it was designated <u>aristo-red</u>.

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 possessed 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 (V111) rather than aristolochic acid (1V).

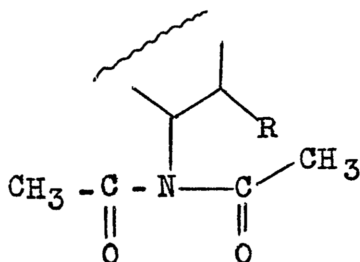
The absence of nitro- and carboxyl groups in aristo-red was shown by the study of infrared absorption curves. Thus, a $C-NO_2$ group shows two strong bands in the ranges 1500-1560cm.$^{-1}$ and 1300-1360cm.$^{-1}$ [40]. These two bands are present in aristolochic acid at 1525 and 1343cm.$^{-1}$ and in 1-methoxy-5,6-methylenedioxy-9-nitrophenanthrene (decarboxylated aristolochic acid) at 1515 and 1343cm.$^{-1}$ 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 organic acids, there are five bands to be considered in detecting a carboxyl group. Three of these bands simply provide confirmatory evidence but the bands in the range 2500-2700cm.$^{-1}$ and near 1700cm.$^{-1}$ are most highly characteristic. The range 2500-2700cm.$^{-1}$ 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 group.[41] Absorption in this region, which is due to the -OH stretching vibrations, consists of one to three bands. A

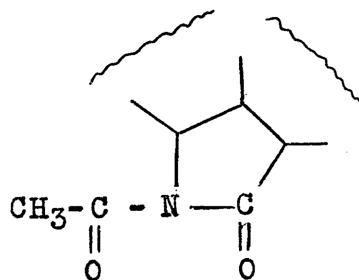
study of the six relevent infrared spectra (Figures 2 - 7) shows that aristolochic acid alone gives broad absorption in this region with two peaks at 2590 and 2640cm.$^{-1}$ whereas decarboxylated aristolochic acid, the lactam, the lactam acetate, aristo-red and its acetate do not absorb and therefore do not possess a carboxyl group.

Strong confirmatory evidence that aristo-red is closely related to aristolochic acid lactam (V111) was obtained from further comparisons of the infra-red and ultraviolet absorption spectra of aristo-red and its acetate with those of the lactam (V111) 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 - 3300cm.$^{-1}$ (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 - 1700cm.$^{-1}$ region (the lactam carbonyl region), aristo-red shows a broad band and peak at 1704cm.$^{-1}$, and a peak at 1652cm.$^{-1}$ which move to 1728cm.$^{-1}$ and 1702cm.$^{-1}$ respectively on acetylation. Similarly the lactam (V111) shows broad absorption in this region with a peak at 1691cm.$^{-1}$, and a second peak at 1655cm.$^{-1}$ which move to 1724cm.$^{-1}$ and 1702cm.$^{-1}$ respectively on acetylation. The two

bands in the region of 1702 and 1724cm.$^{-1}$, shown by both acetates, are typical of NN-diacylarylamines [42,43,44].



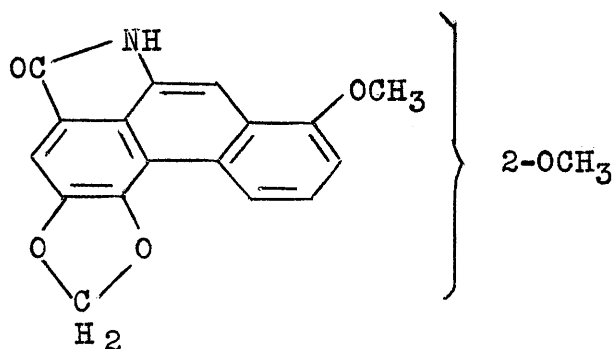
NN-Diacylarylamines



Lactam Acetates

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 (XX1V).



(XX1V)

Figure 2

DECARBOXYLATED ARISTOLOCHIC ACID.

MEDIUM : KBr disc.

CONC^{N} : 0·32 % w/w.

PERCENTAGE TRANSMISSION.

100%

0%

KBr

KBr

739 760 793 817 872 900 944 982 1034 1057 1081 1108 1139 1153 1175 1215 1228 1272 1294 1343 1357 1407 1441 1468 1515 1542 1598 2816 2928

cm.$^{-1}$

Figure 3

ARISTOLOCHIC ACID

MEDIUM : KBr disc

CONC^{N} : 0·28 % w/w.

PERCENTAGE TRANSMISSION.

100%

0%

KBr

KBr

760 797 816 848 881 905 951 1006 1046 1060 1086 1120 1155 1199 1230 1257 1278 1351 1376 1393 1418 1452 1470 1525 1593 1623 1652 1682 1715 2590 2640 2820 2892 2945 3047

Figure 4

LACTAM.

(REDUCED ARISTOLOCHIC ACID)

MEDIUM : KBr disc

CONC^{N} : 0.35 % w/w

PERCENTAGE TRANSMISSION.

100%

0%

KBr

KBr

742 752 788 815 848 875 902 941 980 1009 1055 1075 1165 1179 1213 1273 1316 1334 1367 1419 1441 1453 1473 1507 1542 1622 1655 1691 3075 3195

cm.$^{-1}$

Figure 5

ARISTO-RED

MEDIUM : KBr disc.

CONC^{N} : 0.35 % w/w.

PERCENTAGE TRANSMISSION.

100%

0%

KBr

KBr

745 757 771 783 807 828 850 886 907 956 981 1021 1055 1078 1152 1183 1212 1263 1300 1321 1362 1411 1450 1467 1482 1510 1541 1605 1652 1704 3062 3105 3162

Figure 6

LACTAM ACETATE.

MEDIUM : KBr disc.

CONCN. 0·21 % w/w

749 783 800 816 859 894 924 946 971 995 1052 1080 1096 1127 1160 1197 1214 1269 1288 1339 1369 1406 1431 1469 1500 1537 1605 1640 1702 1724

KBr KBr

100% 0%

PERCENTAGE TRANSMISSION.

cm.$^{-1}$

Figure 7

ARISTO-RED ACETATE.

MEDIUM : KBr disc.

CONCN : 0·24 % w/w

750 783 801 828 855 903 927 965 991 1038 1085 1095 1153 1180 1196 1213 1269 1296 1336 1375 1414 1448 1463 1485 1537 1605 1638 1702 1728

KBr KBr

100% 0%

PERCENTAGE TRANSMISSION.

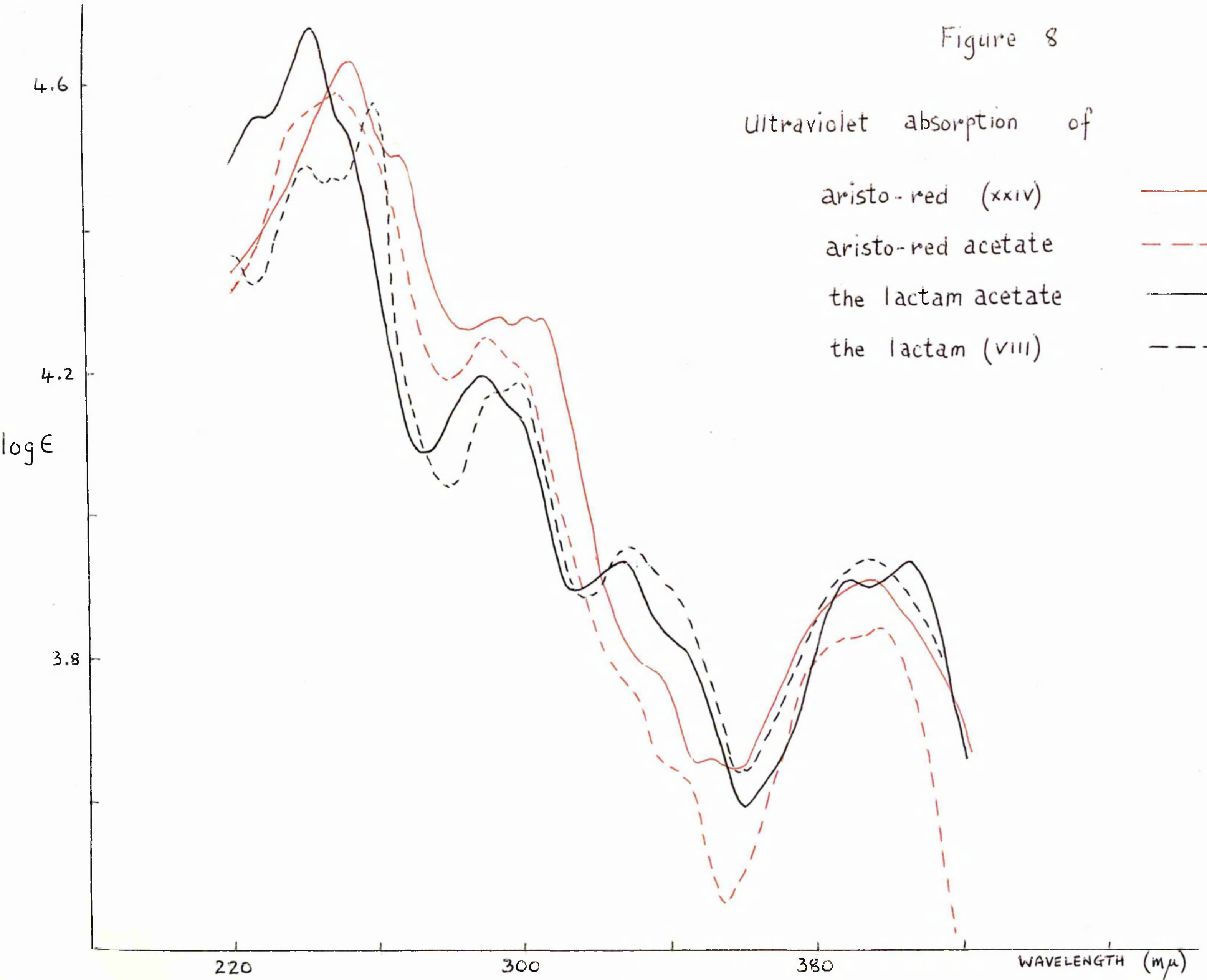


Figure 8
Ultraviolet absorption of
aristo-red (xxiv)
aristo-red acetate
the lactam acetate
the lactam (viii)
4.6
4.2
3.8
log ε
220
300
380
WAVELENGTH (mμ)

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. Sub-limation 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 (V111).

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.

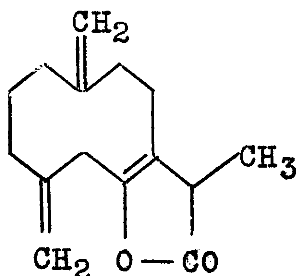
A. longa

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* clears up the controversial reports of both its presence [11] and absence [14] in this source.

General Conclusion

From the present work and on reference to the relevent 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, Stenlake and Williams [45] 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 aristidinic acid, both isolated by Hesse [14] from *A. Argentina*,



(XXV)

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, $C_{15}H_{11-13}O_7N$, m.p. 260 - 270°C, from the same source clearly differs from aristolochic acid.

Table 3.

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

* Present in unstated amount

\- No specific search recorded

0 Substance absent

Recently, Cavallito and Bailey[48] 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

	Substance B	Aristolochic Acid
Appearance	Yellow needles	Yellow needles
m.p.	Darkens between 230-260°C without melting	Darkens similarly, then melts at 275-277°C (decomp.)
Analysis	Found: C 58.2 H 3.5 N 4.55%	Required: C 59.8 H 3.2 N 4.1%
Ultraviolet Absorption Spectrum	$\lambda_{max.}$ (mμ) — ε (Based on $C_{17}H_{11}O_7N$) 250 — 29,325 318 — 12,820 390 — 6,615	$\lambda_{max.}$ (mμ) — ε 223 — 30,000 250 — 29,400 318 — 13,100 390 — 7,300

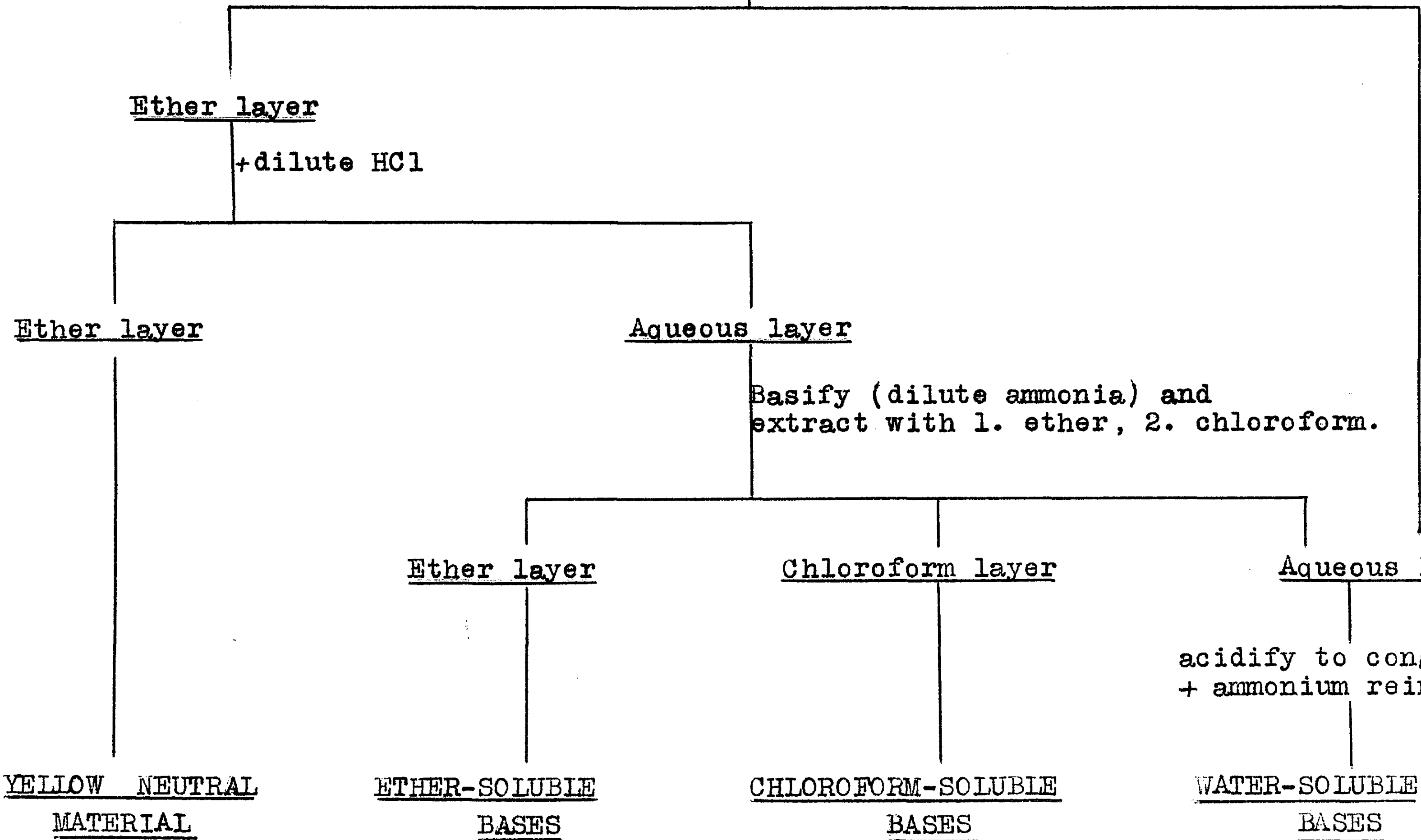
EXAMINATION OF BASIC AND WATER-SOLUBLE MATERIAL

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.

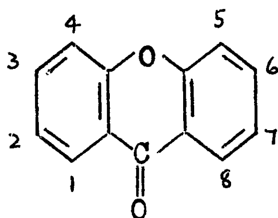
The compound analysed to $C_{13}H_7O_5.OCH_3$. Zinc dust distillation produced an unidentifiable yellow oily distillate which possessed a distinct phenolic odour. Addition of ferric chloride in ethanol to an ethanolic solution of the substance gave a very dark greenish-brown colour which became purplish-brown 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
acidify to congo red
+ ammonium reineckate
YELLOW NEUTRAL
MATERIAL
ETHER-SOLUBLE
BASES
CHLOROFORM-SOLUBLE
BASES
WATER-SOLUBLE
BASES

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[49] 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 $C_{13}H_{8}O_{2}$, such as xanthone (XXVI).

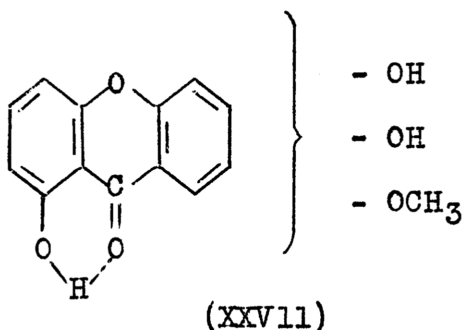


(XXVI)

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

ether-soluble xanthone.

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,x,x,-trihydroxy-x-methoxyxanthone (XXV11) similar to swertianol (51).



(XXV11)

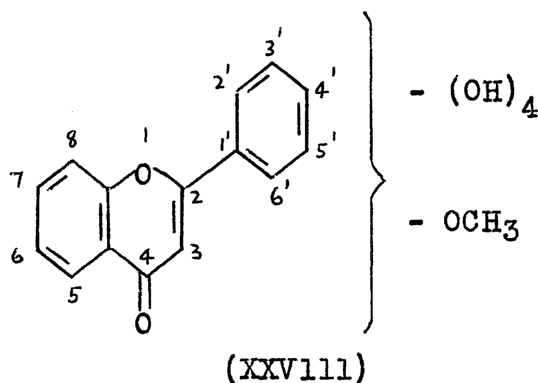
Due to the small quantity of material isolated, other evidence was essential to prove the structure and with this in mind, a study of the ultraviolet absorption curves of many xanthones was made in order to establish a possible structure. The ultraviolet absorption of the yellow neutral material and its methyl ether were compared with the spectra of 32 other

xanthones and, as far as the positions of the maxima and minima were concerned, agreement was excellent. A serious discrepancy, however, did occur for without exception, the log ϵ of the maximum in the 350mμ region in the 32 xanthone spectra studied was much less than the log ϵ of the peak near 255mμ. 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 5

Compound	Max. mμ	log ϵ	Compound	Max. mμ	Log ϵ
1,2,7-trihydroxy-xanthone	398 239	3.57 4.40	1,3,5,6-tetra-methoxyxanthone	305 245	4.30 4.66
1,4,7-trihydroxy-xanthone	410 237	3.67 4.40	(52) 1,3-dihydroxy-xanthone	340 252	3.79 4.48
(52) Aspergillone	364 242	3.12 3.94	(53) 4-hydroxyxanthone	353 250	3.03 3.93
Yellow Neutral Material (Calculated as ($C_{14}H_{10}O_6$)	371 255	4.28 4.27	Methyl ether (Calculated as $C_{16}H_{14}O_6$)	353 254	4.22 4.26

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 (XXV111) therefore, became a distinct possibility as flavones possess many properties similar to those of xanthones.



(XXV111)

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[(54)] that in most cases there are two pronounced peaks at about 250mμ and 350mμ with a less pronounced peak or inflection near

Table 6

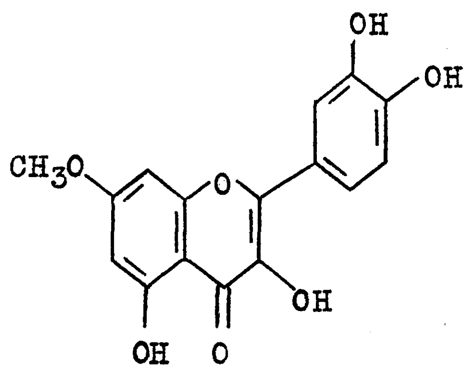
Compound	% C	% H	% O	% OCH_3
Yellow Neutral Material	60.8	3.69	35.9	11.54
$C_{14}H_{10}O_6$ requires	61.3	3.68	35.0	11.32
$C_{16}H_{12}O_7$ requires	60.8	3.82	35.45	9.82
Acetate	60.2	4.50	-	6.65
$C_{20}H_{16}O_9$ requires	60.0	4.01	-	7.75
$C_{22}H_{18}O_{10}$ requires	59.7	4.10	-	7.00
Methyl ether	63.5	5.37	-	-
$C_{16}H_{14}O_6$ requires	63.6	4.67	-	-
$C_{19}H_{18}O_7$ requires	63.7	5.07	-	-

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 [54]. 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

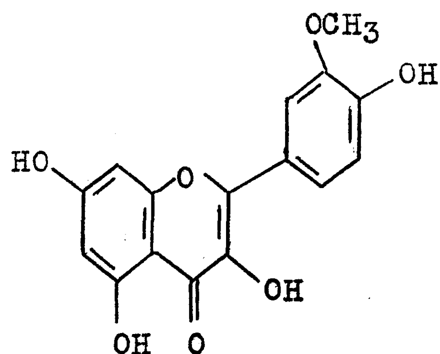
Table 7

Compound	max. (mμ)	log ε
Yellow neutral material ($C_{16}H_{12}O_7$)	255 371	4.32 4.34
Quercetin (54)	258 375	4.32 4.34
Quercetin (55)	257 370	4.31 4.32

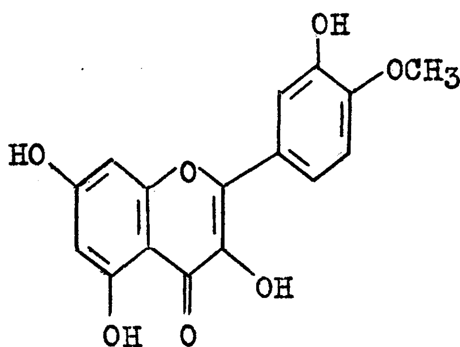
was a monomethyl ether was obtained by comparing the properties of the neutral material trimethyl ether and 5-hydroxy-3,7,3',4'-tetramethoxyflavone (quercetin-3,7,3',4'-tetramethyl ether). Both are pale yellow needles, m.p. 159 - 160°C, and give the same colour with ferric chloride. Furthermore, apart from a small but consistent difference in log values, due presumably to experimental error in weighing the small quantity (< 1mg.) 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 (XX1X), isorhamnetin (XXX), quercetin-3-methyl ether (XXX1) and quercetin-4'-methyl ether (XXX11) - 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,



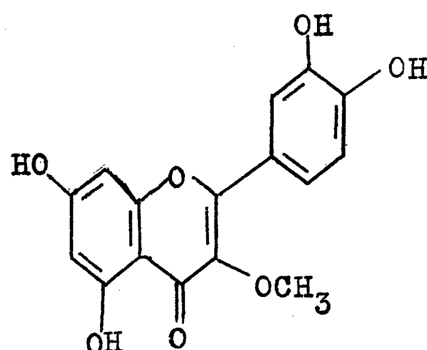
(XXIX)



(XXX)



(XXXII)



(XXXI)

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[(55)] also exclude these two isomers. They

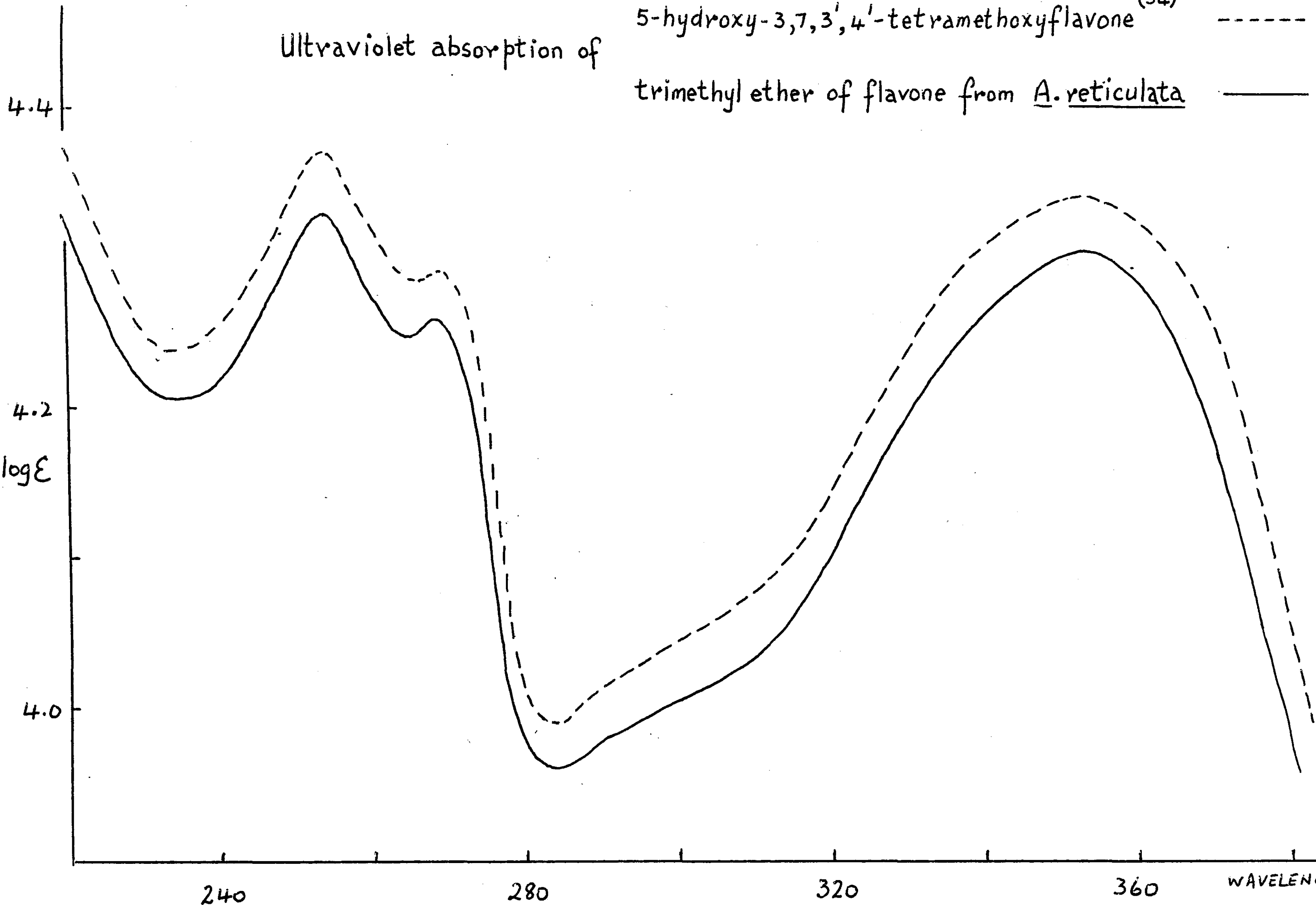


Figure 9
Ultraviolet absorption of
5-hydroxy-3,7,3',4'-tetramethoxyflavone(54)
trimethyl ether of flavone from A. reticulata
4.4
4.2
log ε
4.0
240
280
320
360
WAVELENGTH (mμ)

Table 8.

Substance	m.p. (°C)	Ref.	$\lambda_{max.}$	log ϵ	Ref.	Tetraacetate m.p. (°C)	Ref.
Quercetin-7-methyl ether (rhamnetin).	294-296 292-293 >300 290-294	56 57 58 59	256 371	4.40 4.41	55 55	186-188 186-187 190-192 183-185	56 57 58 59
Quercetin-3'-methyl ether (isorhamnetin).	296 305 307 295	60 62 63 64	255 365-380- (flat)	--	61 61	198-199 205-207 205 198-200	60 62 63 64
Quercetin-4'-methyl ether	240 259-260	65 66				202 203-204	65 66
Quercetin-5-methyl ether.			254 369	4.30 4.25	55 55		
Quercetin-3-methyl ether	272-273	67	258 360	4.31 4.31	55 55		
Quercetin-x-methyl ether (present work).	318-322 (microblock)		255 370- 372	4.32 4.34		214-215 (microblock).	

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 [55] 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 [75] 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 300mμ region [55] whereas the latter has a sharp minimum near 290mμ [68].

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 [69] 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 240mμ (ϵ21,750) and 310mμ (ϵ16,700) in good agreement with the spectrum of the flavone tetraacetate which had peaks at 239mμ (ϵ20,650) and 310mμ (ϵ16,050). This evidence therefore confirmed the identity of the flavone from _A. reticulata_ as isorhamnetin.

Figure 10

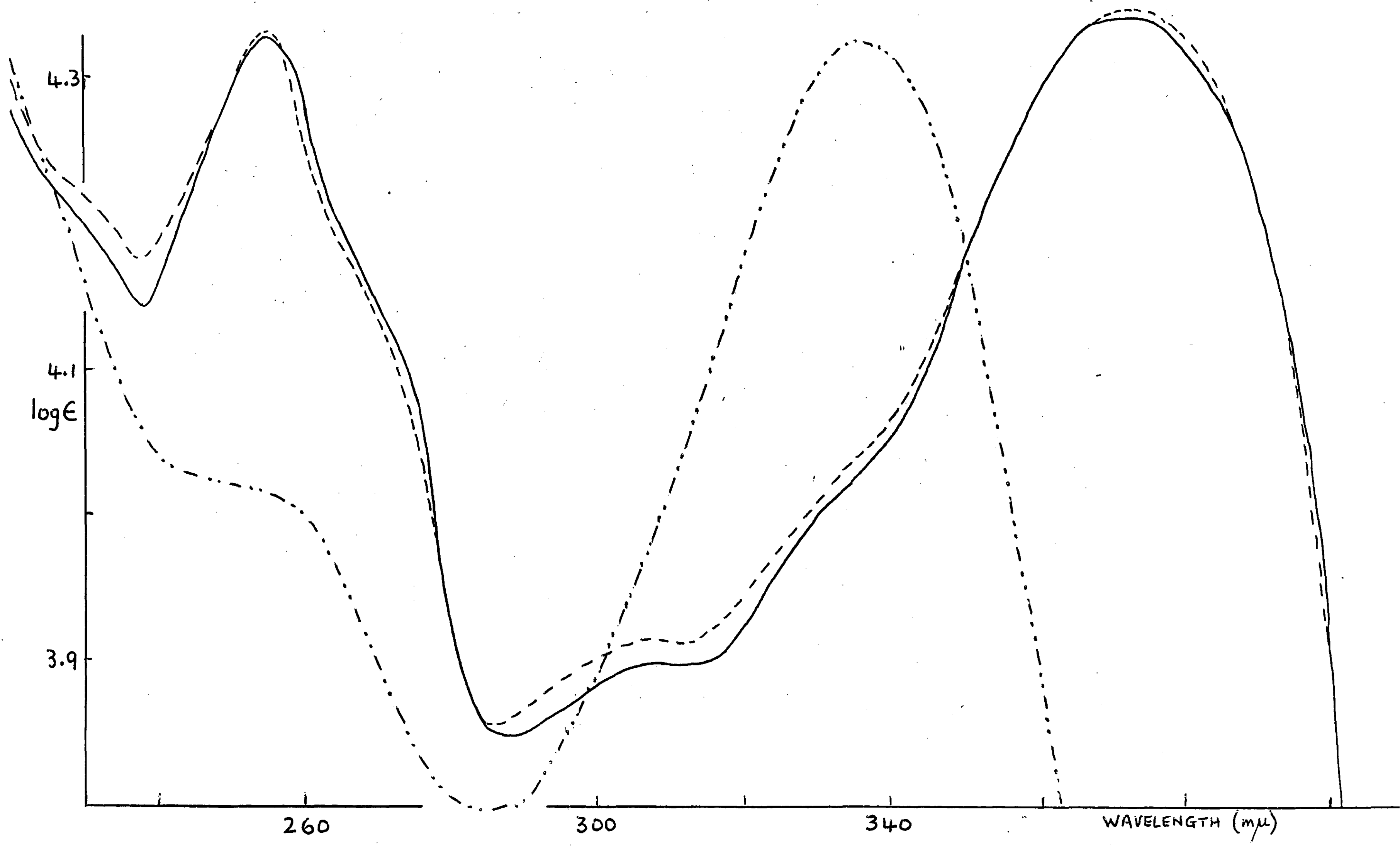
Figure 10.

Ultraviolet absorption

of

Isorhamnetin	---------
Flavone from *A. reticulata*	————
Flavone from *A. reticulata* in ethanolic sodium ethoxide	—..—..—..

Figure 10



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, $C_{17}H_{19}O_3N$, reported to be present[15,35] 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°C from ether[70], 160°C from water[71], 145°C from chloroform[71]]. Elementary analysis indicated a formula $C_{18}H_{15}O_{10}N$ and the ultraviolet absorption spectrum with maxima at 281.5mμ (ε12,030) and 353mμ (ε13,365) was indicative of a berberine-type structure[72]. 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. 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°C (decomp., tube) which was not in agreement with the reported values of 184°C[73] and 190°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°C.

An ethereal solution of the basic material from the second batch of A. serpentaria was obtained as before and extracted with dilute sulphuric acid. The aqueous layer, on standing, deposited orange crystals (0.025%), m.p. 290°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 hydrastine which is variously reported as melting at 132°, 135°, 145°C [74].

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) produced a non-basic dark-brown oil (0.015%), in agreement with the findings of Hesse [14] 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), $C_{17}H_{20}O_3N\left[Cr(SCN)_4(NH_3)_2\right].3H_2O$. This pure reineckate was decomposed by treating a solution in acetone successively with silver sulphate and barium chloride [(76)]. 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, $C_{17}H_{20}O_3NCl$. It was optically active, $[\alpha]_D^{18}$ +50.83°, and its ultraviolet absorption spectrum in ethanol showed peaks at 228mμ ($E_{1cm.}^{1\%}$ 367) and 286mμ ($E_{1cm.}^{1\%}$ 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* (15,35) 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.

EXAMINATION OF NEUTRAL FRACTIONS

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 β-sitosteryl-β-D-glucoside, confirming the observation by Williams[7] that β-sitosterol is present in the plant. The identity of the glucoside was confirmed by conversion to the known tetra-acetate[38] and by hydrolysis with ethanolic hydrochloric acid to β-sitosterol and a reducing sugar, tentatively identified as glucose. β-Sitosteryl-β-D-glucoside was obtained similarly from *A. serpentaria* in 0.044% yield in agreement with the findings of Kind and Celentano[38].

The presence of allantoin has been reported in *A. indica*[15] and *A. cymbifera*[28] 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. serpentaria* 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, $[\alpha]_D^{20}$ -1.11° (in chloroform), d_{20}^{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 9

Source	Weight of Powdered Drug	Volume of Percolate	Weight of Neutral oil	%Yield of Neutral oil	Identification	Remarks
A. reticulata Sample 2	12.5Kg	> 20 litres *	47.0g	0.38	A	Mobile, reddish-Brown
Sample 3	12.8Kg	>20 litres *	85.2g	0.665	B	- do -
Sample 4	9.5Kg	>15 litres *	51.4g	0.54	C	- do -
Sample 2) 4)		*	40.06g		D	Hot extract of powdered drug previously percolated in the cold
A. indica	3.0Kg	10 litres *	4.86g	0.162	-	Greenish-red, fairly viscous n_D^{18} 1.5116
A. serpentaria	4.2Kg	8 litres	0.77g	0.002	-	viscous, reddish-brown
A. longa	3.01Kg	10 litres	3.75g	0.125	-	viscous, dark-red.

* Percolation continued until eluate was very pale yellow in colour.

Table 12

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

Table 13

Fraction	Distillation Temperature °C (0.5mm Hg)	Bath Temp. °C	n_D^{20}	Weight (g)	Remarks
IXA	74 - 85	120 - 132	1.4785	3.211	Colourless oil $[\alpha]_D^{18.5}$ -32.86, d_{20}^{20} 0.973.
IXB	85 -102	132 - 153	1.4881	2.810	Colourless oil $[\alpha]_D^{18.5}$ -12.73, d_{20}^{20} 0.942.
IXC	102 -108	153 - 164	1.4980	1.103	Pale yellow oil
Residue				4.041	

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 IIA 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 V11). The oily filtrates obtained after removal of the crystals were bulked with fractions IIC, IXA, IXB, IXC and termed <u>fraction X1</u>.

<u>Fraction X1.</u>

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

Volume of Eluate (ml.)	Eluant	Wt. of Residue (g.)	Identification	Remarks
100	Light Petroleum	3.575	XIA	Dextro-rotatory colourless oil, n_D^{19} 1.4940
120	Light Petroleum	0.420	XIB	Laevo-rotatory colourless oil, n_D^{19} 1.5022
80	Light Petroleum	-	-	-
360	Light Pet. +5% ethanol	-	-	-
30	Light Pet. +5% ethanol	0.327	XIC	Sweet smelling laevo-rotatory oil, $n_D^{18.5}$ 1.4863 which contained no crystalline material
150	Light Pet. +5% ethanol	2.371	XID	Borneol contaminated with sweet smelling oil

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 differencies in the respective infrared absorption spectra can be readily explained by the presence of some volatile impurity.

Table 15

	Reticulene[7]	Fraction XIA. 1st. Dist$^{n.}$	2nd Dist$^{n.}$	3rd. Dist$^{n.}$
$E^{1\%}_{1cm.}$	148	136	-	-
n_D	1.4955	1.4956	1.4944	1.4942
d^{16}_{16}	0.913	0.919	0.916	0.920
$[\alpha]^{15}_D$	+1.6	+7.0	+7.4	+7.1
$[R_L]_D$	65.2	64.7	-	-

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[77] were able to isolate two isomeric aromadendrenes from Eucalyptus globulus, which they named aromadendrene ($[\alpha]^{20}_D$ +24.5°) and allo-aromadendrene ($[\alpha]^{20}_D$ -21.6°). 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 [7]. Also this fractions 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.

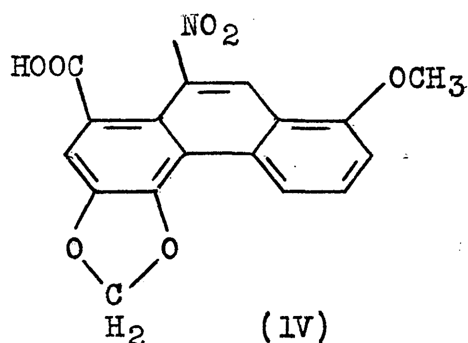
The ability to separate fraction XI into four components was confirmed by later work on reticulene (see Part II, page 136) and the isolation of the laevorotatory fraction XIB, which was not noted by Williams, could partly explain the difference in $[\alpha]_D$ values for reticulene and fraction XIA. 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.

RELATIONSHIP BETWEEN ACIDIC AND BASIC SUBSTANCES IN ARISTOLOCHIA SPECIES

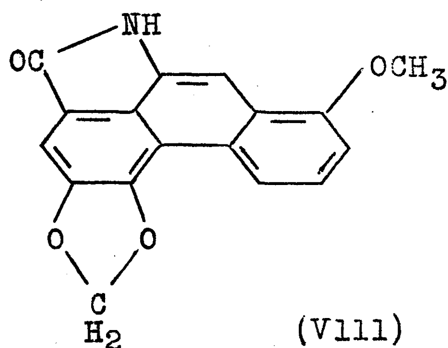
Although Krishnaswamy and his co-workers[15, 35] were able to isolate a basic substance $C_{17}H_{19}O_3N$ from *A. indica*, this was not confirmed in the present work. On theother hand much higher yields of aristolochic acid have been obtained than were reported by the Indian workers (Table 3). It is 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, $C_{17}H_{20}O_3N\,[Cr(SCN)_4(NH_3)_2]\cdot 3H_2O$, from *A. reticulata*, (b) the presence of magnoflorine (XX111), $C_{20}H_{24}O_4N$, in *A. kaempferi*,[3] *A. debilis*[3] and *A. clematitis*[37], a base whose structure bears a formal ressemblance to that of aristolochic acid lactam (V111).

A proposed scheme for the preparation of a basic substance, $C_{17}H_{19}O_3N$, from aristolochic acid (1V) is shown below (page 81) The final product (XXXV11) was considered to be of interest

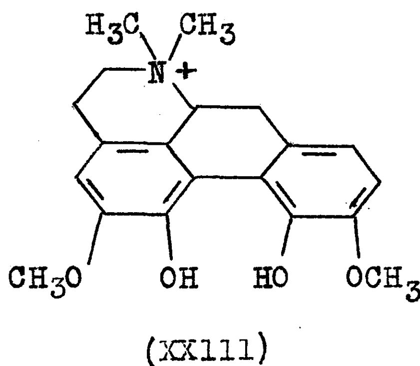
not only because its empirical formula is identical with that of aristolochine (15, 35), but also with morphine (XXX111) with which, together with apomorphine (XXX1V), there are structural similarities.



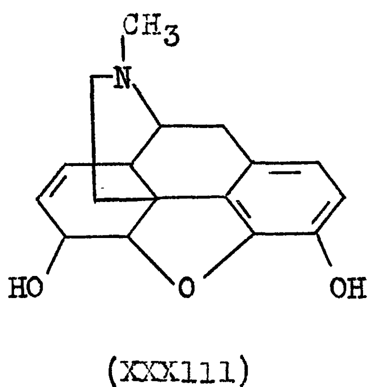
(1V)



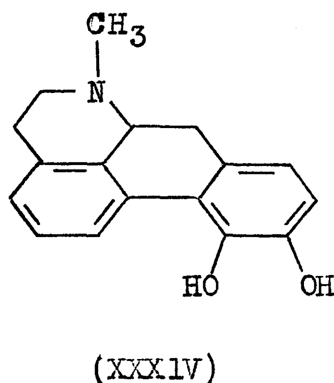
(V111)



(XX111)

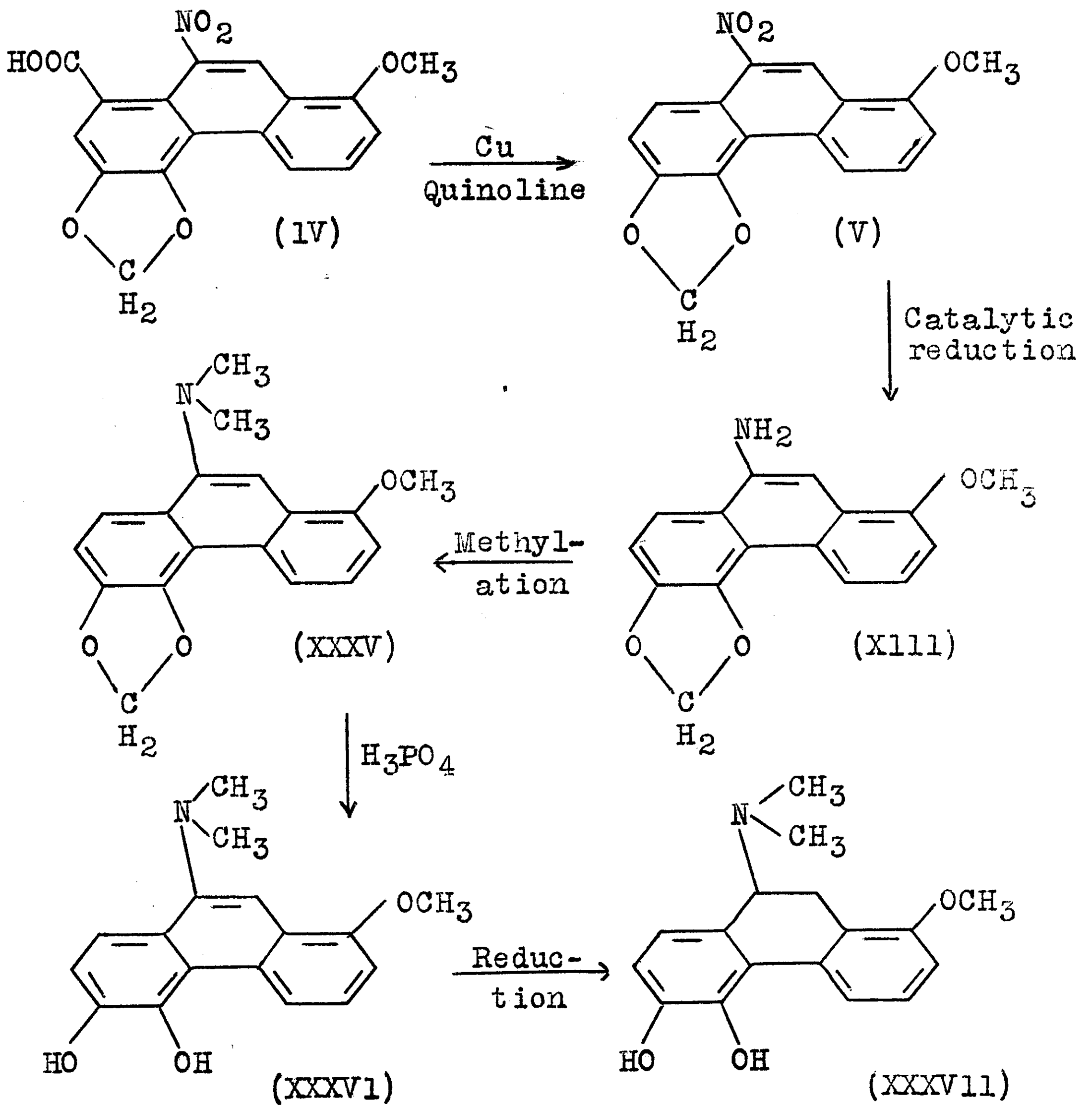


(XXX111)



(XXX1V)

Attempts to prepare the base (XXXV11) by the proposed method have so far failed due to the abnormal properties of the primary



NO2
HOOC
OCH3
(lV)
O
O
C
H2
Cu
Quinoline
NO2
OCH3
(V)
O
O
C
H2
Catalytic
reduction
NH2
OCH3
(Xlll)
Methyl-
ation
N
CH3
CH3
OCH3
(XXXV)
H3PO4
N
CH3
CH3
OCH3
HO
OH
(XXXVl)
Reduc-
tion
N
CH3
CH3
OCH3
HO
OH
(XXXVll)

amine (X111) 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 with the findings of Pailer, Belohlav and Simonitsch[21] 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, $C_{16}H_{13}O_3N$, m.p. 168 - 169.5°C, in agreement with the findings of the Austrian workers[21].

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

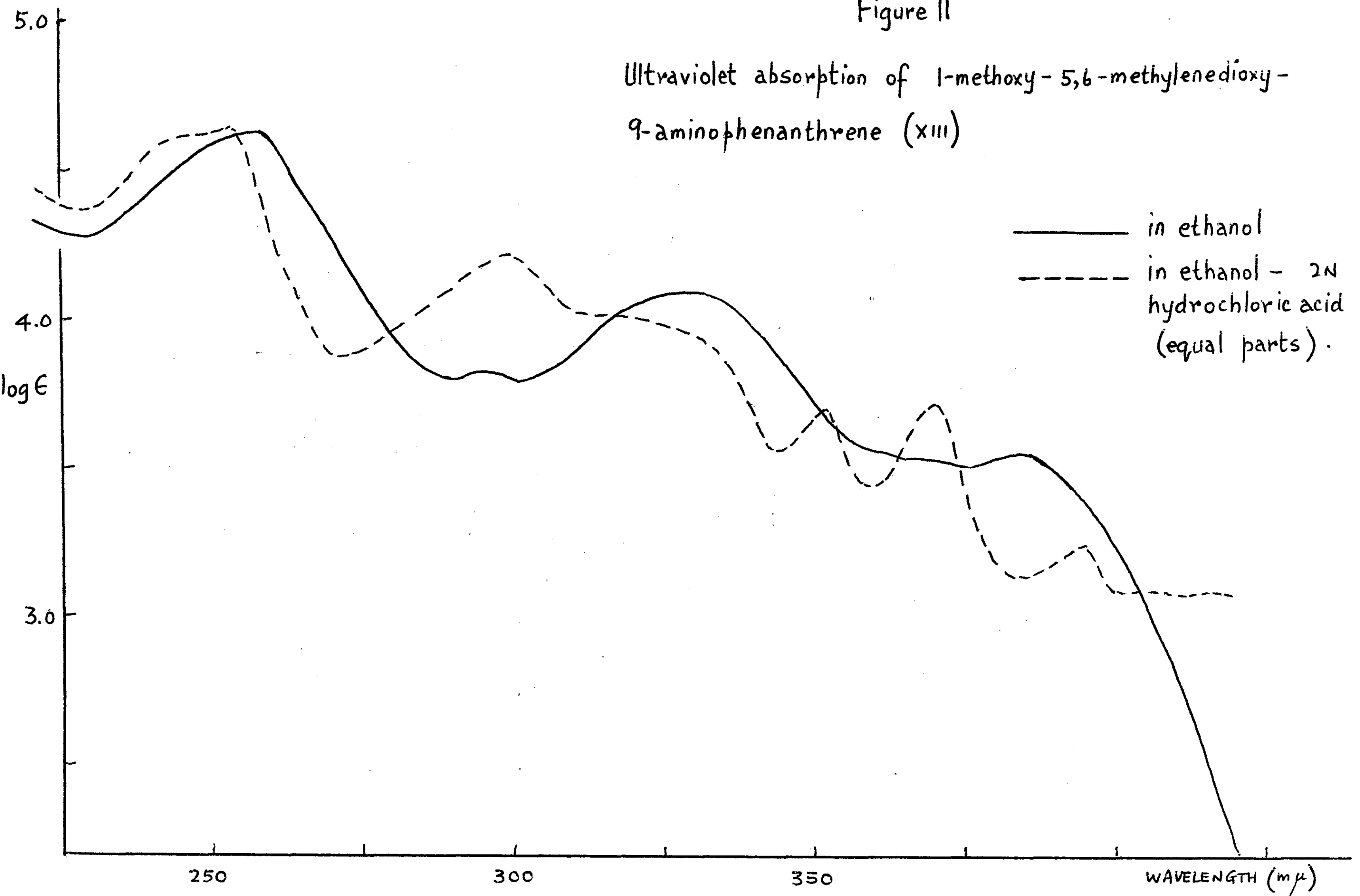
(microblock) which on recrystallisation from squeous methanol were identical with the pale-brown product obtained on catalytic reduction. The yield of product in this case was even less than in the catalytic method which seems the method of choice.

The base (X111) 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 330mμ, together with the introduction of fine structure above 340mμ is in agreement with that displayed by 9-aminophenanthrene in neutral and acid solution[79].

Attempts to methylate the base (X111) 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)



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 aristolochate (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 16

Organism	Observation on Organism Growth	Culture Medium and Incubation Temperature (°C)
BACTERIA		
i. Gram negative.		
Proteus vulgaris	No apparent restriction	Nutrient agar, 37°.
Pseudomonas fluorescens	No apparent restriction	
E. coli	No apparent restriction	
Samonella gallinarum	No apparent restriction	
Shigella paradysenteriae	No apparent restriction	
Acetobacter sp.	Very slight restriction	Malt-wort agar, 25°.
ii. Gram positive.		
Staphylococcus aureus	Slight restriction	Nutrient agar, 37°.
Staphylococcus albus	Slight restriction	
Streptococcus pyogenes	Moderate restriction	
Bacillus cereus	Slight restriction	Nutrient agar, 25°.
Micrococcus citreus	Slight restriction	
YEASTS		
Saccharomyces cerevisiae	Very slight restriction	Malt-wort agar, 25°.
Pichia membranaefaciens	Very slight restriction	Malt-wort agar, 25°.
Candida utilis	Very slight restriction	
Nadsonia fulvescens	Very slight restriction	
MOULDS		
Aspergillus oryzae	Very slight restriction	Czapek-Dox (3% glucose), 25°.
Penicillium notatum	Moderate restriction	
Mucor sp.	Marked restriction of new transfers.	
Rhizopus nigricans		

E X P E R I M E N T A L

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.

ISOLATION OF THE FRACTION SOLUBLE IN COLD ETHANOL

Preparation and extraction of the crude drug

Four samples of drug (total weight 34.8Kg.) were obtained at intervals of several months and each satisfied the pharmacognostical 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[7] 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 β-SITOSTERYL-β-D-GLUCOSIDE

The thick oil obtained from one 6Kg. batch was concentrated further and set aside for 3-4 days when β-sitosteryl-β-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), λ 210mμ (ε 1530, end absorption). Kind and Celentano[38] 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 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, λmax. 223(ϵ30,000), 250(ϵ29,400), 318(ϵ13,100), 390mμ (ϵ7,300). Rosenmund and Reichstein [18] gave m.p. 274-278°C (decomp.) and Pailer, Belohlav and Simonitsch [21] gave m.p. 281-286°C (decomp., microblock).

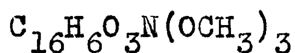
Found:	C 60.2,59.6 ; H 3.3,3.2 ; N 4.1 ; OCH$_3$ 9.15%
Calculated for $C_{17}H_{11}O_7N$:	C 59.8 ; H 3.2 ; N 4.1 : OCH$_3$ 9.1%

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 (80) (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. 286.5°C (microblock), R_F 0.77-0.80 (fluorescent spot), $\lambda_{max.}$ 253(ϵ42,400), 265(ϵ31,500), 294(ϵ19,350), 300(ϵ19,100), 3o5 (ϵ18,800), 395mμ (ϵ8,200) with inflections at 335(ϵ5850) and 352mμ (ϵ5000).

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


$C_{16}H_6O_3N(OCH_3)_3$

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 (Na_2SO_4) and evaporated to give yellow microcrystals of isorhamnetin (0.54g.), m.p. 318-322°C (decomp., microblock) (from dioxan), raised to 324°C on repeated sublimation at 300°C/0.5m.m. $\lambda_{max.}$ 255 (ϵ21,150), 307(ϵ7,950), 370-372mμ (ϵ22,100). A sample of isorhamnetin obtained from G. Tappi (61) had m.p. 318-320°C (decomp., microblock), $\lambda_{max.}$ 255(ϵ21,250), 307(ϵ8,150), 370-372mμ (ϵ22,120).

Found:	C 60.8; H3.7; O 35.9; OCH_3 11.5%.
Calculated for $C_{15}H_9O_6.OCH_3$:	C 60.8; H3.8; O 35.45; OCH_3 9.8%

Ultraviolet absorption spectrum of isorhamnetin in ethanolic sodium ethoxide

This was carried out by the method of Jurd and Horowitz (55) allowing one hour for the reaction. $\lambda_{max.}$ 335(ϵ21,200), 250-

252mμ (ε 10,480 flat).

<u>Ultraviolet absorption spectrum of isorhamnetin in the presence of boric acid and sodium acetate</u>

This was recorded using the method of Jurd[75]. $\lambda_{max.}$ 255(ε 20,350), 307(ε 7,814), 370-372mμ (ε 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°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%

$C_{16}H_{17}O_2N(OCH_3)[Cr(SCN)_4(NH_3)_2].3H_2O$ 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 (Na_2SO_4) and evaporated to give a large yield of neutral oil (Total weight =183.6g.).

ISOLATION OF FRACTION SOLUBLE IN HOT ETHANOL

A portion (22Kg.) of the marc remaining from the cold percolate was continuously extracted in batches of 6Kg. with hot ethanol (14-20 l.) until the percolate was pale yellow (3-6 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 6Kg. batch was further concentrated and on cooling allantoin separated as pale-brown crystals. Recrystallisation from 80% aqueous ethanol with charcoaling gave colourless needles (1.1g.) m.p. and mixed m.p. 232°C (decomp., tube).

Found: C 30.8; H 4.1: N 35.2%

Calculated for $C_4H_6O_3N_4$: 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.

ATTEMPTED ISOLATION OF ISORHAMNETIN FROM RED SQUILL

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, 300ml.) 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 ($Na_2 SO_4$) 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 tendancy to sublime when heated to 320°C.

REACTIONS 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_2SO_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), $\lambda_{max.}$ 247.5(ϵ39,930), 286(ϵ12,510), 310(ϵ9970), 395mμ (ϵ4460). Pailer, Belohlav and Simonitsch[21] gave m.p. 212°C (microblock).

Found:	C 64.9;	H 4.1;	N 4.5%
Calculated for $C_{16}H_{11}O_5N$:	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 80mins. with the absorption

of 19.8ml. (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 (360mg.) was refluxed for 45mins. with zinc powder (1.04g.) and glacial acetic acid (20ml.). The fluorescent solution was filtered hot and on cooling deposited the bulk of the crude product (225mg.). The mother liquors, treated with water (50ml.), yielded a further precipitate which was dissolved in chloroform, washed repeatedly with water, dried (Na_2SO_4) and evaporated to give a further 40mg. of crude product. Sublimation at 240-250°C/0.1mm. gave greenish-yellow crystals of 9-amino-1-methoxy-5,6-methylenedioxy-8-phenanthroic lactam, m.p. 320°C(microblock, inserted at 315°C), $\lambda_{max.}$ 222(ϵ22,900), 242(ϵ30,840), 250(ϵ29,740), 260 (ϵ36,130), 291(ϵ15,050), 301(ϵ15,450), 327(ϵ9220), 346(ϵ7190), 395mμ (ϵ8470). Pailer, Belohlav and Simonitsch[19] gave m.p. 319°C.

Found:	C 69.8; H 4.2; N 4.8%
Calculated for $C_{17}H_{11}O_4N$:	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-methylene-dioxy-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), $\lambda_{max.}$ 227(ϵ36,200), 242(ϵ47,750), 252(ϵ34,700), 288(ϵ15,800), 300(ϵ13,500), 328 (ϵ8630), 344(ϵ6,600), 388(ϵ8,130), 406mμ (ϵ8630). Rosenmund and Reichstein[18] 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%

$C_{19}H_{13}O_5N$ requires: C 68.1; H 3.8; N 4.2; OCH_3 9.25%

b) Aristolochic acid (42mg.) was refluxed for 1hr. 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,x,x,-trimethoxy-5,6-methylenedioxy-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(ϵ35,730), 250.5 (ϵ38,860), 291(ϵ17,960), 302(ϵ15,690, shoulder), 330(ϵ5,580, inflection), 343(ϵ4410), 385(ϵ6680), 400mμ (ϵ6740).

Found:	N 4.2 %
$C_{21}H_{17}O_7N$ 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_2SO_4) and evaporated to give a small quantity of a reddish-brown material which could not be crystallised from glacial acetic acid. Paper chromatography gave 2 spots, the main one fluorescent with R_F value 0.80 (aristo-red).

REACTIONS 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 253mμ.

Preparation of tetraacetate

Isorhamnetin (40mg.) was refluxed for 30mins. 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 recrystallised from ethanol had m.p. 214-215°C (microblock), $\lambda_{max.}$ 239(ϵ20,650), 310mμ (ϵ16,050). A sample of isorhamnetin-3,4',5,7-tetraacetate obtained from G. Tappi[61] had m.p. 210-211°C (microblock), $\lambda_{max.}$ 240(ϵ21,750), 310mμ (ϵ16,700) and displayed similar fluorescent properties.

Found: C 60.2; H 4.5; OCH_3 6.65%

Calculated for $C_{22}H_{18}O_{10}$: C 59.7; H 4.1; OCH_3 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[49]. 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. $\lambda_{max.}$ 254 (logϵ 4.33), 269(logϵ 4.26), 353mμ (logϵ 4.305). Gomm and Nierenstein[81] gave m.p. 159-160°C. Briggs and Locker[54] gave $\lambda_{max.}$ 254(logϵ 4.37), 269(logϵ 4.29), 352mμ (logϵ 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_2SO_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% w/v, 35.0ml.), followed by an equivalent volume of a solution of barium chloride (1.062% w/v $BaCl_2.2H_2O$, 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 $[\alpha]_D^{18}$ +50.83° (C 0.6), $\lambda_{max.}$ 228($E_{1cm.}^{1\%}$ 367), 286mμ ($E_{1cm.}^{1\%}$ 122).

Found: C 61.0; H 9.2; N 5.6%

The expected base chloride, $C_{17}H_{20}O_3NCl$ 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% w/v, 4ml.). Recrystallisation of the bulky product from ethanol was accompanied by decomposition and gave crystals (4mg.), m.p. 178-179.5°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% w/v $HAuCl_4.3H_2O$) 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% w/v $H_2PtCl_6.6H_2O$) 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% w/v, 2ml.). The supernatent 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% $^{w}/_{v}$ sodium hydroxide). The crude acetate (93mg.) was recrystallised from aqueous ethanol as lustrous plates, m.p. 166.5-167.5°C (microblock), $[\alpha]_{D}^{17°c}$ -23.91° (c=0.92 in chloroform). Kind and Celentano(38) gave m.p. 167.5-168.5°C, $[\alpha]_{D}$ -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 (8ml.) 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),

$[\alpha]_D^{19^\circ C}$ -37.75° (c=0.5 in chloroform). Kind and Celentano[38] gave m.p. 140° C, $[\alpha]_D$ -37° and -38°.

Found: C 82.4; H 12.1 %

Calculated for $C_{29}H_{50}O \cdot \frac{1}{2}CH_3OH$: C 82.2; H 12.2 %.

Attempted preparation of osazone

The filtrate (10ml.) obtained on removal of β-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[82]. 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 (0.228g.) 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., $n_D^{20°c}$ 1.5184), termed fraction I (Table 10). The main band (1.28 l.) 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.,

$n_D^{19.5°C}$ 1.4924, $d_{20°C}^{20°C}$ 0.963, $[\alpha]_D^{20°C}$ -1.11 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 (Na_2SO_4) and evaporated to give an orange aromatic oil (4.00g., $n_D^{20°C}$ 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 (Na_2SO_4) gave a dark red oil which was refluxed (1½ hours) with 2N ethanolic potassium hydroxide (200ml.). The solution was cooled, diluted with water (600ml.) and extracted with ether (total 700ml.), the ether dried (Na_2SO_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 (Na_2SO_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., $n_D^{19.5°C}$ 1.5088). Identical treatment of fractions D gave a similar oil (28.93g., $n_D^{19.5°C}$ 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_2SO_4) and evaporated and gave a pale yellow oil (13.41g., $n_D^{20°C}$ 1.4907, $d_{20°C}^{20°C}$ 0.935, $[\alpha]_D^{20°C}$ +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., $n_D^{18°C}$ 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., $n_D^{18°C}$ 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

4.33g ($n_D^{20°C}$ 1.5184) I

a) 62.84g

b) 6.46g

c) 5.42g

d) 1.75g

e) 0.49g

(b–e): Completely immobile dark red oils.

a) 62.84g → Steam distillation

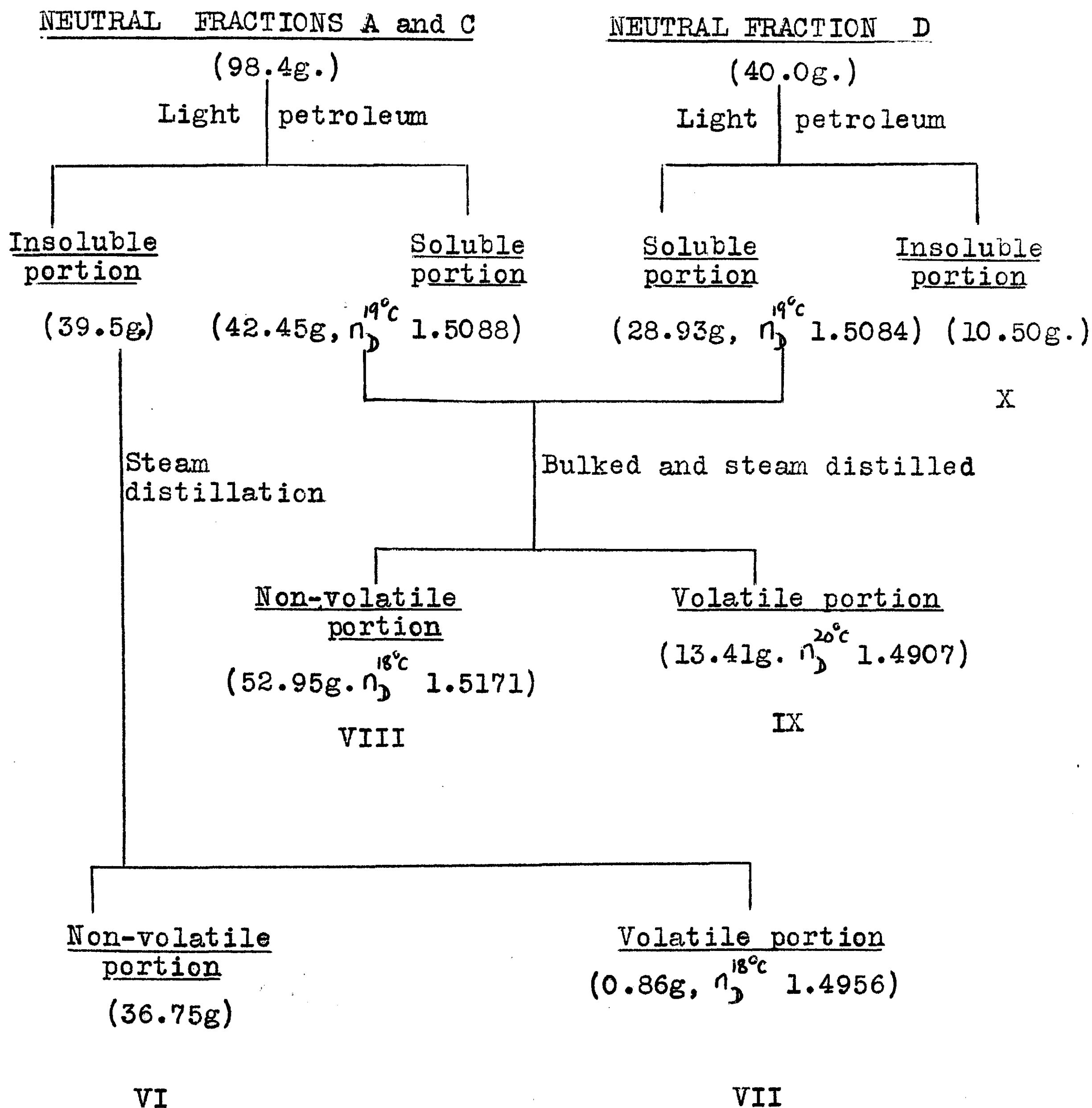
Volatile portion

- 11.06g ($n_D^{19.5°C}$ 1.4924) II
- 4.00g ($n_D^{20°C}$ 1.5095) III

Non-volatile portion → Saponification

- Alcohols and non-saponifiable material (22.07g.) IV
- Resin Acids (26.16g.) V.

Table 11



EXAMINATION OF FRACTIONS I - X

Fractions I, III, V, VI, VIII, and X were left at room temperature then at $0^{\circ}C$ 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 tendancy to crystallise. They were not examined further.

Fraction VII was a pale yellow oil (0.86g.) with an odour ressembling 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 IX A, 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 17.

Fraction	Volume of eluant (ml.)	Eluant	Weight of residue (g.)	Remarks
1.	50	light petroleum	1.083	Small yellow band preceding main band.
2.	250	light petroleum	6.428	Main band. Red-brown → yellow.
3.	100 100 100	light petroleum light petroleum + Benzene (½%). light petroleum + Benzene (5%).	0.576	Pale yellow band.
4.	400	benzene	2.615	Dark red band.
5.	700	benzene + ethanol (½%-2%).	1.746	Reddish-yellow band.
6.	400	benzene + ethanol (5%).	0.620	Pale yellow band.
7.	300 400	benzene + ethanol (50%). ethanol	0.400	Pale yellow band.
8.	100	ethanol + glacial acetic acid (1%).	0.822	Red band.

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[83] 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 30mins. 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 (Na_2SO_4) 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 (Na_2SO_4) 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 ($[\alpha]_D^{15^\circ C}$ +7.0° (c=4.05), $d_{16^\circ C}^{16^\circ C}$ 0.919, $n_D^{20^\circ C}$ 1.4956, $E_{1cm.}^{1\%}$ at 212mμ = 136) which possessed a reticulene-like odour. Redistillations failed to alter appreciably these physical constants (see Table 15, page 77).

Fraction XIB.

This oil (0.420g.), $n_D^{19^\circ C}$ 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 (80mg.) was dissolved in pyridine (2ml.) and gently refluxed for 30mins. with p-nitrobenzoyl chloride (100mg.). After cooling, the addition of water (10ml.) 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-methylenedioxy-9-aminophenanthrene (XIII).

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Method I

Decarboxylated aristolochic acid (1-methoxy-5,6-methylenedioxy-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 flouresced 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).

<u>Method III a</u>

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(logϵ 4.635), 297(logϵ 3.835), 330(logϵ 4.10), 383mμ (logϵ 3.55) - in ethanol.

$\lambda_{max.}$ 245(logϵ 4.60, shoulder), 253(logϵ 4.64), 300(logϵ 4.21), 330(logϵ 3.95, shoulder), 352(logϵ 3.71), 370(logϵ 3.72),

395mμ (logϵ 3.24) - in 2N hydrochloric acid/ethanol (equal parts). Pailer, Belohlav and Simonitsch[(21)] gave m.p. 170°C.

Found: C 71.9; H 5.15; N 5.35%

Calculated for $C_{16}H_{13}O_3N$: 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 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 %
$C_{16}H_{13}O_3N$.HCl requires: C 63.2; H 4.7 %

Methylation of 1 methoxy-5,6-methylenedioxy-9-aminophenanthrene

Method I

The base (X111) (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 (X111) (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[49]. 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 (X111) (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 1V

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

Method V

The base (X111) (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%

$C_{17}H_{15}O_3N.HI$ requires: C 49.9 ; H 3.92%

Method V1

The base (X111) (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%

$C_{17}H_{15}O_3N.HI$ 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.

A. SERPENTARIA LINN.

ISOLATION AND TREATMENT OF THE FRACTION SOLUBLE IN COLD ETHANOL

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 (7days). 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 (25·1 g.) 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), $\lambda_{max.}$ 281.5(ϵ 12,030), 353mμ (ϵ 13,356).

Found: C 53.6; H 3.75; N 3.6%

$C_{18}H_{15}O_{10}N$ 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 [73]. After recrystallisation from methanol the base had m.p. 132°C (tube), $\lambda_{max.}$ 297mμ ($E^{1\%}_{1cm.}$ 196). El Ridi, Khalifa and Mamoon [84] gave $\lambda_{max.}$ 297mμ ($E^{1\%}_{1cm.}$ 200), m.p. 132°C.

Found: C 65.6; H 5.5; N 3.7%

Calculated for $C_{21}H_{21}O_6N$: 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 $C_{21}H_{21}O_6N.C_6H_2(NO_2)_3OH$: C 52.95; H 3.95%.

Sample b

The second sample of defatted root and rhizome (4.20Kg., 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 (1.037g.), m.p. 288-290°C (decomp., microblock), after recrystallisation from ethanol/ether. $\lambda_{max.}$ in 88% ethanol 267($E^{1\%}_{1cm.}$ 648), 351mμ ($E^{1\%}_{1cm.}$ 609), El Ridi, Khalifa and Mamoon (84) gave $\lambda_{max.}$ 270 ($E^{1\%}_{1cm.}$ 610), 350mμ($E^{1\%}_{1cm.}$ 600) for berberine hydrochloride.

Found: C 55.0; H 4.2; N 3.3; S 7.2%

Calculated for $C_{20}H_{17}O_4N.H_2SO_4$: 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 (1.097g.), m.p. 132°C (tube) (from methanol), 145°C (tube) (from aqueous methanol). Both melting points have been reported (74) 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 *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), $\lambda_{max.}$ 223(ϵ 29,300), 250(ϵ 32,300), 317.5(ϵ12,900), 391mμ(ϵ 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_2SO_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 (Na_2SO_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, $\lambda_{max.}$ 250(ϵ 30,600), 317(ϵ 11,500), 390mμ (ϵ 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.

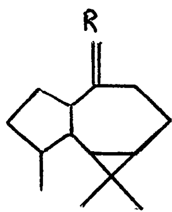
RETICULENE

HISTORICAL INTRODUCTION

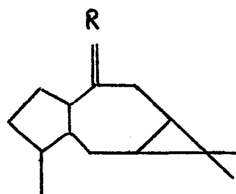
The sesquiterpene reticulene was first isolated by Stenlake and Williams (7,8) from the light petroleum-soluble portion of the roots and rhizomes of <u>A. reticulata</u>. On concentration of the percolate, a dark green oil was obtained from which aristolactone, free acids and carbonyl compounds were 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, $C_{15}H_{24}$, $[\alpha]_D^{15°C}$ +1.6; $d_{16°C}^{16°C}$ 0.913; $n_D^{16°C}$ 1.4955; λ 212mμ (ϵ 2260, 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., $[\alpha]^{17°C}$ +4.0: $d_{15°C}^{15°C}$ 0.900; $n_D^{17°C}$ 1.4826; λ 208mμ (ϵ 1681, in $[\alpha]_D^{17°C}$ +4.0; $d_{15°C}^{15°C}$ ams 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 (85) who observed that bromination of aromadendrene (XXXV111 or XXX1X, 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.



(XXXV111)



(XXX1X)

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 dinitrophenylhydrazone 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 (ϵ2260 at 212mμ) and dihydroreticulene (ϵ1681 at 208mμ) did not compare favourably with that reported by Birch and Lahey[86] for apoaromadendrone (XXXV111 or XXX1X, R=0) (ϵ95 at 212mμ) 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[87] precipitated aristolactone from a concentrated extract of _A. reticulata_ 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[7]. 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[88] 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.

A COMPARISON OF SOME PHYSICAL AND CHEMICAL PROPERTIES OF RETICULENE, AROMADENDRENE AND THEIR REDUCTION PRODUCTS.

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[89]. 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 1646cm.$^{-1}$ in the spectrum of reticulene

Table 20

SUBSTANCE	$[\alpha]_D$	n_D	d	ϵ 209mμ *	ϵ 212mμ *	Ref.	Remarks
Reticulene	+ 0.96°	1.4970	0.915	3998	3040	Present work	
-do-	+ 1.6°	1.4955	0.913			7	
Aromadendrene	+ 12.4°	1.4950	0.909	2835	2040	Present work	One vinylidene group
-do-	+ 7.54°	1.4953	0.911			86	
-do-	+ 0.8°	1.4990	-			90	
Dihydroisoreticulene	-	1.4842	0.902	1970†	1770	Present work	† at 210mμ
-do-	+ 4.0°	1.4826	0.900			7	
Dihydroaromadendrene	-	-	-	331	266	Present work	Fully saturated
-do-	- 12.14°	1.4850	0.900			85	

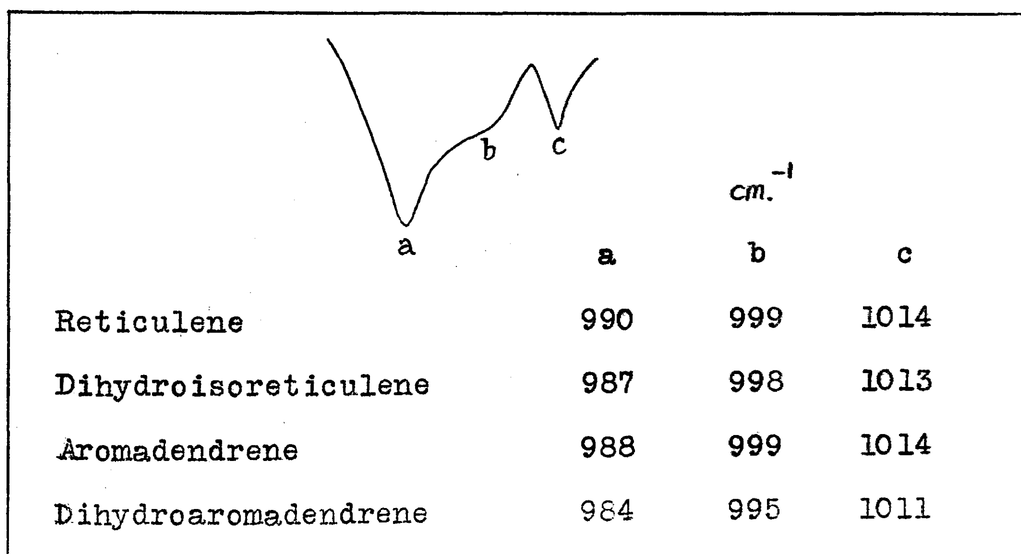
* The molecular extinction coefficients were determined under controlled conditions and so the values obtained by other workers are not quoted.

\- 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 $-CH_2-$ group. Cole (91) 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 21



	a	b (cm.$^{-1}$)	c
Reticulene	990	999	1014
Dihydroisoreticulene	987	998	1013
Aromadendrene	988	999	1014
Dihydroaromadendrene	984	995	1011

Furthermore, Rees and Shoppee[92] quote that the cyclopropane ring absorbs in the 884 to 888 and 1020cm.$^{-1}$ regions. The vinylidene group of reticulene and aromadendrene would mask the cyclopropane absorption in the 880-890cm.$^{-1}$ region but dihydroisoreticulene has a peak at 884cm.$^{-1}$ and dihydroaromadendrene shows twin absorption peaks at 881 and 888cm.$^{-1}$. 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.$^{-1}$ in reticulene and aromadendrene respectively deserve comment. Cole[91] has found that if a cyclopropane ring, which possesses an unsubstituted $-CH_2-$ group, is present in the molecule then characteristic small peaks are shown in the region 3040-3060cm.$^{-1}$. In the many examples he quotes, these peaks are almost identical with those shown by reticulene and aromadendrene. Bellamy[93], on the other hand, records that the $\backslash C=CH_2$ group also absorbs in the region 3075-3095cm.$^{-1}$ 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 catalytic 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 22

Compound *	No. of double bonds.	Reference	Remarks
Dihydroaromadendrene	0.06	Present work	1 cyclopropane ring. 0 double bonds.
Hederagenin [94]	0.83	Present work	trisubstituted double bond.
Hederagenin methyl ester diacetate. [94]	0.90	87	trisubstituted double bond.
Cyclo-eucalanyl acetate [95]	0.07	Present work	1 cyclopropane ring. 0 double bonds.
2,3-dimethyl-2,3 methylene-1,4 naphthaquinone [96]	0.00	Present work	1 cyclopropane ring. 0 double bonds.
Iso-Ketal from aristolactone [87]	1.98	87	Two double bonds.
α-angelicalactone	0.90	87	trisubstituted double bond.
Reticulene	1.97	Present work	
	1.90	87	
Dihydroisoreticulene	0.70	7	

* References are to molecular structure.

Table 23

Compound	No. of Double Bonds reaction time (hrs) 6	24	48	Remarks
Cholesterol	0.98	1.10		1 trisubstituted double bond.
Hederagenin	0.26	0.39		1 trisubstituted double bond.
Cyclo-eucalanyl acetate	0.03			1 cyclopropane ring.
Aristolactone	2.28	2.56	2.63	3 double bonds.
Reticulene	1.48	1.54	1.56	
Dihydroisoreticulene	0.43	0.52		
Reticulodione	0.10	0.15		

OXIDATION OF RETICULENE

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 24

Substance	R_F in phenol 80%, formic acid 1%
Crystals, m.p. 170°C	0.935
Oil (1)	0.954
Oil (2)	0.977
Crystals, m.p. 49-53°C	0.14
Pimelic acid	0.905
Adipic acid	0.856
Glutamic acid	0.77
Succinic acid	0.66

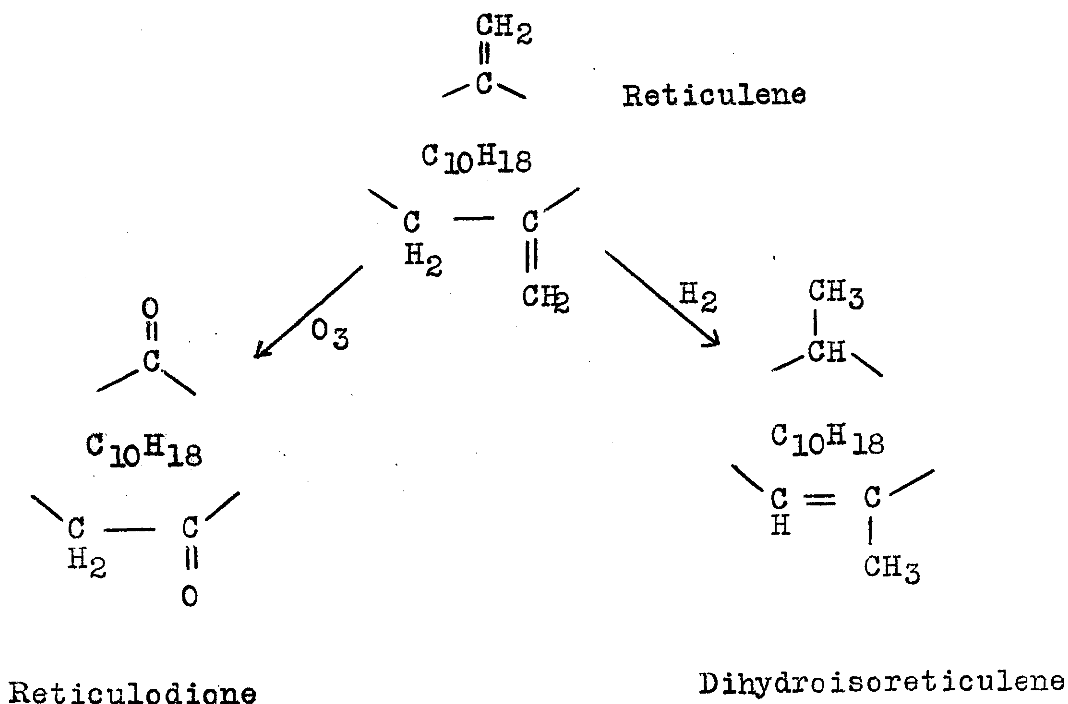
OZONOLYSIS OF RETICULENE

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, $C_{13}H_{20}O_2$, 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 ($E^{1\%}_{1cm.}$ at 290mμ 5.12) and showed end absorption (ε at 218mμ 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, $C_{13}H_{24}$, would also be fully saturated. To satisy 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).

THE NATURE OF THE DOUBLE BONDS IN RETICULENE AND ITS DIHYDRO-DERIVATIVE

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.

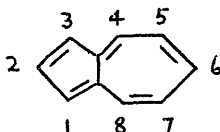
Excellent confirmation of this postulate is given by a peak at 814cm.$^{-1}$ in the infrared absorption curve of dihydro-isoreticulene, characteristic of a trisubstituted double bond. This peak is absent from the absorption curve of reticulene.



DEHYDROGENATION OF DIHYDROISORETICULENE

Williams[7] 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[97] but was not identical with any of the relatively few published spectra.

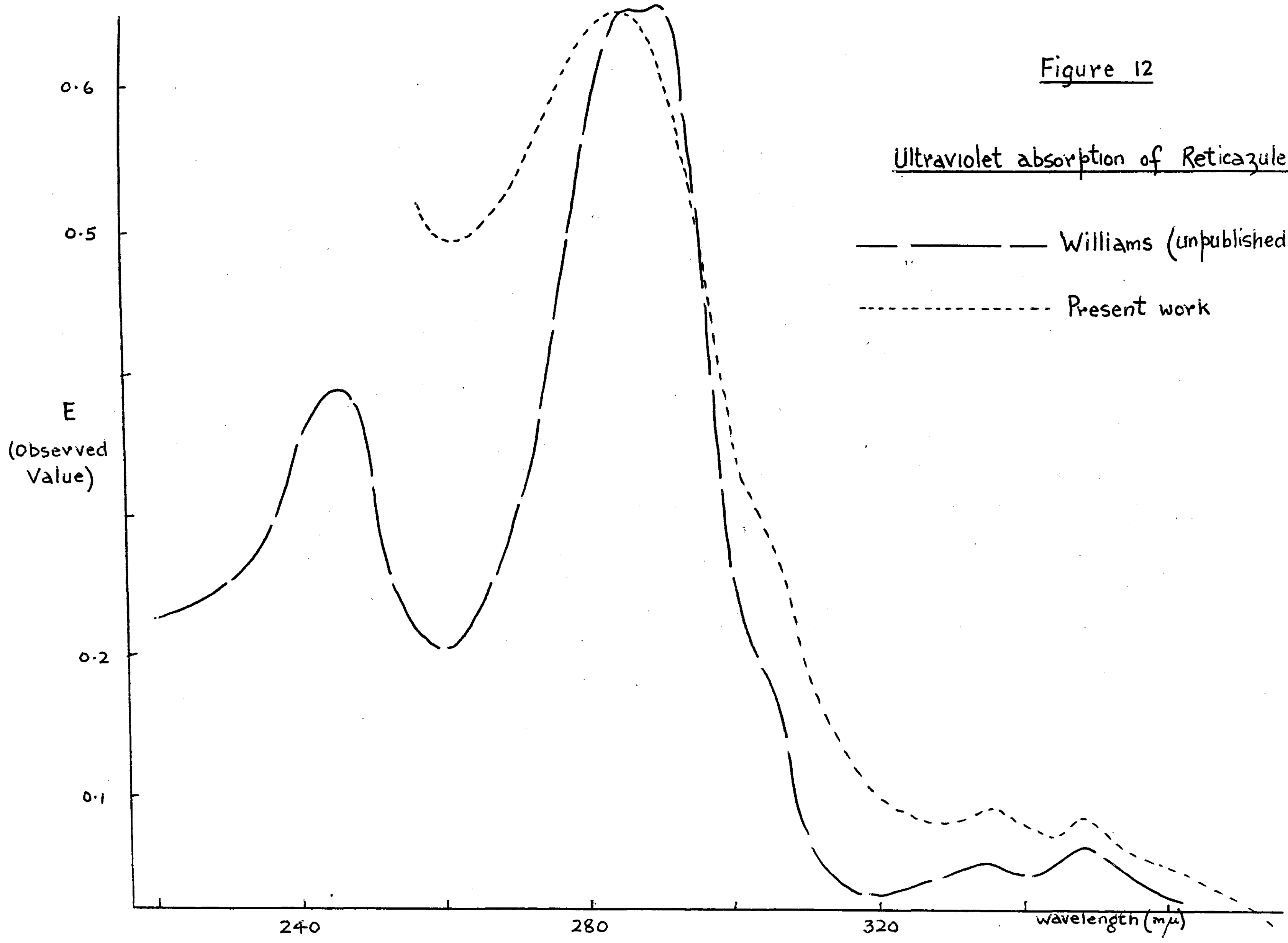
Steele[87] recorded in tabular form the characteristic wavelengths of maximum absorption of many azulenes and comparison showed that reticazulene was not identical with aristazulene (Table 25). The latter possessed an additional maximum at 306mμ and was violet in colour. Reticazulene is blue and therefore is not a 2-alkylazulene,[98,99]



Azulene

Figure 12

Ultraviolet absorption of Reticazulene.



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

Table 25

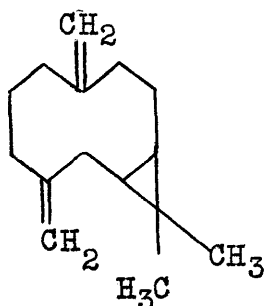
Compound	Wavelength of Maximum Absorption (mμ).	Ref.
Aristazulene	245,* 279, 289, 306, 333, 348	87
1,4,8-trimethyl-azulene	246, 286, -, 335, 348	100
1,4-dimethyl-8-isopropylazulene	246, 287, 305 (infl.), 335, 348	100
Reticazulene	245, 285-290, 305 (infl.), 335, 348	101
Reticazulene	245,* 284, 305 (infl.), 335, 348	Present work

* Readings in this region not reproducable.

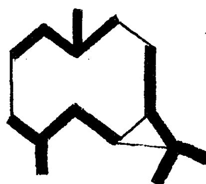
The spectra of 1,4,8-trimethylazulene and 1,4-dimethyl-8-isopropylazulene differ slightly. The former has an additional inflection near 232mμ while the latter also possesses an additional inflection but in this case near 305mμ (100). Reticazulene has no inflection near 232mμ but one is present at 305mμ. This evidence indicates that reticazulene is a 1,4,8-trialkylazulene, probably 1,4-dimethyl-8-isopropylazulene.

A TENTATIVE STRUCTURE FOR RETICULENE

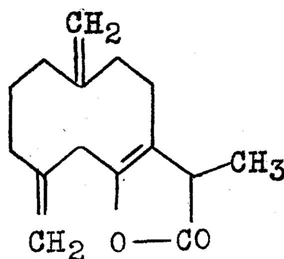
From the preceding evidence, a tentative structure for reticulene can be proposed (XL). Such a structure conforms to the isoprene rule as shown in XL1 and would explain the inability to obtain small identifiable fractions on oxidation. It is closely related structurally to aristolactone (XXV) which could conceivably be its precursor in agreement with the findings of Cekan, Herout and Sorm (102) who isolated both a hydrocarbon and a lactone from Matricaria chamomilla L. and showed that the latter was the precursor of the former.



(XL)

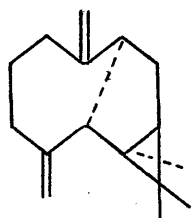


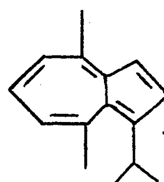
(XL1)



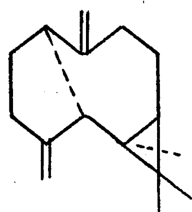
(XXV)

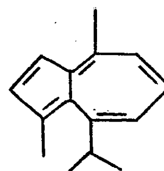
The proposed structure for reticulene also explains why reticazulene was formed only slowly, as a ring closure is necessary similar to that found in the preparation of aristazulene (87). This ring closure could occur in two ways, each of which would give a 1,4,8-trialkylazulene (XL11, XL111).





(XL) (XL11)

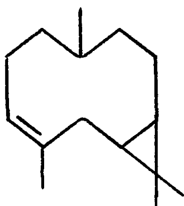




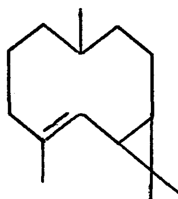
(XL) (XL111)

On the basis of structure XL for reticulene and by analogy with aristolactone (45,87), dihydroisoreticulene can be represented by either structure XL1V 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 210mμ 1970), in agreement with the findings

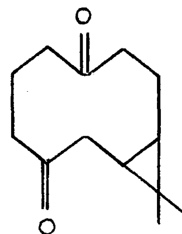
in the umbellulone series of compounds [103,104].



(XL1V)



(XLV)



(XLV1)

In the infrared spectrum of reticulodione, which is therefore XLV1, a carbonyl peak at 1703cm.$^{-1}$ (in CCl_4) and at 1708cm.$^{-1}$ (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 hindrance 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.

ISOLATION OF RETICULENE AND RELATED MATERIALS FROM OILY MIXTURES

a) The oily mixture obtained from Steele (see page 136) (15.136g.), which still contained some solvent, was chromatographed 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 chromatographing 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 (8.682g.), $n_D^{18°C}$ 1.4970.

Combined fraction B: Removal of solvent gave a colourless laevorotatory oil (1.857g.), $n_D^{18.5°C}$ 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°C, the oil failed to crystallise. Distillation at 110-115°C (bath temp.)/18mm. Hg, gave a colourless oil (2.40g., $n_D^{19°C}$ 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 18

Eluant	Fraction	Volume of Eluate (ml)	Optical Rotation	Remarks
Light petroleum		100	0	light petroleum
	1	20	+0.03	Fractions 1-7 bulked (Fraction A)
	2	10	+0.11	
	3	10	+0.11	
	4	10	+0.11	
	5	10	+0.11	
	6	10	+0.10	
	7	10	+0.04	
	8	10	0	
	9	10	-0.06	Fractions 9-10 bulked (Fraction B)
	10	10	-0.04	
	11	10	+0.01	Fractions 11-14 No residue on evaporation
	12	20	+0.02	
	13	20	+0.02	
	14	20	+0.02	
Light petroleum 10% ethanol		50	0	No residue
	15	10	-0.23	Fractions 15-27 bulked (Fraction C)
	16	20		
	17	20	-0.22	
	18	20		
	19	20	-0.37	
	20	20		
	21	20	-1.41	
	22	20		
	23	10	-1.37	
	24	20	-0.26	
	25	20		
	26	20		
	27	10		
	28	10	0	Fractions 28-32 No residue on evaporation
	29	10		
	30	10		
	31	10		
	32	10		

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 (Na_2SO_4) 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, $n_D^{15°C}$ 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

°C, b.p. 18mm. Hg	$n_D^{19.5°C}$	Weight (g.)	Identification	Remarks.
106-108°	1.4780	7.515	A	bornyl formate
108-116°	1.4817	7.154	B	mainly bornyl formate
120-130°	1.4932	7.259	C	mainly reticulene

$n_D^{18°C}$ 1.4952, with an odour resembling that of reticulene.

RETICULENE

The reticulene fractions isolated as reported in the previous pages were bulked and distilled under reduced pressure (18mm. Hg) at 130-135°C (bath temp.) to give a colourless oil, $n_D^{18^{\circ}C}$ 1.4970, $d_{18^{\circ}C}^{18^{\circ}C}$ 0.915, $[\alpha]_D^{18^{\circ}C}$ +0.964 (c=5.29), $[R_L]_D$ 65.3, λ 209mμ(ϵ 3998), infrared absorption - peaks at 888, 1646, 3060cm.$^{-1}$ (vinylidene); 990, 999, 1014cm.$^{-1}$ (cyclopropane). Williams[7] gave $n_D^{14^{\circ}C}$ 1.4972, $d_{15^{\circ}C}^{15^{\circ}C}$ 0.914, $[\alpha]_D^{15^{\circ}C}$ +1.1 (c=4.2), $[R_L]_D$ 65.22 for reticulene.

REACTIONS OF RETICULENE

Reduction

Reticulene (1.253g.) was dissolved in ethanol and hydrogenated at a platimum catalyst (0.219g.) until hydrogen uptake ceased (2 hours, 0.98 ethylenic double bonds). After filtering, the solvent was removed under pressure to give dihydroisoreticulene (1.235g., $n_D^{18^{\circ}C}$ 1.4859). Distillation at 128-135°C (bath temp.)/18mm. Hg, gave the dihydro-compound as a colourless oil, $n_D^{20^{\circ}C}$ 1.4842, $d_{16^{\circ}C}^{16^{\circ}C}$ 0.902, which gave an orange colour with tetranitromethane. λ 210mμ(ϵ 1970), Infrared absorption - 987, 998, 1013cm.$^{-1}$ (cyclopropane); 814cm.$^{-1}$ (trisubstituted double bond). Williams[7] gave b.p. 130-135°C (bath temp.)/18mm. Hg, $d_{15^{\circ}C}^{15^{\circ}C}$ 0.900, $n_D^{17^{\circ}C}$ 1.4826 for "dihydroreticulene."

Oxidation

Reticulene (1.026g.) was shaken with Beckmann's chromic acid mixture [108] (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_2SO_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_2SO_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

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

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.

<u>Treatment of Fraction 2</u> 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 27

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

* R_F values determined on Whatman No. 1 paper using phenol: water: formic acid (80:19:1) as solvent.

<u>Fraction 2A</u> 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, R_F 0.935, using same system as before (Table 27). (Contrast succinic acid which has m.p. 184°C with sublimation, R_F 0.66, in same system)

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

<u>Ozonolysis</u>.

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 <u>in vacuo</u> 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(ϵ394), 240(ϵ337, small maximum), 290mμ (ϵ105, inflection), infrared absorption - peaks at 1703, 1738cm.$^{-1}$ (in CCl_4); 1707, 1737cm.$^{-1}$ (in CS_2); 1707, 1733cm.$^{-1}$ (thin film) (diketone); 986,996,1016 (in CS_2) (cyclopropane).

Found	C 74.60, H 9.75%
$C_{13}H_{20}O_2$ requires	C 74.95, H 9.70%

<u>Attempted dehydrogenation</u>

Reticulene (75mg.) was mixed with 20% palladium on charcoal (22mg.) and the mixture gently refluxed for 30mins. in an apparatus similar to that used by Williams [(7)] in the dehydrogenation of aristolactone. The distillate did not become blue or violet.

REACTIONS OF DIHYDROISORETICULENE

Attempted ozonolysis

The oil (0.637g.) was dissolved is 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.), $n_D^{21°C}$ 1.4860, λ 212mμ (ϵ 1300), 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% w/v, 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 (Na_2SO_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[97] 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: $\lambda_{max.}$ 225, 268-270, 372mμ.

AROMADENDRENE

The sample was obtained from Dr. Sutherland[89] and possessed the physical constants shown in Table 20. Infrared absorption - peaks at 885, 1640, 3065cm.$^{-1}$ (vinylidene); 988, 999, 1014cm.$^{-1}$ (cyclopropane).

REACTIONS OF AROMADENDRENE

Dehydrogenation

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

Reduction

The oil (0.55g.) was dissolved in ethanol and hydrogenated at a platinum oxide catalyst (97mg.) until hydrogen uptake ceased (30mins.). The volume of hydrogen utilised (81ml. 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, λ 209mμ(ϵ 331), infrared absorption - peaks at 984, 995, 1011cm^{-1} (cyclopropane).

DETERMINATION OF ULTRAVIOLET ABSORPTION SPECTRA OF RETICULENE, AROMADENDRENE AND THEIR REDUCTION PRODUCTS.

To ensure reproducable 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% w/v, 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:

$$\text{No. of double bonds} = \frac{(a - b) \times m}{w \times 2000 \times 10}$$, 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[109] 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[109] 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% w/v, 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.

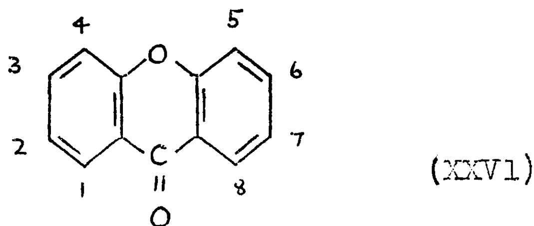
Table 23 (page 145) summarises the results.

APPENDIX I.

STUDIES ON THE ULTRAVIOLET ABSORPTION SPECTRA OF XANTHONES

Direct comparison of xanthone spectra was used by Mull 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 Dalal (110) and by Lund (52) 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 (XXV1) in ethanol (53,112,113) shows the presence of a shoulder at 232mμ (S), and maxima at 238mμ (A), 260mμ (B),



286mμ (C) and 337mμ (D), peaks B and D being broad. Diagrammatically, the spectrum has the following composition (Figure 13).

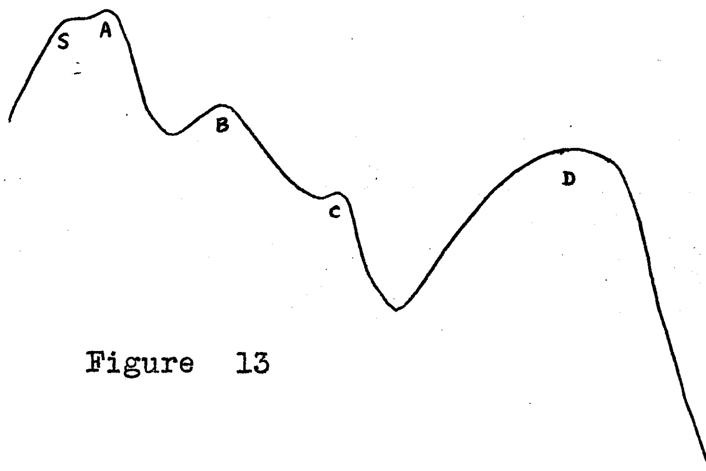


Figure 13

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 230mμ region. It is significant that none of these compounds are substituted in the 1-position of the xanthone molecule. Of the remaining

Table 28

Substance	S Max.	Min.	A Max.	Min.
1. Xanthone	230(sh)	-	238	251
	232(sh)	-	238	251
2. 1.OH Xanthone	228	237	251	267
3. 1.OCH_3 Xanthone	228	238	251	267
4. 1.OH 3.CH_3 Xanthone	234	240	251	269
5. 3.OH Xanthone	230(sh)	-	235	253
6. 2.OH Xanthone	225(sh)	-	237	-
7. 4.OH Xanthone	233(sh)	-	250	273
8. 4.OCH_3 Xanthone	233(sh)	-	246	-
9. 1.OH 5.CH_3 Xanthone	232	240	249	272
10. 1:3$(OH)_2$ Xanthone	233	248	252	268
11. 1:5$(OH)_2$ 3.CH_3 Xanthone	233	243	250	-
12. 1:6$(OH)_2$ Xanthone	228	241	248	-
13. 1:7$(OH)_2$ 3.CH_3 Xanthone	236	246	249	-
14. 1:8$(OH)_2$ Xanthone	228	236	251	-
15. 1:2:7$(OH)_3$ Xanthone	-	-	239	254
16. 1:4:7$(OH)_3$ Xanthone	-	-	237	249
17. 1:4$(OH)_2$ 7.OCH_3 Xanthone	-	-	237	249
18. 1:3:5$(OH)_3$ Xanthone	218	(220)	240	-
19. 1;3:6$(OH)_3$ Xanthone	226	240	-	-
20. 1:3:7$(OH)_3$ Xanthone	234	245	257	269
21. 1:4:8$(OH)_3$ 3.CH_3 Xanthone	233	238	259	-
22. 1:5:6$(OH)_3$ 3.CH_3 Xanthone	227	240	249	-
23. 1:6:7$(OH)_3$ 3.CH_3 Xanthone	230	241	246	-
24. 1.OH 6:7$(OCH_3)_2$ 3.CH_3 "	230	238	254	-
25. 1:6:8$(OH)_3$ 3.CH_3 Xanthone	228	234	250	-
26. Rubrofusarin.	224	-	250(sh)	-
27. Nor Rubrofusarin	225	236	247	258
28. Aspergillone.	-	-	242	261
29. Decussatin.	240	246	260	278
30. Swertinin.	236	252	267	282
31. 1.OH 3:5:6$(OCH_3)_3$ Xanthone	215(sh)	218	243	265
32. 1:3:5:6$(OCH_3)_4$ Xanthone	-	219	245	260
† 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 28

	B Max.	Min.	C Max.	Min.	D Max.	Reference	
	260	281	286	300	337	53, 112	1.
	260	281	287	298	336	113	
	275	-	300 (sh)	317	360	53	2.
	279	-	298(sh)	316	360	53	3.
	288(sh)	-	298 *	321	361	53	4.
	261 - 268	278	306 *	-	332(sh)	53	5.
	250 (sh)	282	297	315	354	52	6.
	280	-	295(sh)	310	353	53	7.
	280 (flat)	-	298(sh)	306	346	53	8.
	282	288	294	317	353	52	9.
	-	-	306 *	328	340	52	10.
	267(sh)	273	300 *	-	345(sh)	52	11.
	265(sh)	273	303 *	332	355	53	12.
	265(sh)	276	303 *	330	343	52	13.
	278(sh)	288	333	365	380	53	14.
	269	-	325(sh)	352	398	Present work	15.
	271	-	332(sh)	352	410	Present work	16.
	271	-	325(sh)	348	400	Present work	17.
	270 (sh), 283(sh)	294	307 *	-	345(sh)	52	18.
	264(sh)	-	281 *	319	336	52	19.
	281(sh)	-	312 *	333	367	52	20.
	269(sh)	290	339 *	378	>390	53	21.
	-	273	296 *	-	323(sh)	52	22.
	264(sh)	273	297 *	330	341	52	23.
	286(sh), 306(sh)	-	325 *	-		52	24.
	-	276	324 *	-		52	25.
	278(sh)	318	325	349	>400	53	26.
	279	-	325(sh)	352	399	53, 52	27.
	279	-	316(sh)	343	364	52	28
	295(sh)	-	315 *	350	375	110	29.
	-	-	329 *	360	390	110	30.
	284	290	315 *	-	360 (sh)	Present work	31.
	285(sh)	-	305 *	-	340 (sh)	Present work	32.
	270 (sh)	285	305	310	371		
	268	285	300 (sh)	-	355		

* 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 230mμ and correspondingly, a minimum is introduced near 240mμ. 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 (XXV1) yet no minimum is apparent in the 240mμ region. Again, this contradicts Mull and Nord [53] and the Indian workers [110] 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 240mμ, 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 [110] 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 ϵ.

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 285-330mμ (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 300mμ. (Table 28.)

1,8-dihydroxyxanthone also shows a large bathochromic shift of maximum C to 333mμ. 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 340-360mμ. Eleven derivatives, however, have this maximum above 365mμ (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 365mμ. 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 400mμ (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 330mμ 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-trihydroxyxanthone agreed with this conclusion (Figure 17) indicating that nor-rubrofusarin was probably a 12,7-trihydroxy-x-methylxanthone. This contradicts the postulate of Mull and Nord[53].

Subtractive Spectra

Deduction of xanthone structures from spectral evidence has always been by direct comparison of spectra[52,53,110] 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 of molecules such as naphthalene, quinoline or isoquinoline (114) 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 ϵ 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 ϵ. (Table 29 and Figure 18).

Table 29

The Ultraviolet Absorption Spectrum of 3-hydroxyxanthone

	Calculated $m\mu$	Actual $m\mu$
Maximum	236	235
Minimum	252	253
Maximum	261 - 265	261 - 268
Minimum	278	278
Maximum	310	306

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

	1,3,5,6-tetra-methoxy-xanthone.	Calculated curve of 1,3,5,6-tetra-hydroxyxanthone.	1-hydroxy-3,5,6-trimethoxy-xanthone.
	mμ	mμ	mμ
Maximum	245	249	245
Minimum	260	260	265
Small maximum or shoulder	285	285	285
Minimum	-	290	290
Maximum	305	305	315

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- (≡ 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.

	Actual curve (mμ)	Calculated curve (mμ)
Maximum	235	235
Minimum	240	243
Maximum	252	256
Minimum	271	271
Shoulder	280	280
Maximum	304	308
Minimum	332	332
Maximum	> 360	368

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 (52) but, once again the

calculated positions of the minima and maxima were reasonably close (Figure 21, Table 32.)

Table 32

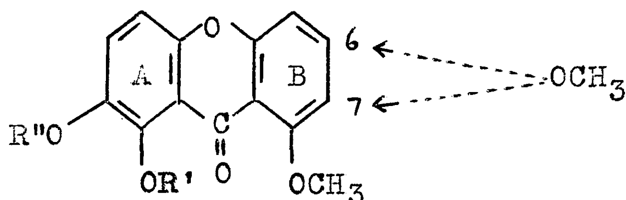
Ultraviolet Absorption Spectrum of 1,5,6-trihydroxy-3-methylxanthone

	Actual Curve (mμ)	Calculated Curve (mμ)
Maximum	231	231 ⁎
Minimum	240	240
Maximum	251	250
Minimum	278	270
Maximum	306	300
Shoulder	320	-

⁎ Inflection

Decussatin and Swertinin

By direct comparisons of ultraviolet absorption spectra, Shah, Kulkarni and Dalal (110) deduced that the structures of decussatin (R'=H, R"=CH_3) and swertinin (R'=R"=H) were as shown (XLV11) with the fourth substituent, a methoxyl group, in ring B but not in position 5. Decussatin and swertinin were therefore 1,2,6,8- or 1,2,7,8-tetrasubstituted xanthones.



(XLV11)

On the wrong assumption (Table 28) that the minimum and maximum at 370 and 380mμ 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 (XLV11). 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 1,2,6,8-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

	Decussatin (mμ)	Swertinin (mμ)	Calculated curve of 1,2,7,8-tetra-hydroxyxanthone (mμ)	Calculated curve of 1,2,-6,8-tetra-hyd-roxyxanthone (mμ)
Maximum	240	240	239	237
Minimum	246	252	251	245
Maximum	260	267	267	259
Minimum	278	282	298	273
Maximum	315	320-330	310-325	312
Minimum	350	360	358	338
Maximum	375	390	$>$ 375	370

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-trihydroxy-3-methylxanthone) but they were dissimilar (Table 34).

1,4,7-Trihydroxyxanthone

The theoretical curve for 1,4,7-trihydroxyxanthone was also quite unlike the actual curve (Figure 22). The curve was calculated from 1,3,7-trihydroxyxanthone, 3-hydroxyxanthone

Table 34

Ravenelin	(mμ)	Calculated curve of 1,4,8-trihydroxyxanthone	(mμ)
Maximum	233		
Minimum	238	Minimum	236
Maximum	258	Maximum	250
Minimum	290	Minimum	265
Maximum	339	Maximum	297
Minimum	377	Minimum	329
Maximum	> 390.		

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.

<u>1,2,7-Trihydroxy-3-methylxanthone</u>.

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 300mμ 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 xanthones 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 (53), 1-hydroxy-3-methylxanthone (53) and 1-hydroxy-5-methylxanthone (52) showed that, apart from differences in log ϵ 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 1,4,8- , 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.

EXPERIMENTAL

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[52] were in terms of frequency and were therefore replotted in terms of wavelength as were the spectra given by Mull and Nord[53]. 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[112,113] (see Table 28).

Calculation of Theoretical Spectra

As most published spectra are accurate only to the second decimal place in terms of log ϵ, the resultant theoretical spectra cannot be more accurate. In some cases the calculated value of ϵ 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 ϵ at various wavelengths, an arbitary constant can be added to each before conversion into log ϵ. 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 repitition, a work sheet is recorded for one typical example.

Theoretical ultraviolet absorption curve of 3-hydroxyxanthone

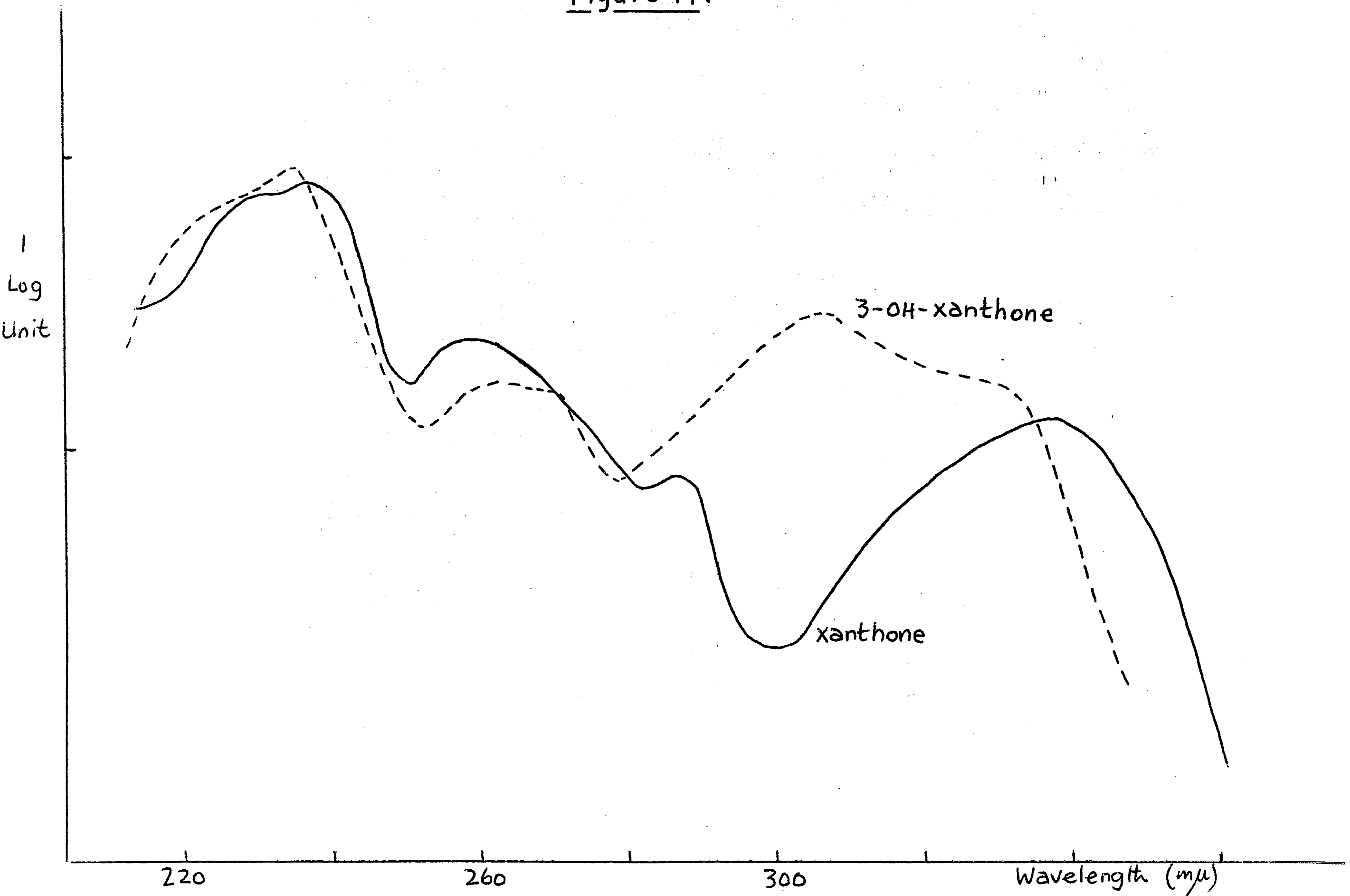
	a.	b.	c.	d.	e.	
	1,6-dihydroxyxanthone	1-hydroxyxanthone	Contribution due to a 3-hydroxy group (b - a)	xanthone	3-hydroxyxanthone (theoretical) (c + d)	3-hydroxyxanthone (theoretical)
mμ	ε	ε	ε	ε	ε	log ε
214	19050	11750	7300	17780	25080	4.41
219	24550	17780	6770	21380	28150	4.45
225	30900	26920	3980	37150	41130	4.61
231	28180	28180	0	42660	42660	4.63
236.5	20420	21880	-1460	46770	45310	4.66
243	17780	25700	-7920	28840	20920	4.32
246	17780	26920	-9140	15850	6710	3.83
248	17780	27540	-9760	11220	1460	3.17
250	17380	27540	-10160	9772	-388	-
252	15850	26920	-11070	10230	-840	-
255	13490	20420	-6930	12590	5660	3.75
257	12300	15490	-3190	13180	9990	4.00
262	10470	7500	2970	12880	15850	4.20
265	9772	6457	3315	12020	15335	4.19
268	8128	6310	1818	10000	11818	4.07
273	6166	7762	-1596	7943	6347	3.80
278	6683	8128	-1445	5129	3684	3.56
281	7244	7762	-518	4266	3748	3.57
283	7762	7413	349	4365	4714	3.67
285	8128	7244	884	4571	5455	3.74
290	9550	6918	2632	3715	6347	3.80
291	9550	6918	2632	3631	6263	3.77
300	12020	5623	6397	1202	7599	3.88
305	12020	3162	8858	1514	10372	4.02
310	10840	1023	9817	3020	12837	4.11
315	8913	955	7958	3802	11760	4.07
322	6166	1202	4964	4677	9641	3.98
333	5012	2042	2970	6918	9888	3.99
340	5623	2818	2805	6761	9566	3.98
346	6166	3802	2364	3890	6254	3.80
360	5495	4898	597	462	1059	3.03.

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 ϵ so that all new values of ϵ became positive.

$m\mu$	ϵ_1 (ϵ + 5000)	log ϵ_1	$m\mu$	ϵ_1 (ϵ + 5000)	log ϵ_1
214	30,080	4.49	273	11,447	4.06
219	33,150	4.52	281	8,748	3.94
225	46,130	4.66	291	11,262	4.05
231	47,660	4.68	300	12,599	4.10
236.5	50,310	4,70	310	17,837	4.25
243	25,920	4.41	322	14,641	4.16
250	4,612	3.66	333	14,888	4.17
257	14,990	4.17	346	11,254	4.05
265	20,335	4.31	360	6,060	3.78

Figure 14.



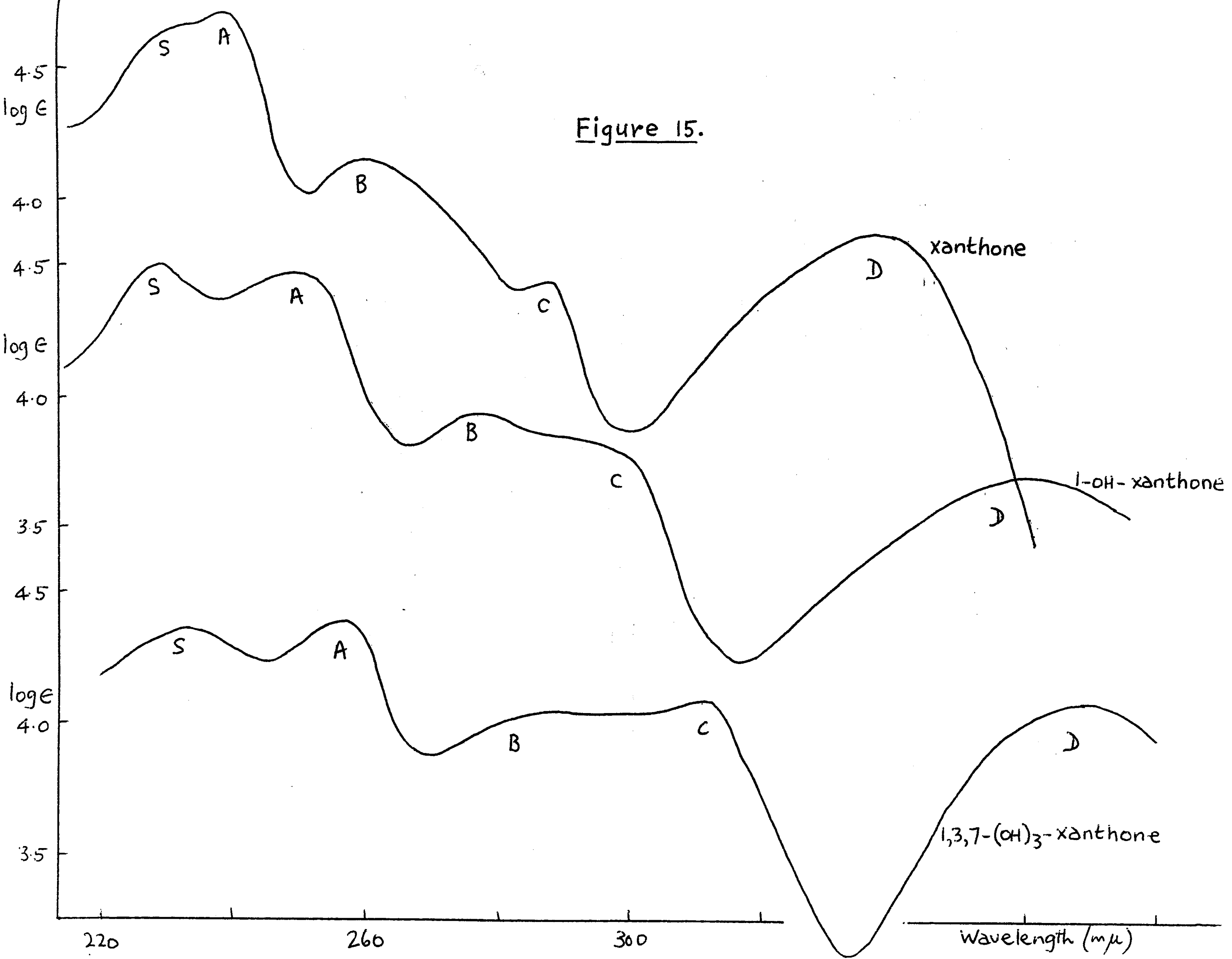


Figure 15.
xanthone
1-OH- xanthone
1,3,7-(OH)3-xanthone
S
A
B
C
D
log ϵ
4.5
4.0
3.5
220
260
300
Wavelength (mμ)

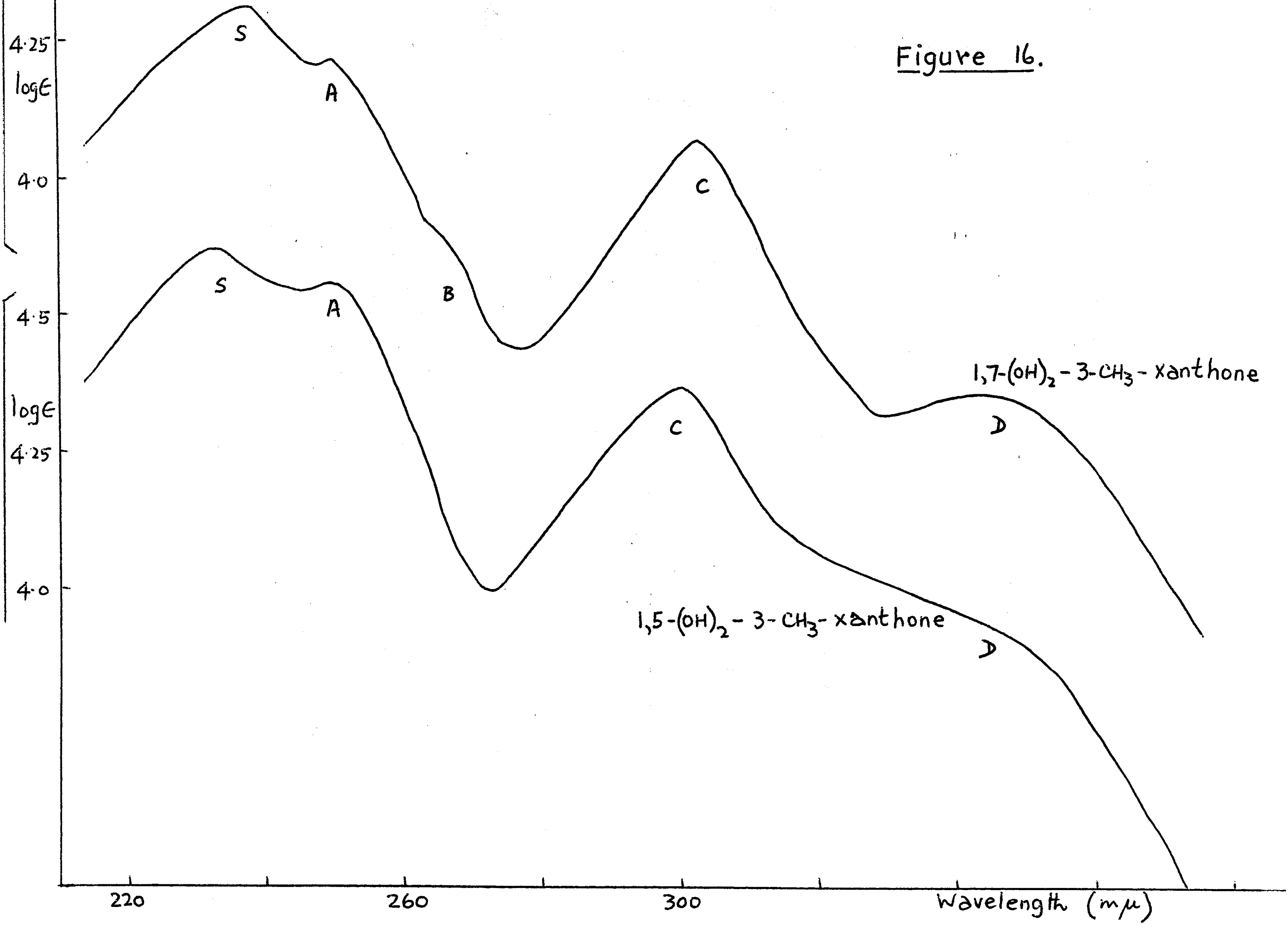
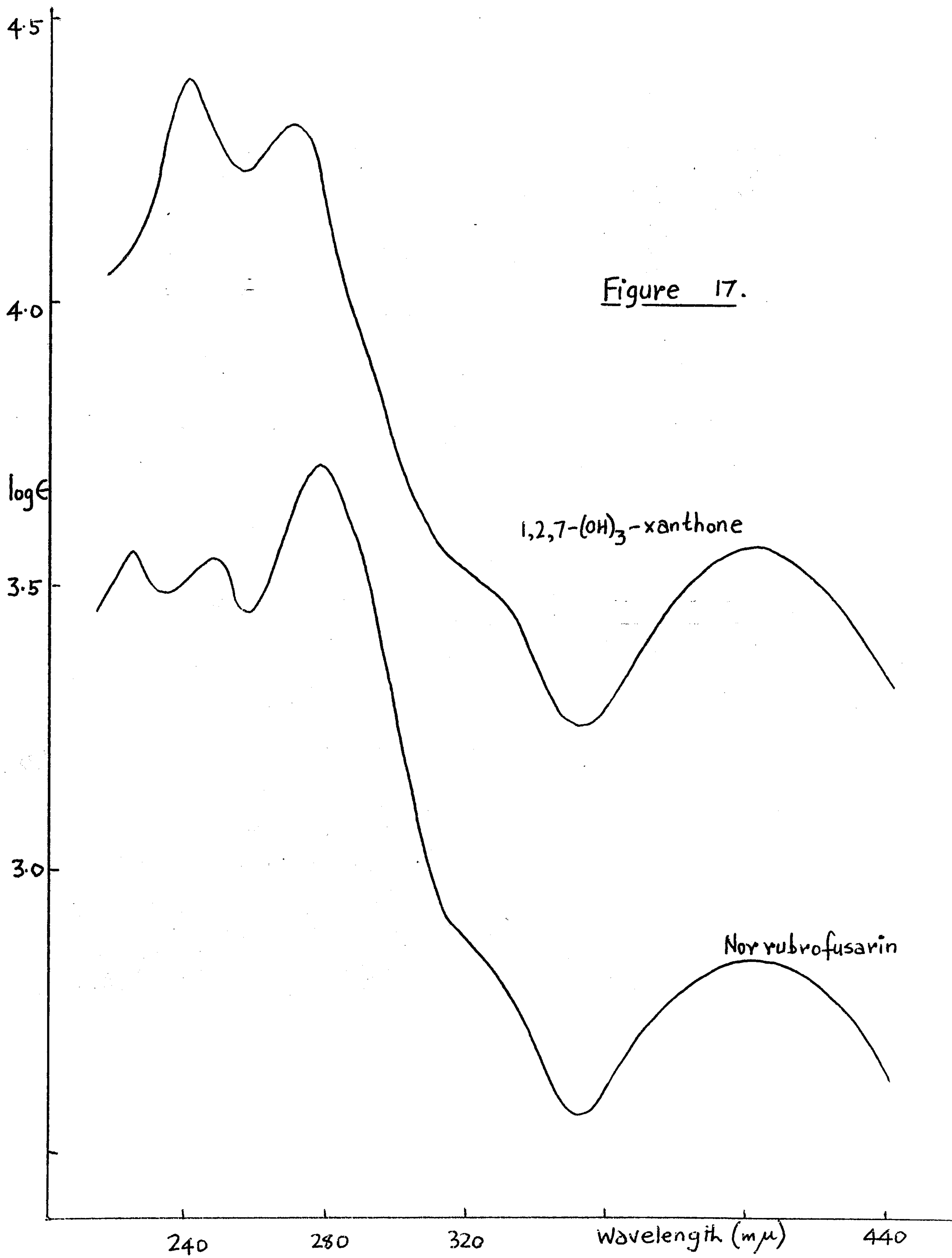


Figure 16.
4·25
log ε
4·0
4·5
log ε
4·25
4·0
S
A
B
C
D
S
A
C
D
1,7-(OH)$_2$ - 3-CH$_3$ - xanthone
1,5-(OH)$_2$ - 3 - CH$_3$ - xanthone
220
260
300
Wavelength (mμ)


4.5
4.0
log ε
3.5
3.0
240
280
320
Wavelength (mμ)
440
Figure 17.
1,2,7-(OH)3-xanthone
Norrubrofusarin

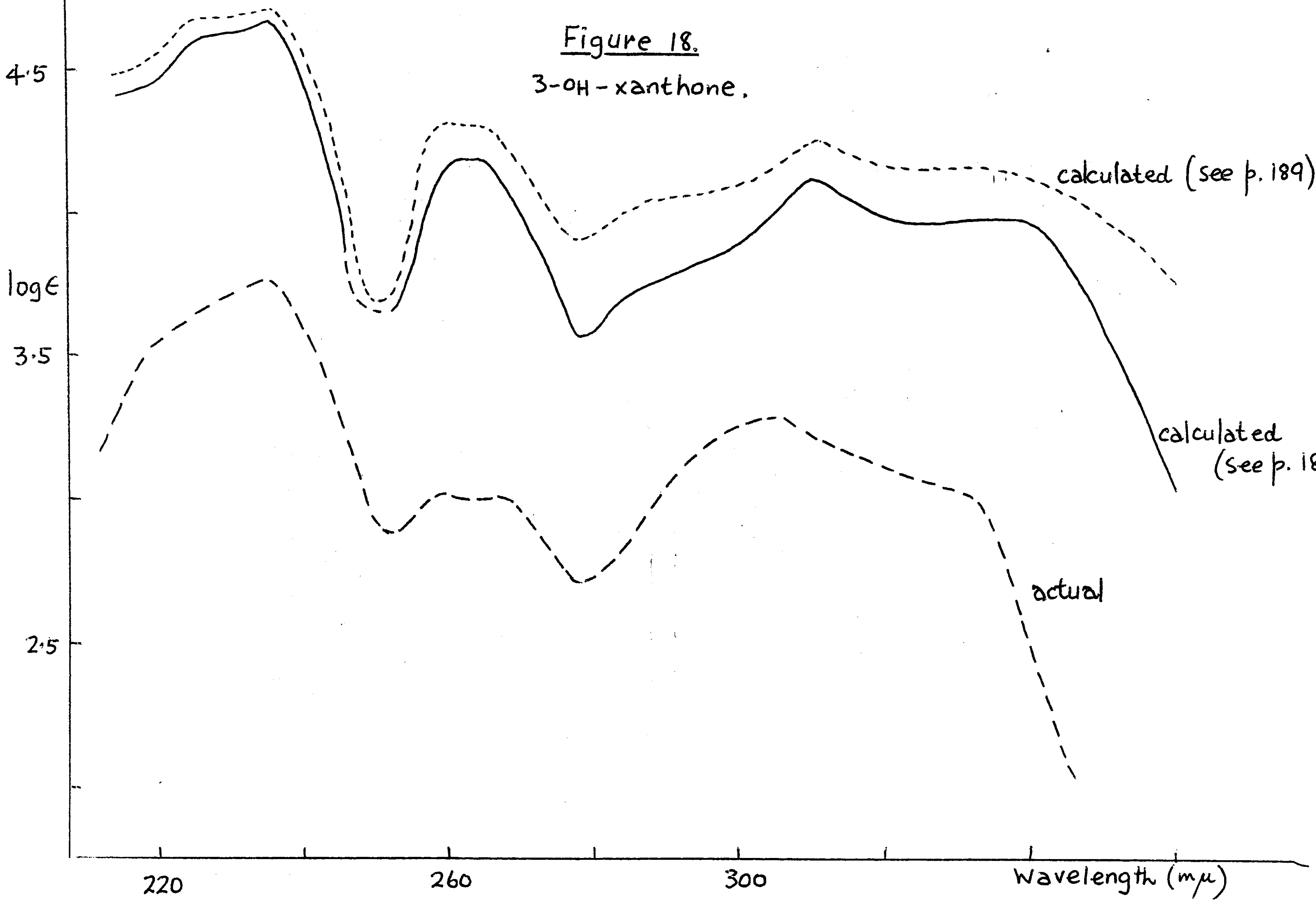


Figure 18.
3-OH-xanthone.
calculated (see p. 189)
calculated
(see p. 188)
actual
4.5
log ε
3.5
2.5
220
260
300
Wavelength (mμ)

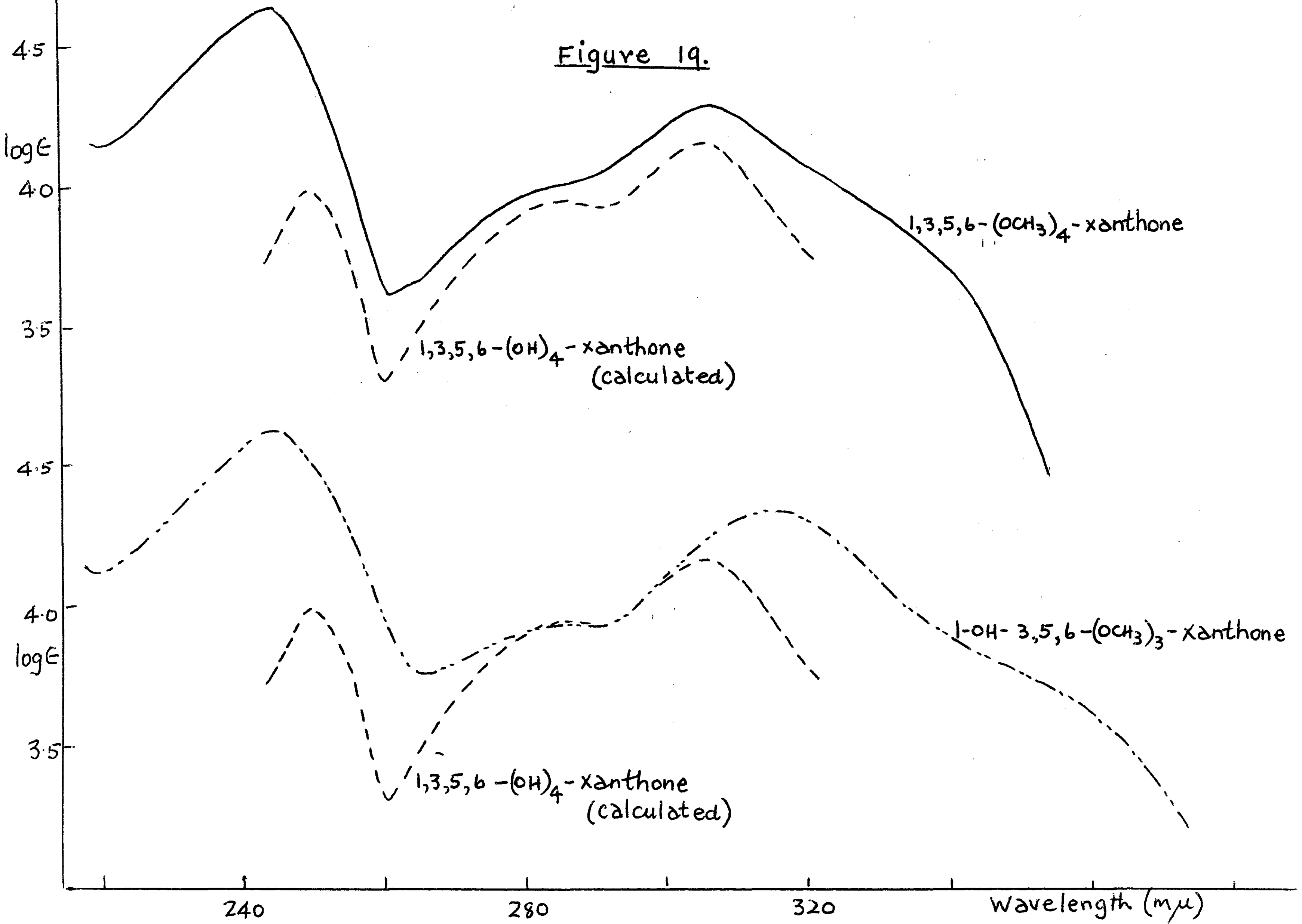


Figure 19.
log ε
4.5
4.0
3.5
4.5
4.0
log ε
3.5
1,3,5,6-$(OCH_3)_4$-xanthone
1,3,5,6-$(OH)_4$-xanthone
(calculated)
1-OH-3,5,6-$(OCH_3)_3$-xanthone
1,3,5,6-$(OH)_4$-xanthone
(calculated)
240
280
320
Wavelength (mμ)

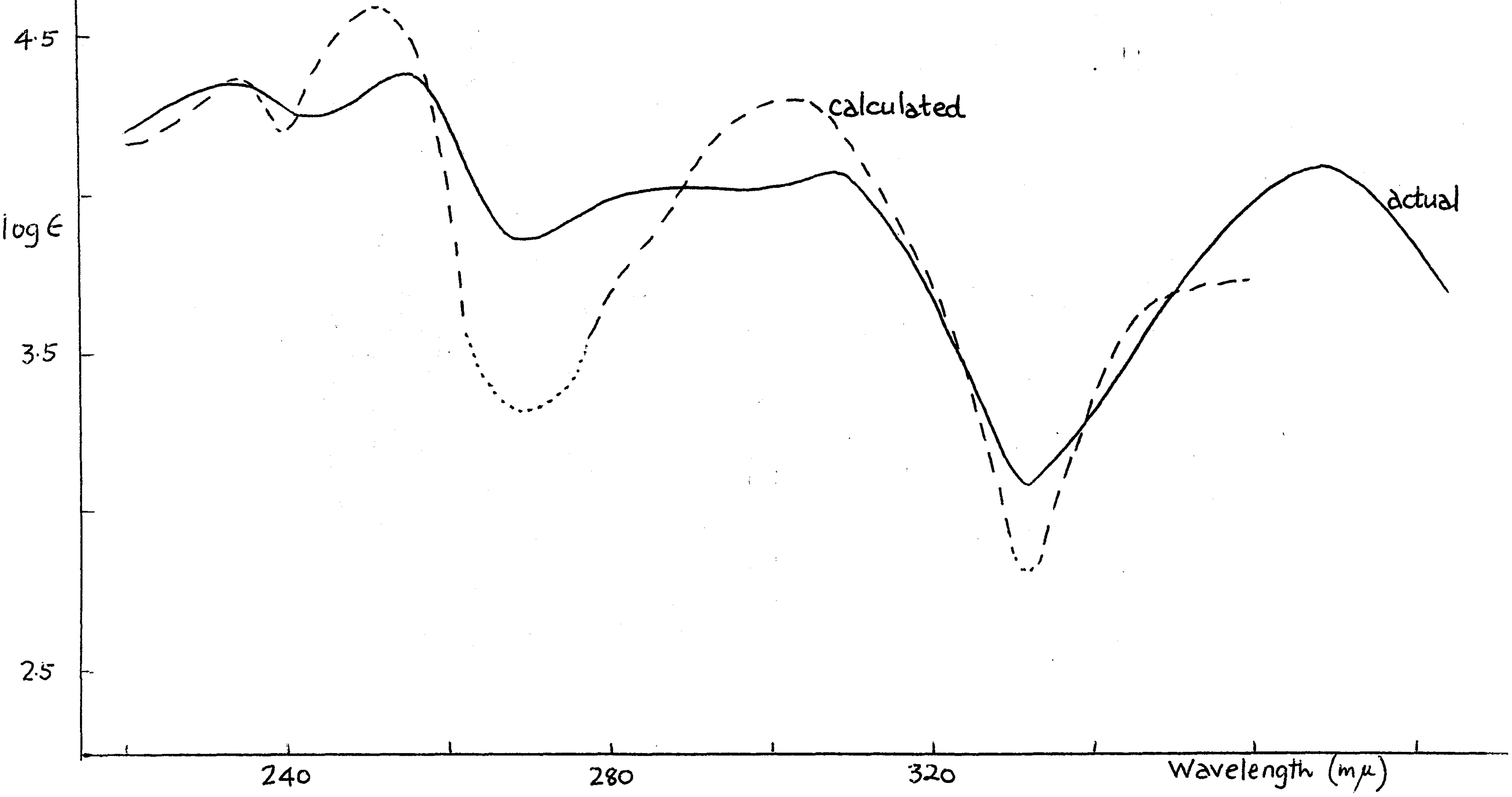


Figure 20.
1,3,7-(OH)3-xanthone
calculated
actual
4.5
log ϵ
3.5
2.5
240
280
320
Wavelength (mμ)

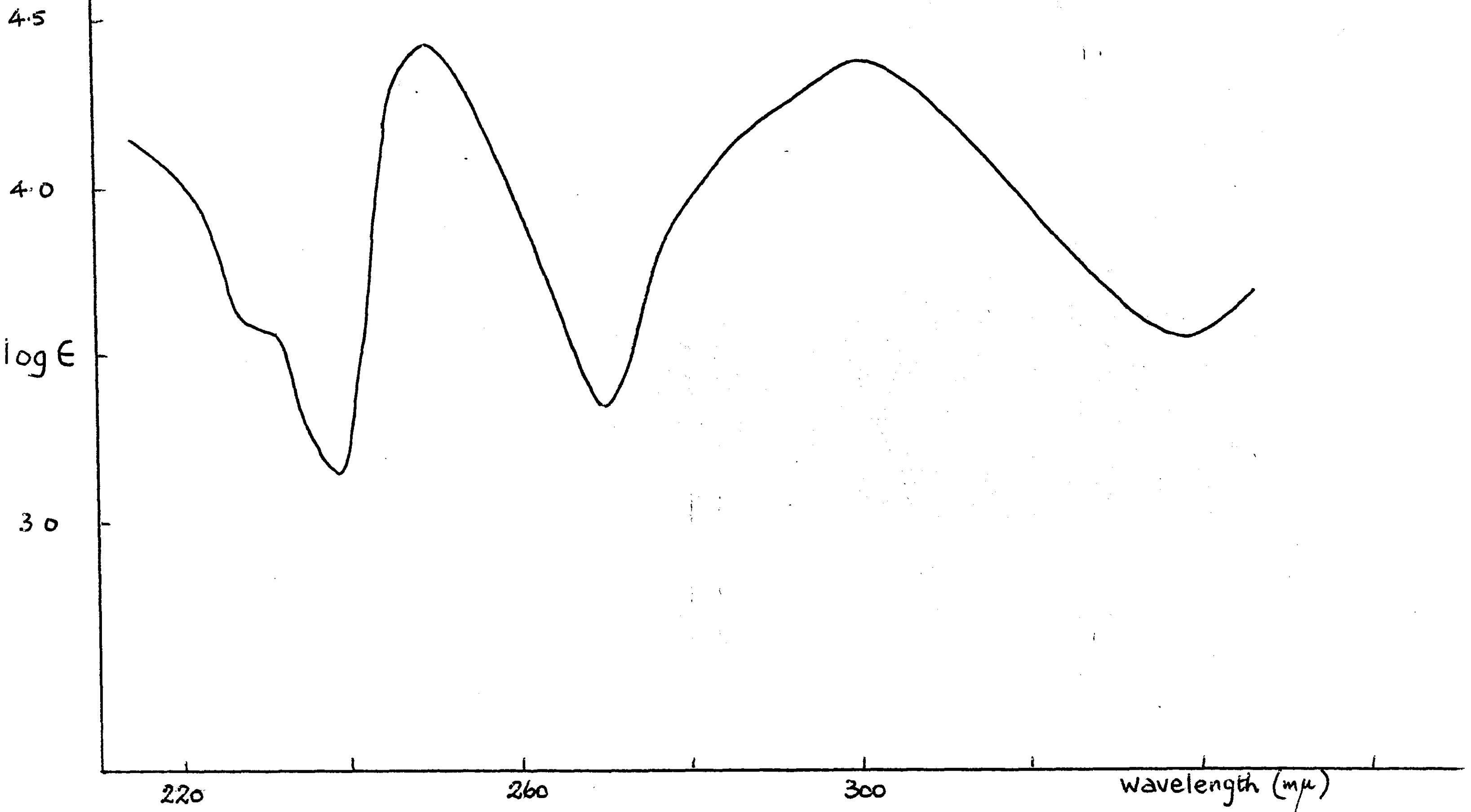


Figure 21.

Calculated curve of 1,5,6-$(OH)_3$-3-CH_3-xanthone

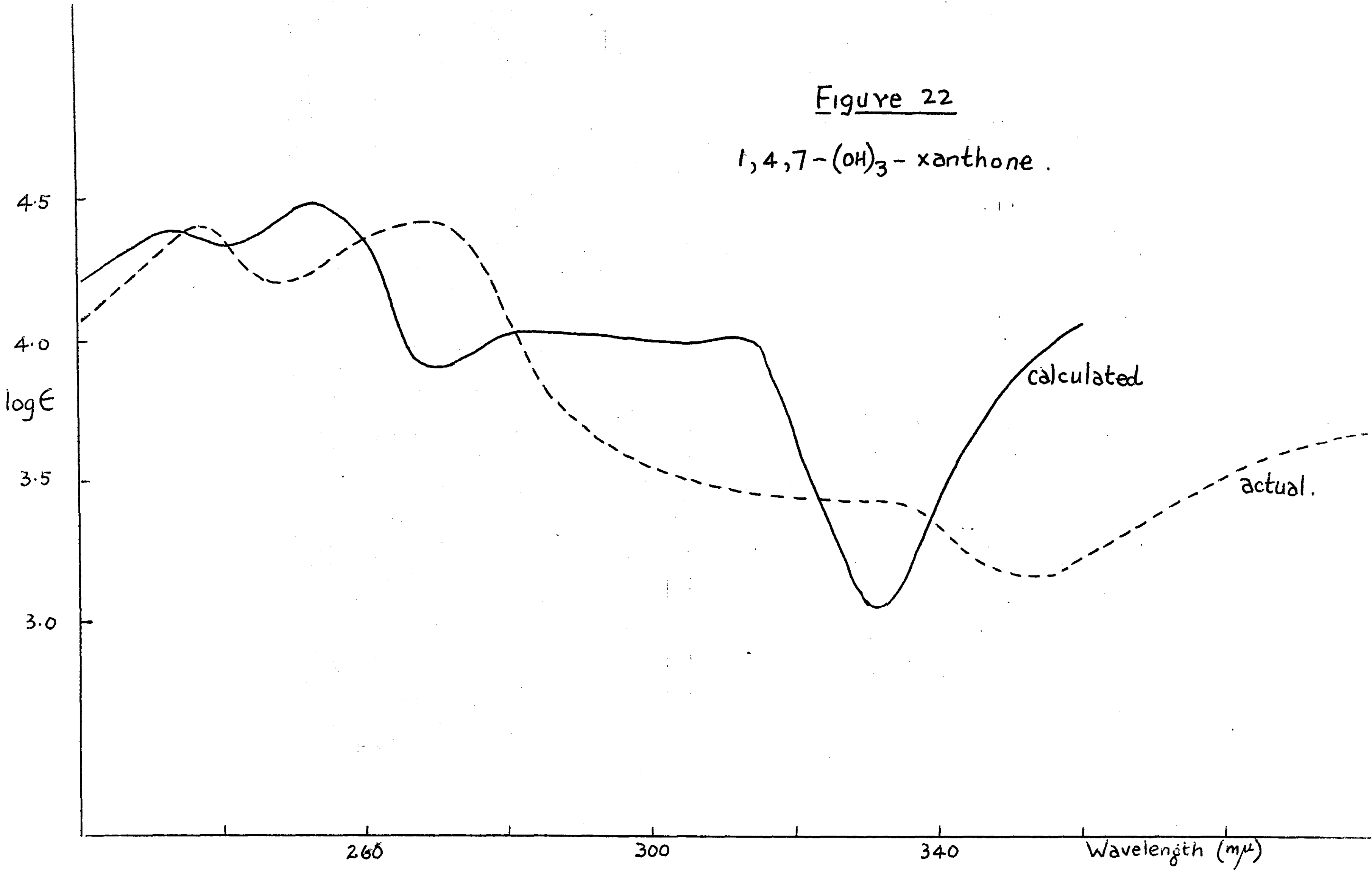


Figure 22

1,4,7-$(OH)_3$- xanthone.

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