A THESIS

submitted to

The UNIVERSITY of GLASGOW

by

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in fulfilment of the
requirements for the degree of

DOCTOR of PHILOSOPHY

December 1955

The School of Pharmacy
Royal Technical College
Glasgow.

THE CHEMISTRY OF *ARISTOLOCHIA* SPECIES

THE LIGHT PETROLEUM - SOLUBLE FRACTION

OF

A. RETICULATA

The author wishes to thank Professor J.P.Todd and Professor F.S.Spring for useful advice, and Dr. J.B.Stenlake under whose direct guidance this work was carried out, and whose wealth of helpful suggestions and criticisms has proved invaluable.

Thanks are also due to the British Drug Houses Ltd. for gifts of raw materials, and to Mr. J.Chilton for help in the preparation of the drug.

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PART I

CONSTITUENTS OF THE LIGHT PETROLEUM EXTRACT OF *A. RETICULATA*

HISTORICAL INTRODUCTION

The genus Aristolochia contains the major proportion of plants belonging to the family Aristolochiaceae, comprising about 180 out of 200 species, the remaining twenty being distributed among five other genera. Plants of this genus are widely distributed, representatives being found in all five continents.

Most of the members are herbs or climbing plants with woody stems. Oil-secreting cells, sometimes forming transparent dots on the leaves, are found throughout the family, but the parts of the plants most frequently used are the roots and rhizomes. The common commercial drug, Texan or Red River snakeroot, is the dried root and rhizome of A. reticulata, and is collected in the woods of Texas, Louisiana, Arkansas and Oklahoma. The drug has fallen into disuse and was deleted from the British Pharmacopoeia 1948, and also from the Pharmaceutical Codex 1954.

Aristolochia species have been used from very early times, and in a review by Dawson[1] its medical history has been traced to the 4th. century B.C. to the time of Theophrastus (370-285 B.C.) who mentioned it in his History of Plants. The number of species then in common use was small, Dioscorides (40-90 A.D.) describing three, and Pliny (23-79 A.D.) four, all being characterised by the type of roots in which the particular virtues of the drug were known to reside even at that early time. They were described as

(a) round tubers..... A. rotunda

(b) elongated root ..... A. longa

(c) slender and long ..... A. clematitis

(d) fibrous roots ..... A. pistolochia [2]

It is of interest that (d) accurately describes the American drugs A. reticulata and A. serpentaria equally well, although the latter was not introduced into Europe until 1633[3]. Dawson's researches indicated that some of the drugs in common use at the beginning of the 17th. century were A. rotunda, A. longa and A. clematitis, but he omits any mention of the American drug, although it tended more and more to replace the European varieties because of its more powerful activity. Later, other species again were recommended, but being considered less valuable than the North American variety, they gradually lost favour until at the beginning of the 18th. century A. serpentaria was the drug used in most countries.

Plants of the genus Aristolochia were held in high esteem by the ancient Greek and Roman doctors on account of its value in childbirth, its wholesome influence on wounds and abscesses, its use against fevers and the bites of poisonous snakes and its use as a bitter with tonic properties. The common name Birthwort for the plant and the term Snakeroot are obviously derived from these traditional uses. According to Rosenmund and Reichstein[3] A. cymbifera Mart.

still serves as a household remedy for snakebite in Brazil. Professional Roman snake-catchers used a species of Aristolochia as a protective agent in their trade and A. indica was reputed to be a valuable antidote to the bites of snakes and poisonous insects[4]. This however has not been substantiated by Mhaskar and Caius (quoted in reference 4), and Bonsmann[5] in his experiments with three Aristolochia species (A. pandurata, A. maxima, and A. ringens) found that extracts had no protective action against the venom of nine different species of poisonous snakes using mice and guinea pigs as the experimental animals.

Some credence to the use of Aristolochia in childbirth is given by the work of Shaw[6] who reported that A. elegans contained an alkaloid which caused contraction of the uterus. Further details were not given as the work formed only a part of a long term investigation of alkaloids obtained from Australian flora. An extract of this species was also found by Barnard[7] to possess C-mitotic activity when tested on the root tips of germinating onion seeds.

Extracts of Aristolochia species have been observed to inhibit cultures of Staphylococcus aureus[8], whilst two substances, A and B, were isolated from the Mexican plant, Raiz de Indio[9], and shown to be active against the following organisms:

Organism	Inhibitory Concentration
Micrococcus pyogenes	1·2 - 1.7 μg. per ml.
M. citreus	2.4 μg. per ml.
B. anthracis	3.0 μg. per ml.

Neither A nor B contained N or S and both were inactivated by 1% cysteine and 10% serum. The sole difference between A and B lay in the fact that B was less soluble in acetone and ether.

Many of the properties attributed to Aristolochia species are derived from folklore and these must be clearly differentiated from those which have resulted from the detailed investigation of the species which has been carried out in more recent times. These studies can be considered under the two headings of chemical constituents, and physiological activity.

CHEMICAL CONSTITUENTS

General examination

A general examination and estimation of the constituents of A. reticulata involving a painstaking analysis of the whole drug, was carried out by Ferguson[10]. His results were summarised in the following form:

Solvents in order of use	Extractive	Percentage of whole drug	Total extractive with each solvent
Petroleum spirit	Volatile oil	1.00	
	Resin	3.20	4.20
Ether	Resin	1.90	1.90
Absolute alcohol	Aristolochine (soluble in water)	0.03	
	Tannin	0.82	
	Phlobaphene	0.95	1.80
Distilled water	Mucilage	0.60	
	Dextrin	0.80	
	Glucose	0.72	
	Malic acid and extractive matter	5.88	8.00
Sodium hydroxide solution	Albuminoids	0.60	
	Extractive matter	2.50	3.10

Solvents in order of use	Extractive	Percentage of whole drug	Total extractive with each solvent
Hydrochloric Acid 1%	Pararabin	1.80	
	Starch	6.48	
	Calcium oxalate	0.53	
	Albuminoid and extractive matter	15.59	24.40
Chlorine treatment			5.78 (Loss)
Residue	Cellulose and lignin		23.09
Ash			11.4
Moisture			10.7
Loss			5.63

The ethereal oil fraction

The presence of ethereal oil in Aristolochia species was noted from the first chemical investigations. Bucholz[11] found 0.5% of volatile oil as well as 2.85% yellowish brown oleo-resin, 1.7% extractive matter, 62.4% wood fibre and 14.1% water in the roots of A. serpentaria. Spica[12] examined the volatile oil from A. serpentaria and identified borneol. The oil from A. reticulata was found

by Peacock[13] to contain borneol and an acid which was presumed to be $C_5H_9O_2$. This formula was based not upon an analysis of the acid itself, but upon that of the parent bornyl ester which had also been shown to contain acetic acid; it must therefore be treated with reserve. A terpene (b.p. 157°C. at 769.6 m.m.; S.G. 0.865 at 15.5°C.) which absorbed bromine readily, was also found. Higher boiling fractions included a greenish yellow oil (S.G. at 15.5°C., 0.9888). This oil was obtained by distillation of a mixture, so that the formula, $C_{18}H_{29}O$, must be accepted with caution because of the doubtful homogeneous nature of the product.

Krishna Rao, Manjunath and Menon[14] examined the essential oil obtained by Krishnaswamy, Manjunath and Venkato Rao[4] from the roots of A. indica. The latter authors obtained a pale yellow oil by extracting the powdered roots with hot alcohol, removing the solvent by distillation and steam distilling the concentrated extract. Two main fractions, A and B, were isolated from this oil. Fractional distillation of fraction A at different pressures was unsuccessful, and after removal of oxygenated compounds by boiling with sodium, a colourless liquid was obtained, b.p. 130-132°C./10 m.m.; $D^{25°C.}_{25°C.}$ 0.9227; $n_D^{25°C.}$ 1.5035; $\alpha_D^{25°C.}$ -42.37°. This oil, a sesquiterpene, was called ishwarene. It gave a liquid monohydrochloride, b.p. 128-130°C./1 m.m.; $d^{30°C.}_{30°C.}$ 1.0200; $n_D^{30°C.}$ 1.5107; $[\alpha]_D^{30°C.}$ -18.7° (in alcohol).

Although ishwarene gave rise to a dark blue liquid on dehydrogenation with selenium no crystalline product or derivative was obtained, and most of the original material was recovered. Fraction B was obtained in larger quantity from the unsaponifiable matter extracted with light petroleum from the residues left from the steam distillation of the total alcoholic extract of the roots, and yielded a new sesquiterpene ketone, $C_{15}H_{22}O$, which was purified by means of its semicarbazone m.p. 240°C. It was a colourless liquid b.p. 118-120°C./1 m.m.; $d^{30°C.}_{30°C.}$ 1.0290; $n_D^{30°C.}$ 1.5122 $\alpha_D^{30°C.}$ -46.47°, and was named ishwarone. The residues from fraction B were fractionally distilled to give a new sesquiterpene alcohol, ishwarol, as a pale yellow viscous oil, $d^{30°C.}_{30°C.}$ 0.9926; $n_D^{30°C.}$ 1.5098; $[\alpha]_D^{30°C.}$ -7.29° (in alcohol). No derivative was obtained and Simonsen and Barton[15] comment that the homogeneity of ishwarene and ishwarol appears doubtful.

The non-volatile oil fraction.

Pohl[16] found that evaporation of the light petroleum extract from the powdered seeds and roots of A. clematitis, A. longa and A. rotunda yielded chlorophyll, an oil and a semicrystalline nitrogen-free substance which was not investigated further as it had no physiological activity. The results obtained by Hesse[17] on A. argentina suggest

that identity of the crystalline substance, which he also mentions, and aristolactone is unlikely. Hesse exhausted the finely powdered roots with ether which was then saturated with ammonia gas to precipitate the ammonium salts of acids. These were filtered off, and evaporation of the filtrate yielded a greenish-brown oily mass which on strong cooling deposited a crystalline, neutral, physiologically inert substance which was identified as a palmityl phytosterolin, $C_{42}H_{74}O_2$ m.p. 82°C., $[\alpha]_D^{15°C.}$ -15.8° (c = 3 in chloroform). Hesse gave the results of his analysis in the form:

"0.1735 g. melted at 110°C. gave 0.522 g. CO_2 and 0.1915 g. H_2O."

Aristolactone melts at 110-111°C but identity with Hesse's neutral substance is clearly ruled out by the analysis and optical rotation. Aristolin, $C_{15}H_{28}O_3$, m.p. 265°C., was also present in the crystal mass and Hesse regarded it tentatively as an alcohol.

Krishnaswamy, Manjunath and Venkato Rao[4] isolated a small quantity of a phytosterolin m.p. 285-290°C. from the non-volatile residue of the alcoholic extract of A. indica. It gave an acetyl derivative, m.p. 162-163°C., and hydrolysis of the original substance gave a phytosterol, m.p. 146°C. The authors suggested that the phytosterolin was a glucoside of the type of ipuranol. The non-volatile

residue was dried on some of the powdered root and extracted with light petroleum, ether, chloroform, ethyl acetate and ethanol in that order. Saponification of the light petroleum extract led to the identification of glycerol and cerotic, lignoceric, linoleic, oleic, palmitic and stearic acids. Ceryl alcohol and a phytosterol were isolated from the unsaponifiable matter. The constants given for the sterol (m.p. 137°C., acetyl derivative, m.p. 127°C.) indicate that it could be ß-sitosterol.

Celentino and Kind[18] identified ß-sitosterol and ß-(ß-sitosteryl)-D-glucoside in the unsaponifiable matter from a light petroleum extract of A. serpentaria, the sterol being isolated by means of the digitonin complex, and the glucoside as a solid during the ether extraction of the unsaponifiable matter.

Green, Eugster and Karrer[19] obtained crocetin dimethyl ester $C_{22}H_{28}O_4$, m.p. 211-212°C., directly from a light petroleum extract of the roots of A. cymbifera Mart. A part of the light petroleum extract (after separation of the ester) was shaken with sodium bicarbonate solution, the aqueous liquor acidified and extracted with ether. Evaporation of the ether gave fraction I. Fraction II consisted of the light petroleum remaining after shaking with sodium bicarbonate solution, and fraction III was the other part of the original light petroleum extract.

Chromatography of these fractions on a column of zinc carbonate 50% and Celite 50% gave two zones, one being red and the other yellow. Crystalline isobixin was ultimately isolated from the red zone, whilst the yellow zone yielded crystals, m.p. 297-298°C., possibly a salt which was decomposed by 4N hydrochloric acid to aristolochic cymbifera acid, $C_{20}H_{32}O_2$ or $C_{21}H_{34}O_2$, m.p. 107°C. It contained two carbon-methyl groups and gave a monomethyl ester and a diethylamide as thick oils.

Allantoin was also identified in the ethanol extract of the residue which remained after extraction of the drug with light petroleum.

The bitter principles

These are usually regarded as alkaloidal, but for clarity this section can be divided into two subsections dealing with acidic and basic principles respectively.

Acidic principles

The presence of a yellow bitter substance in *Aristolochia* species was noted from the early investigations of Chevallier[20], Winkler[21], Frickhinger[22], and Walz[23], all of whom obtained some yellow crystalline or resinous material, the homogeneous nature of which appeared doubtful.

Pohl[16] obtained a nitrogen-containing acid in the form of radiating yellow needle crystals with an empirical formula of $C_{32}H_{22}O_{13}N_2$, m.p. 215°C to charring at 220°C. Its simple chemical properties were studied in some detail, in particular the formation of salts such as the barium compound. When treated with zinc dust and acetic acid it gave a pale yellow physiologically-inactive reduced compound. The crystals were only very weakly acidic, being liberated by carbon dioxide from the salts, so that Pohl adopted the name aristolochine rather than aristolochic acid for the substance, and judged that a carboxyl group was absent. Typical analyses were given as follows:

aristolochine	C 59.94%;	H 3.48%;	N 4.35%
reduced compound	C 68.95%;	H 4.36%;	N 4.66%

Hesse[17] pointed out that these figures for aristolochine fit the formula $C_{17}H_{11}O_7N$ equally well and thus brought aristolochine into line with the three acids that he had isolated from the roots of A. argentina. These acids were obtained as the ammonium salts as described on page 9, and separated by fractional crystallisation from glacial acetic acid, or by means of the different solubilities of their potassium salts, that of aristinic acid being the least soluble. This acid, $C_{18}H_{13}O_7N$, formed greenish-yellow leaflets and needles, m.p. 275°C. (decomp). It gave a methyl ester $C_{19}H_{15}O_7N$, m.p. 250°C., and a methoxyl content

of 1.5% was ascribed to an impurity. Aristidinic acid $C_{18}H_{13}O_7N$, m.p. 260°C., was obtained from the mother liquors of the above as the potassium salt and contained one methoxyl group. Aristolic acid $C_{15}H_{11}O_7N$ or $C_{15}H_{13}O_7N$, m.p. 260-270°C. (after darkening at 220°C.), was obtained when the alkaline residues remaining after isolation of aristinic and aristidinic acids, were acidified. It formed orange-red needles when crystallised from ethanol.

Hesse suggested that Pohl's aristolochine should be called aristolochic acid, and that the substances isolated by Chevallier, by Walz and by Frickhinger were impure specimens of aristolochic acid. Support for these suggestions came from the isolation of aristolochic acid from A. sipho L'Hérit by Castille[24]. The acid of molecular formula $C_{17}H_{11}O_7N$ was monobasic and gave a methyl ester m.p. 260-261°C. (decomp.) so that it appeared to be identical with Pohl's aristolochine. Analysis of the ester gave the formula as $C_{19}H_{15}O_7N$ which indicated that two methyl groups were taken up, whilst reduction of the acid with zinc and acetic acid gave a result similar to that obtained by Pohl. Fusion with solid potassium hydroxide at 250°C yielded ammonia and a residue showing the properties of anthraquinone and of a phenolic substance.

Krishnaswamy, Manjunath and Venkato Rao[4] isolated an intensely bitter micro-crystalline yellow acid, $C_{17}H_{11}O_7N$,

m.p. 275°C., by ether extraction of the dried ethanol extract from A. indica. (page 10). The acid was called isoaristolochic acid to distinguish it from Pohl's acid which melted at 215°C. It did not contain methoxy, methylenedioxy or enolic groups, but one active hydrogen was present. It could not be acetylated and did not react with the usual reagents for carbonyl compounds. Benzoylation gave a small yield of a yellow micro-crystalline powder, m.p. 170-171°C. (decomp.), $C_{24}H_{15}O_8N$, and methylation with dimethyl sulphate gave a methyl compound, $C_{18}H_{13}O_7N$, m.p. 267°C. (decomp.). As the methyl compound remained unaffected by boiling with ethanolic potassium hydroxide for four hours it was considered to be a methyl ether, indicating, in agreement with Pohl, that a carboxyl group was absent. Oxidation of isoaristolochic acid with alkaline hydrogen peroxide gave a colourless dibasic acid, $C_{16}H_{13}O_9N$, m.p. 164.5°C., which lost 1 mol. of water at 120°C.

Rosenmund and Reichstein[3], who in addition to correlating and surveying the literature on Aristolochia species in a most lucid manner, reported their own incomplete investigations on aristolochic acid obtained from A. sipho and described it as forming intensely yellow needles, $C_{17}H_{11}O_7N$, m.p. 274-278°C. It was optically inactive and the methoxyl content of 1.3% was attributed to partial cleavage of a N-dimethyl group. Diazomethane gave a methyl

ester, $C_{18}H_{13}O_7N$, m.p. 280-282°C. (decomp.), which was saponified with difficulty and suffered decomposition in the process. This observation agrees with the earlier observations[4] and suggests a tertiary or hindered carboxyl group.

Oxidation of aristolochic acid with alkaline permanganate gave a crystalline substance, but insufficient for characterisation, whilst in acid solution oxidation appeared to be complete, as no product could be isolated by ether-extraction of the aqueous liquid. Chromic acid in pyridine gave no crystalline material, but this may have been due to the small amount of material available.

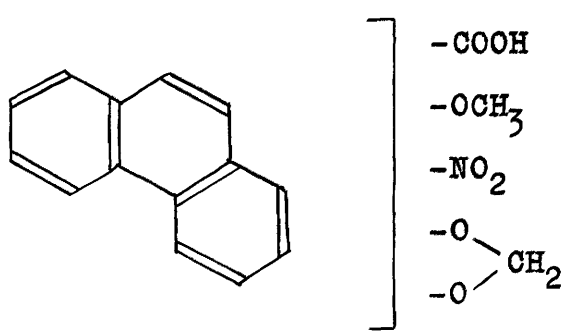
Hydrogenation of the methyl ester with platinum oxide in glacial acetic acid gave a bright yellow substance, $C_{18}H_{13}O_4\ N\ \frac{1}{2}H_2O$, m.p. 312-315°C. This formulation must be accepted with reserve as the substance was unstable and difficult to purify. Acetylation of this product gave a diacetate, $C_{22}H_{15}O_6N$ or $C_{22}H_{17}O_6N$ approximately, m.p. 306-308°C. Direct reduction and acetylation of the methyl ester with acetic anhydride, pyridine and zinc dust gave the same diacetate. The ultra-violet absorption spectra of the methyl ester and the reduced, acetylated product are not markedly different, so that reduction would appear to involve groups containing oxygen rather than ethylenic bonds.

The presence of a carboxyl group in the acid was shown by the elimination of one mol. of carbon dioxide on warming with quinoline and copper powder, the product being a neutral orange-yellow substance, $C_{16}H_{11}O_5N$, m.p. 206-212°C. It was not bitter and gave no crystalline products after reductive acetylation or hydrogenation with platinum oxide in glacial acetic acid.

Definite decisions could not be drawn from the results obtained but the authors postulated the presence of a quinonoid group which could be converted to an unstable phenol. They also submitted the suggestion that Frickhinger's aristolochia-yellow, Hesse's aristinic acid, Castille's aristolochic acid and the isoaristolochic acid of Krishnaswamy, Manjunath and Venkato Rao were all one and the same substance. They pointed out that the empirical formula of aristinic acid could also be calculated as $C_{17}H_{13}O_7N$, and the analysis of the methyl ester also fitted the formula $C_{18}H_{13}O_7N$. Differences in melting points of the esters were ascribed to impurities whilst the difference in melting point between aristolochine (215°C.) and isoaristolochic acid (275°C.) was thought to be due to the fact that they are decomposition melting points, the rate of heating and presence of impurities having some influence on the final observed melting point.

The most recent work on aristolochic acid (from

A. clematitis L.) is that by Pailer, Belohlav and Simonitsch[25], who, while confirming many of Rosenmund and Reichstein's results, have established the presence of one methoxy and one methylenedioxy group in aristolochic acid by suitable modifications in the method of estimation. The nitrogen of aristolochic acid has been shown to be present as a nitro-group, and the structure of the acid as based upon that of phenanthrene. As a diphenic acid was obtained by oxidation of the decarboxylated acid, the position of the nitro-group was tentatively assigned to the 9 or 10 position, since the product still contained the methoxyl and methylene-dioxy groups and all the C atoms of the starting material. The bright yellow substance obtained by Rosenmund and Reichstein on hydrogenation of the methyl ester, and assigned the formula $C_{18}H_{13}O_4N \cdot \frac{1}{2}H_2O$, was found to be $C_{17}H_{11}O_4N$, m.p. 317-319°C. which was in accord with the reduction of the nitro-group and the formation of a lactam of the corresponding amino-acid. The partial structure of aristolochic acid thus appeared to be as shown (I)



(I)

A second acid, $C_{16}H_9O_6N$, m.p. 209°C., similar to aristolochic acid but containing no methoxyl group was also isolated from the rhizomes and called noraristolochic acid.

Basic principles

Investigations into the basic constituents are not so detailed as those into the acidic fraction, probably because of the difficulty of obtaining a pure substance. Chevallier[20] by precipitating a decoction of the root of A. serpentaria with lead acetate and extracting the precipitate with hot ethanol obtained a substance which he called aristolochin or serpentarin. Feneulle[26], isolated a substance in a similar manner but from the filtrate obtained in the above procedure. The original papers were not available but these substances were regarded as being the same (cf. Wiesner[27]).

Ferguson[10] in his investigations of A. reticulata tried both these methods and found that Feneulle's method yielded a basic principle in the form of light yellow needles which were odourless and bitter and which evolved ammonia on heating with soda-lime. The crystals gave a reddish brown colour with sulphuric acid, a reaction which distinguished this base, which Ferguson called aristolochine, from that isolated by Hesse[17] from A. argentina. The latter base was also called aristolochine (German, Aristolochin), but it

gave a dark green colour with sulphuric acid. It formed an amorphous precipitate when its solution in acid was made alkaline. Chemical Abstracts[28] state that a second base-aristine, isolated as gold-coloured needles m.p. 270°C. (decomp.) - was also obtained, but the original paper (Hesse,[29]), showed that aristine formed red compounds with alkali and could be precipitated from the red solution by acids. These properties would suggest that aristine is probably related to aristolochic acid.

Peckolt[30] obtained dull-white, flaky crystals which were odourless and tasteless but with a nauseating after-taste, from A. cymbifera genuina Masters. Dymok and Warden[31] found indications of a basic substance in the roots of A. indica but could not obtain it in crystalline form. However, crystalline material was obtained from this source by Krishnaswamy, Manjunath and Venkato Rao[4], the first two authors[32] making a more detailed study of the alkaloid which was called aristolochine. After purification by means of the hydrochloride, m.p. 268°C. (decomp.), the base was obtained as a crystalline powder $C_{17}H_{19}O_3N$, m.p. 215°C., $[\alpha]_D^{25°C.}$ -268.5°. It formed crystalline compounds with benzene and toluene m.p. 163°C. (decomp.) and 159°C. (decomp.) respectively. Aristolochine was found to be sparingly soluble in most of the common solvents, but dissolved readily in alkalies from which it was precipitated by carbon dioxide.

It gave no colour with ferric chloride, and carbonyl and methylenedioxy groups were absent. It contained one methoxy group and a N-dimethyl group.

PHYSIOLOGICAL ACTIVITY OF ARISTOLOCHIA SPECIES

The first experimental investigation was probably that of Murray[33] who examined the action of A. anguicida L. against snakes. He observed that the odour from the sap of the root caused them to flee from it, and a few drops served to stupify them or send them into convulsions.

Orfila[34] found that on dogs, a large amount of aqueous extract or root caused general paralysis and finally death. He described the action as an effect on the nervous system and Aristolochia as a narcotic poison. Pohl[16] criticised the report of Orfila's experiments on the grounds that they merely demonstrated toxicity without allowing any definite conclusion on the point of attack of the toxins to be drawn. He carried out experiments on frogs, rabbits and dogs, using a crystalline principle, which he called aristolochine, isolated from A. clematitis, A. rotunda and A. longa. Aristolochine in this case was the weakly acid material and not the basic principle. He found that frogs were unaffected by it, and concluded that the action of Aristolochia species on cold-blooded animals was not due to this constituent. He went on to demonstrate that the oil distilled from the root narcotised and rapidly killed frogs, and showed that the poisoning was accompanied by a gradual slowing of the heart.

Aristolochine was extremely toxic to rabbits, the first symptom being a marked diuresis following subcutaneous injection of 0.01g. per kilo bodyweight. Later, the volume of urine became less and contained albumin and epithelial cells. If the dose was not too large (0.007g. per kilo body weight) the animal recovered, otherwise paralysis followed by death, occurred. In pregnant rabbits aristolochine caused abortion two days after administration of the drug.

Dogs were almost unaffected by subcutaneous injections of aristolochine, the only untoward symptoms being watery stools and an increase in temperature to 40.2°C. Injection into the jugular vein however, was followed by death in one day after a weakened, sleepy and very thirsty condition. Pohl classified his aristolochine as a renal poison, it being at least ten times as potent as aloin.

Lalanne and Mathou[35] investigated the botanical characters and physiological effects of a plant which they suggested was identical with, or closely related to A. eurystoma Duchartre. They examined two preparations of the roots and rhizomes. A soft extract obtained by extraction with ethanol, caused abortion in pregnant guinea pigs when injected subcutaneously, whilst the oil distilled from the roots and rhizomes was merely an irritant, but regularly caused loss of hair in the experimental animals.

The abortive character of the soft extract was not due to a specific action on the uterus but was attributed to its drastic purgative action causing inflammation of the pelvic organs. The natives in Guadeloupe used either the whole plant, or more commonly the roots and rhizomes in the form of decoctions or infusions, as an emmenagogue and abortifacient.

Ryo[36] isolated aristolochine from A. debili and his report of the pharmacological action of this substance was substantially the same as that described by Pohl, except that it caused cardiac and respiratory paralysis in frogs.

Summary of *Aristolochia* species and constituents

Species	Constituents	Formula	M.p. °C.	Remarks	Ref
argentina	Aristolochine	-	-	basic	
	Palmityl phytosterolin	$C_{42}H_{74}O_2$	82		
	Aristinic acid	$C_{18}H_{13}O_7N$	275		17
	Aristolic "	$C_{15}H_{11}O_7N$	260-270	or $C_{15}H_{13}O_7N$	
	Aristidinic "	$C_{18}H_{13}O_7N$	260		
clematitis	*nor*Aristolochic acid	$C_{16}H_9O_6N$	209		25
clematitis / *longa* / *rotunda*	Aristolochine	$C_{32}H_{22}O_{13}N_2$	215	Probably identical with aristolochic acid $C_{17}H_{11}O_7N$	16
cymbifera Mart.	Compound	$C_{18}H_{28}O$	137		
	Crocetin dimethyl ester	$C_{22}H_{28}O_4$	211-212		
	Isobixin	$C_{25}H_{30}O_4$	214.5-215.5		19
	Allantoin	$C_4H_6O_3N_4$	221		
	Aristolochic cymbifera acid	$C_{20}H_{32}O_2$	107	or $C_{21}H_{34}O_2$	
indica	Phytosterolin				
	Aristolochine	$C_{17}H_{19}O_3N$	215	(basic)	4
	*iso*Aristolochic acid	$C_{17}H_{11}O_7N$	275		
	Ishwarene	$C_{15}H_{24}$		sesquiterpene	
	Ishwarone	$C_{15}H_{22}O$			
	Ishwarol	$C_{15}H_{23}(OH)$			
	Oleic acid				
	Linoleic acid				
	Palmitic acid				

Species	Constituents	Formula	M.p. °C	Remarks	Ref
indica	Stearic acid				
	Lignoceric acid				
	Cerotic acid				
	Glycerol				
	Ceryl alcohol				
	Phytosterol		137-138		
reticulata	Borneol				
	Terpene	$C_{10}H_{16}$			13
	Acetic acid				
	Water insoluble acid	$C_5H_9O_2$	about 65°	$C_{10}H_{16}O_2$ (this work)	
	Aristolochine	-	-	basic	10
serpentaria	Borneol				12
	Aristolochine	-	-		20,26
	ß-sitosterol	$C_{29}H_{50}O$	140		
	ß(ß-sitosteryl-D-glycoside)				18
sipho L'Hérit.	Aristolochic acid	$C_{17}H_{11}O_7N$	274-278		24 3

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

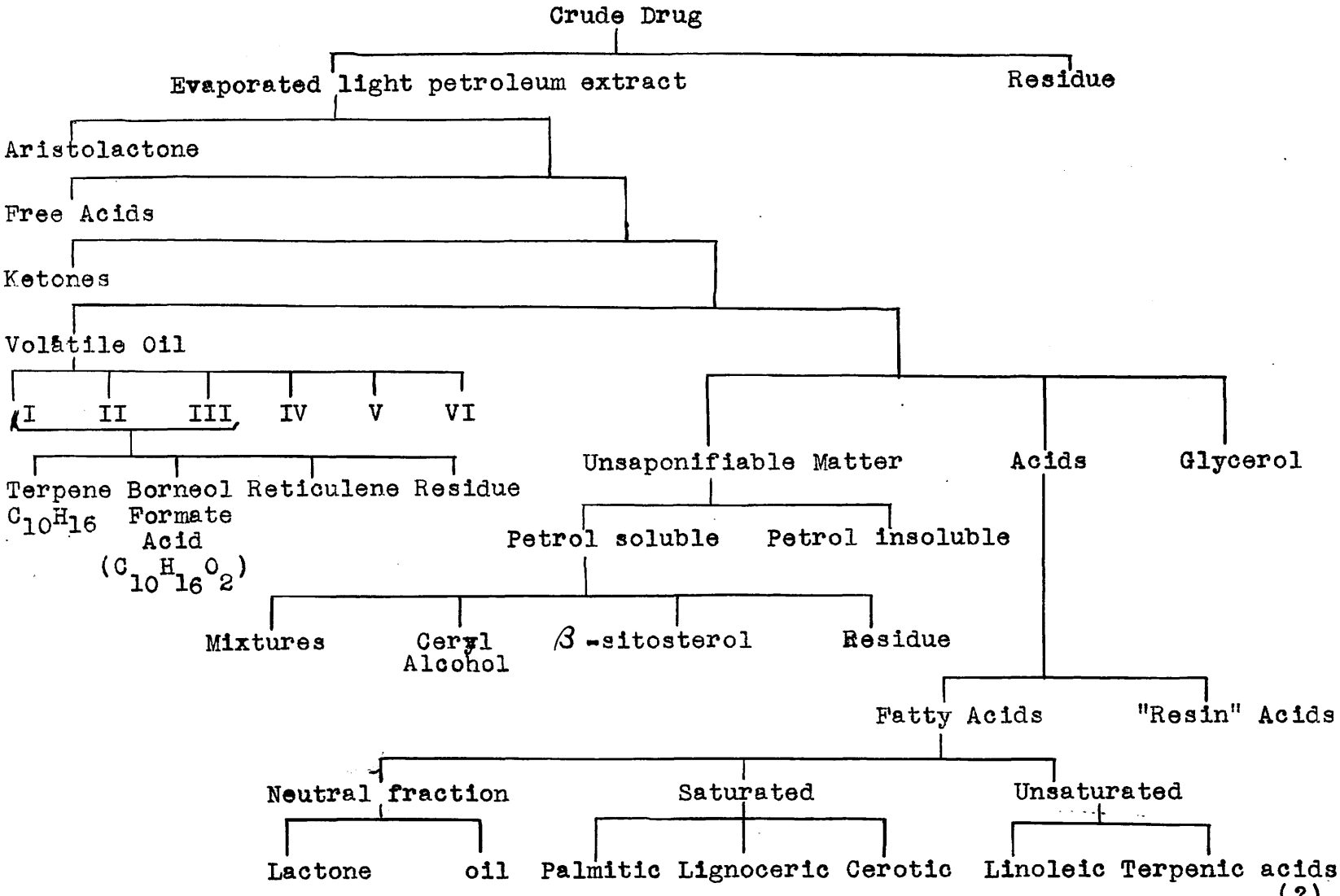
The object of the investigation was the isolation and identification of the chemical constituents of A. reticulata. Three batches of drug, which were shown to be authentic A. reticulata, were extracted in fine powder with light petroleum by cold percolation. Cold extraction and rapid evaporation of the percolate was used to minimise decomposition of unstable substances and to avoid loss of volatile constituents. The light petroleum recovered was optically inactive, indicating that loss by co-distillation, if any, was small.

Examination of the light petroleum extract was sufficiently promising for attention to be confined solely to that fraction, the alkaloidal bitter principles being left for future examination. With the crystallisation of aristolactone from the light petroleum extract the work fell naturally into two parts,

Part I, a general examination of light petroleum soluble extractive, including the isolation and identification, where possible, of its constituents, and

Part II, a study of aristolactone.

The appended scheme summarises the experimental approach to the problem and the main products isolated.


Crude Drug
Evaporated light petroleum extract
Residue
Aristolactone
Free Acids
Ketones
Volatile Oil
I
II
III
IV
V
VI
Terpene $C_{10}H_{16}$
Borneol Formate Acid ($C_{10}H_{16}O_2$)
Reticulene
Residue
Unsaponifiable Matter
Acids
Glycerol
Petrol soluble
Petrol insoluble
Mixtures
Ceryl Alcohol
β-sitosterol
Residue
Fatty Acids
"Resin" Acids
Neutral fraction
Saturated
Unsaturated
Lactone
oil
Palmitic
Lignoceric
Cerotic
Linoleic
Terpenic acids (?)

EXAMINATION OF THE LIGHT PETROLEUM-SOLUBLE FRACTION

Isolation of aristolactone

The oil obtained after evaporation of the solvent was a dark greenish-brown colour, and of pleasant odour, similar to that of the root itself. The yields varied from 3.6% to 5.5% of the air-dried drug, the difference between batches 2 and 3 being very marked (Table 7). As Pohl[16] and Hesse[17] had obtained crystals on strong cooling of similar extracts, the oils were kept in a refrigerator for two days, but crystals were not precipitated. Steam distillation of a small portion of the oil from which free acids and carbonyl compounds had been removed, had yielded in the later runnings a small quantity of a colourless crystalline solid, and when this was used to seed the main bulk of the oil a much larger quantity of the same material was slowly deposited. This substance, a hitherto unknown lactone, $C_{15}H_{20}O_2$, designated aristolactone, was recrystallised in needles from light petroleum, m.p. 110.5°-111°C. Batch 3 of the drug was of interest in that the *A. serpentaria* taken from it gave an oil and crystals of aristolactone when treated in the same way as that used for *A. reticulata*.

Isolation of free acids

Free acids were present in comparatively small amount and were recovered as a glassy resinous solid (0.25% of the oil), which exhibited a violet fluorescence under ultra-violet light. They were not examined further.

Isolation and examination of carbonyl compounds

The carbonyl material present in the oil did not form a sodium bisulphite addition complex, the small amount of solid obtained remaining as a pale yellow powder on heating with a 10% solution of sodium carbonate.

The presence of a ketonic fraction was shown by the use of Girard's reagent[37]. Reagent T (II) was selected

$$H_2N \cdot NH \cdot CO \cdot CH_2 \cdot N(CH_3)_3^{+} Cl^{-}$$

(II)

because of its greater solubility under the experimental conditions used. Aldehydes also react to form derivatives which, it was thought, could not be cleaved to the original aldehyde, but this view is open to doubt in view of the results obtained by various authors[38]. In any case this did not present a serious obstacle as the result of the bisulphite extraction indicated the absence of aldehydes.

The use of the reagent did involve one disadvantage in that acetic acid was necessary, so that the presence of

acetates in subsequent fractions could not necessarily identify them as a constituent of the original oil.

Ketonic material was extracted as a brownish oil (3%) which was strongly dextrorotatory ($[\alpha]_D^{17°C.}$ + 170°). A small portion of this oil was further resolved into two components, a brownish-yellow viscous semi-solid, ($[\alpha]_D^{13°C.}$ +62°), insoluble in light petroleum, and a pale yellow oil ($[\alpha]_D^{13°C.}$ + 179°), soluble in light petroleum. The insolubility of the former fraction in light petroleum is of interest and suggests that it may be an artefact, though the possibility that it was solubilised in the original extract by the presence of terpenes cannot be discounted entirely. The usual derivatives were not obtained in crystalline form, but only as amorphous powders of indefinite melting points e.g. semicarbazone m.p. 80-90°C. The oil exhibited high intensity ultraviolet absorption, $\lambda_{max.}$ 250 mμ, $E^{1\%}_{1cm.}$,650 so that the presence of an α,β-unsaturated carbonyl group is highly probable.

This fraction was not examined further.

Isolation and examination of the volatile oil

Steam distillation of the ketone-free oil recovered during the isolation of the ketone gave volatile products of which those from extract A (first batch of drug), were collected in twenty-seven fractions (Table 11, page 64), with

the idea of constructing a refractive index/density diagram for the tentative recognition of terpenes (cf.[39]). The presence of crystals in some of the fractions made this unreliable so that in distilling the comparable oily fraction from extract B (second batch of drug) the fractions collected were fewer in number but larger in volume to avoid the use of solvents.

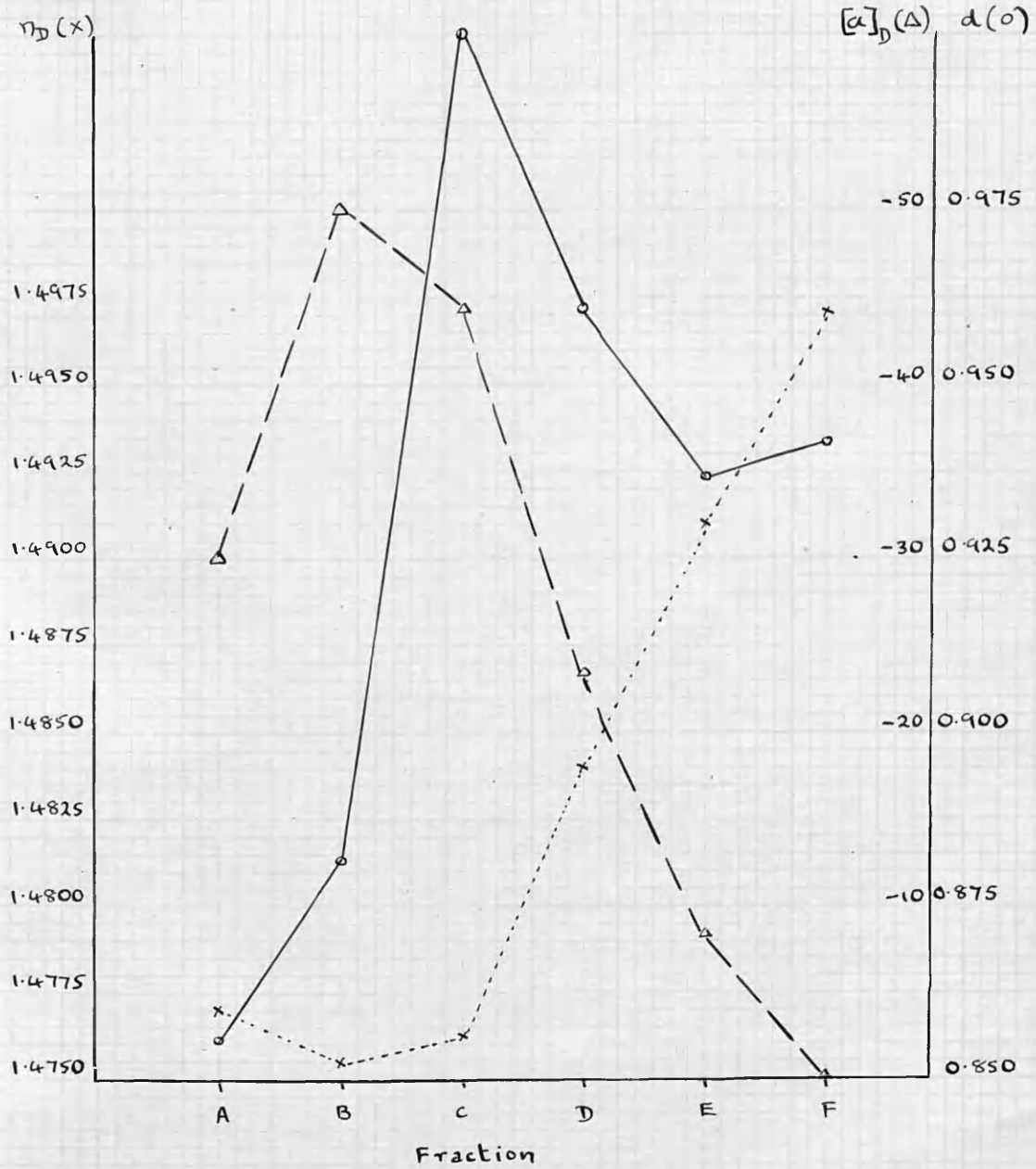
The small quantity of oil obtained in the first experiment was used for preliminary tests and as a result the analysis of fractions I, II and III (Table 12, page 65) was much simplified. The oil present in fraction I could not be separated from solvent by fractional distillation and was isolated as a dibromide after treatment with bromine. The product, a colourless oil, yielded a small quantity of a colourless crystalline solid, m.p. 87-88°C. when chromatographed from light petroleum on alumina. This substance was undoubtedly the same as the dibromide subsequently isolated from fractions A and B as described below.

Repeated refractionation of fractions II and III gave further fractions as shown in Table 13, page 67). Unfortunately, fractionation on this small scale was not complete and most of the fractions were found to be mixtures. Graphical analysis (Fig.1), of the physical data recorded for fractions A-F (Table 13) suggested the presence of at least three components. Fractions A and B are distinct and

Fig. 1

Graphical analysis of the fractions obtained

by redistillation of fractions II and III



could be monoterpenes (from boiling point, refractive index and density). Few monoterpenes have densities as high as those for fractions A and B but contamination with fractions C and D was possible. The high densities of these latter fractions suggested the presence of oxygen-containing compounds, probably esters. Fractions E and F have densities which could be attributed to sesquiterpenes.

The trends of the graph show that fraction A could be a mixture of fraction B with some other terpene, whilst fractions E and F could be mixtures of fraction D and a sesquiterpene or sesquiterpenes.

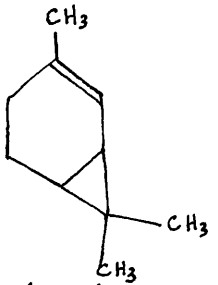
Fraction A

Bromination of the oil and chromatography of the product by the method adopted for fraction I yielded three fractions, two of which were oils and one crystalline. The crystals were those of a dibromide $C_{10}H_{16}Br_2$, m.p. 89°C., $[\alpha]_D^{17°C.}$ + 98°, identical (m.p. and mixed m.p.) with that isolated from fraction I. The oils were not examined.

Fraction B

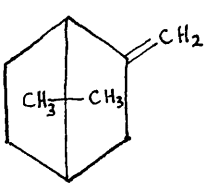
Contamination with fraction C was suspected, but saponification revealed the presence of only traces of esters, and chromatography of the neutral material gave an oil, $d_{15°C.}^{15°C.}$ 0.859, $n_D^{15.5°C.}$ 1.4742, $[\alpha]_D^{17°C.}$ -59°, $[R_L]_D$ 44.59. These constants are substantially in agreement with those for (+) - Δ^4 - carene (III) which are given[40] as $d_{30°C.}^{30°C.}$ 0.8552,

$n_D^{30°C.}$ 1.474, $[\alpha]_D^{20°C.}$ +62.2° $[R_L]_D$ 44.69, the optical exaltation of the latter (0.5), over the theoretical value for a



(III)

bicyclic monoethenoid terpene $C_{10}H_{16}$, $[R_L]_D$ 44.19 [40] being due to conjugation of the ethylenic bond and cyclopropane ring. The presence of a single double bond in the molecule of the terpene was confirmed by an iodine value and by the low intensity ultraviolet absorption maximum at 210 mμ(ε, 4200) characteristic of a trisubstituted ethylenic bond [41][42]. This latter characteristic, the optical exaltation of the molecular refraction and the doubtful occurrence of the fenchenes in nature, eliminated the possibility of the terpene being α-fenchene (IV), d 0.869, n_D 1.4724,



(IV)

$[\alpha]_D$ -32.12, dibromide $C_{10}H_{16}Br_2$ m.p. 89°C.

Unfortunately Δ^4-carene does not yield any well-authenticated crystalline derivatives. Oxidation with permanganate in acetone solution (Simonsen [40]) gives a

ketone and thence a crystalline semicarbazone but this was not attempted with the very small quantity of terpene available.

Reaction with bromine is said to give a viscid oil which shows no sign of crystallising. Bromination of the purified monoterpene similarly gave a colourless viscous oil, but which, after chromatography yielded a colourless crystalline dibromide, identical (m.p. and mixed m.p.) with those obtained from fractions I and A. Attempts to obtain an authentic sample of Δ^4-carene have so far proved abortive although the Imperial Institute were most helpful in suggesting possible sources, among them, the grass Andropogon Jwarancusa.

Further work on this terpene fraction is at present restricted from lack of material, but it is interesting to note, in connection with its tentative recognition as (-)-Δ^4-carene that, to date, only the dextro-rotatory form of this terpene has been isolated from natural sources.

Fraction C

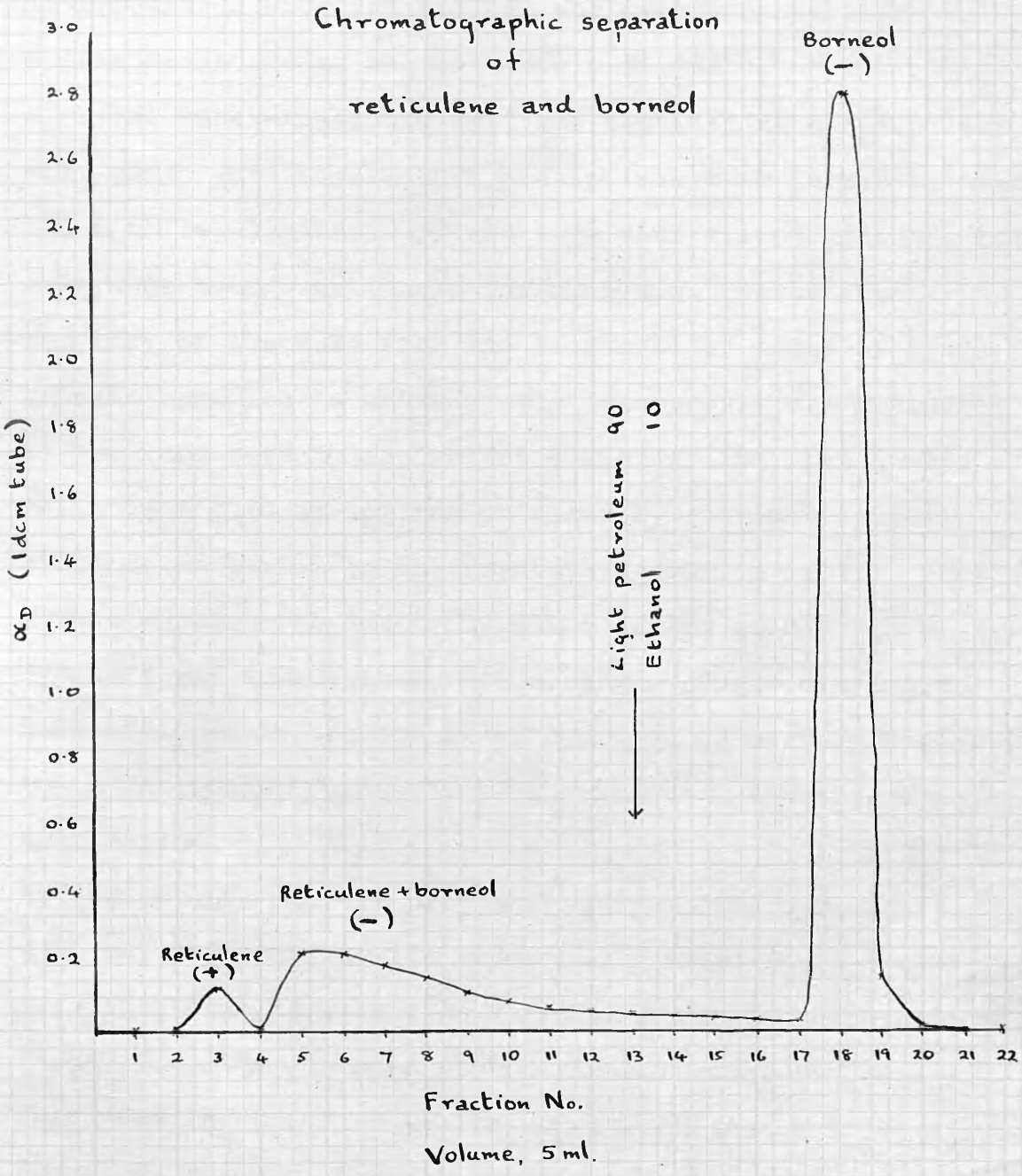
Constants for this fraction were in reasonable agreement with literature figures[43] for (-)-bornyl formate ($d_{4^{\circ}C.}^{20^{\circ}C.}$ 1.006, $n_D^{15^{\circ}C.}$ 1.4708, $[\alpha]_D$ -49°), but saponification with ethanolic potassium hydroxide gave the equivalent weight as 276, equivalent to only about 70% of bornyl formate, assuming the remainder consisted of inert material. The presence of this

material was confirmed by extraction of neutral substances after saponification. These were separated chromatographically (Fig.2) into a small quantity of a colourless oil, $C_{15}H_{24}$, and (-)-borneol which was confirmed by conversion to its p-nitrobenzoate, and by oxidation to camphor, the latter being confirmed by preparation of its 2:4-dinitrophenylhydrazone.

Physical constants for the oil, $C_{15}H_{24}$ ($d^{16°C.}_{16°C.}$ 0.913, $n_D^{16°C.}$ 1.4955, $[\alpha]_D^{15°C.}$ +1.6°, $[R_L]_D$ 65.2) were characteristic of a bicyclic sesquiterpene ($[R_L]_D$ 66.1) with two ethylenic bonds (Simonsen and Barton[44]), and the presence of these two ethylenic bonds was indicated by an iodine value. The constants of this terpene did not agree with those of any known bicyclic sesquiterpene and it was therefore designated reticulene. It will be considered in more detail later in this work.

The aqueous liquors remaining after the extraction of borneol and reticulene exhibited reducing properties typical of formic acid. However, the immediate formation of a buff precipitate on addition of ferric chloride was atypical and acidification of the solution caused the deposition of a second (water-insoluble) acid. The latter was extracted with light petroleum, whilst the light petroleum-insoluble formic acid was isolated from the residual liquors by steam distillation. Neutralisation and concentration of the steam

Fig. 2


Chromatographic separation
of
reticulene and borneol
Borneol
(−)
Light petroleum 90
Ethanol 10
Reticulene + borneol
(−)
Reticulene
(+)
α_D (1dcm tube)
3·0
2·8
2·6
2·4
2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Fraction No.
Volume, 5 ml.

distillate gave crystalline sodium formate, identified by conversion to the corresponding p-bromophenacylester, by a determination of sulphated ash and by its conversion to sodium oxalate when heated rapidly to 360°C.

The water-insoluble acid was obtained as a colourless oil, which gave a pale buff precipitate with ferric chloride and a white gelatinous precipitate with silver nitrate, both compounds being unsuitable for analysis. Attempted recovery of the acid from the iron salt by means of hydrochloric acid led to considerable decomposition. Titration of the acid gave an equivalent weight of 165, but a more reliable figure of 169 was obtained by gravimetric determination of barium in the crystalline barium salt. This figure agrees with the formulation of the acid as $C_{10}H_{16}O_2$. The acid was unsaturated, containing a single ethylenic bond as shown by the uptake of hydrogen on microhydrogenation, and by an iodine value, both experiments indicating one double bond.

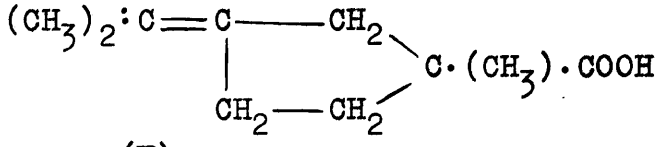
Constants for the oily acid ($d^{15°C.}_{15°C.}$ 1.050; $n_D^{20°C.}$ 1.5019; $[R_L]_D$ 47.29), and for its reduction product ($d^{15°C.}_{15°C.}$ 1.030) suggested that it should be formulated with a monocyclic $[R_L]_D$ 47.25 rather than an open chain structure $[R_L]_D$ 49.45 . This view is supported by the molecular formula $C_{10}H_{16}O_2$. Benzyl isothiourea hydrochloride gave a crystalline salt with the partially neutralised acid, and after recrystallisa-

tion from ethanol, this melted at 136.5°-137°C. This solvent (ethanol) caused very little decomposition of the salt, which melted at a temperature significantly higher than that found when water was used as solvent (124-125°C) (cf. Donleavy[45]). Analysis of the salt agreed with the formulation of the acid as $C_{10}H_{16}O_2$, or $C_{10}H_{14}O_2$. The action of diazomethane on the acid yielded a pleasant smelling (camphoraceous) liquid ester, unfortunately, insufficient in quantity for proper characterisation.

The low intensity ultraviolet absorption maximum at 215 mμ(ε, 2970) which was absent from the spectrum of the saturated acid suggests that this oily acid may well be a mixture of related α,β - and β,γ -unsaturated acids. This view is supported by the fact that on long standing, the oil slowly crystallised to yield an acid, which after chromatography on a mixture of charcoal (1 part) and cellulose (3 parts) melted at 72-73°C. This substance exhibited a high intensity ultraviolet absorption at 206 mμ(ε, 9980) characteristic of an isolated tetrasubstituted double bond[41,42], or an α,β -unsaturated acid. The wavelength of maximum absorption is low for α,β -unsaturation unless the system is unsubstituted as in acrylic acid (cf. Ungnade and Ortega[46]. The crystalline acid is similar to and is possibly identical with the acid isolated by Peacock[13]. This melted about 65°C. and gave a flesh-coloured precipitate

with ferric chloride.

That the acid is not tiglic acid, m.p. 64.5°C. was clearly shown by its formula, and although ß-fencholenic acid (V) melts at 72-73°C., gives an insoluble iron salt and possesses a tetrasubstituted double bond, further degradative work on the acid is necessary before suggesting a structural formula.



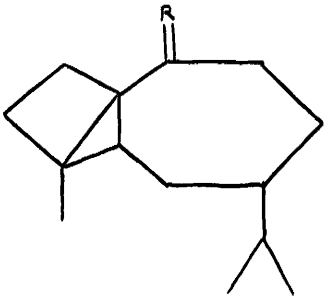
(V)

Fractions D, E, F and reticulene

Saponification of fractions D and E and treatment as above yielded further small quantities of the water-insoluble acid. The neutral fractions, isolated after saponification contained considerable proportions of the sesquiterpene reticulene. Fraction F was almost pure reticulene.

The physical constants of reticulene suggested a bicyclic structure and apparent confirmation was obtained by bromination, which indicated the presence of two ethylenic bonds. However, Naves and Perrotet[47] have observed that bromination of aromadendrene (VI) R = CH_2, and dihydro-aromadendrene caused the absorption of two and one mols. of bromine respectively. The authors suggested that this was due to the opening of the cyclopropane ring, and found that

catalytic hydrogenation of aromadendrene gave the dihydro-



(?)

(VI)

compound without abnormal reactions.

The behaviour of reticulene under the above conditions was identical in every respect with that of aromadendrene, and the possibility that reticulene was also tricyclic seemed worthy of investigation. Comparison of the physical constants of reticulene and dihydroreticulene with those of aromadendrene and dihydroaromadendrene (Tables 1 and 2) showed a marked similarity.

The exaltation of the molecular refraction of dihydroaromadendrene over the calculated value (64.87) was a point in favour of its homogeneous nature as Pfau and Plattner[48] had considered the parent hydrocarbon to be a mixture of bicyclic and tricyclic terpenes. Dihydroreticulene showed a similar optical exaltation and a further point in favour of the homogeneous nature of reticulene is the fact that the terpenes isolated from fractions C, D, E and F, (Table 16 page 71), which are markedly different in composition, possessed almost identical constants.

Table 1.

Author	$[\alpha]_D$	d	n_D	b.p/(m.m.)	$[R_L]_D$	Ref.
Smith	+4.7	0.9222	1.4964	124-125/10	64.89	49
Briggs and	-6.1	0.9116	1.4978	121/10	65.58	50
Short	± 0	0.9157	1.4993	121-121.4/10	65.44	
Naves and Perrotet	+4.86	0.9169	1.4982	114/6	65.37	47
Radcliffe	+0.8	-	1.4990	120-121/10	-	51
and Short	+15.6	0.9197	1.5024	125-127/10	65.58	
Birch and Lahey	+7.54	0.9111	1.4953	122/10		52
Reticulene	+1.6	0.913	1.4955		65.2	

Table 2.

Author	$[\alpha]_D$	d	n_D	b.p./(m.m.)	$[R_L]_D$	Ref.
Naves and Perrotet	-12.14	0.9001	1.4850	104-104.5/4	65.02	47
Briggs and Short	-	0.9014	1.4817	121-122/10	65.83	50
Dihydro-reticulene	+4.0	0.900	1.4826		65.3	

Reticulene gave no crystalline derivatives and ozonolysis of the terpene by methods essentially the same as those used for aromadendrene by Naves and Perrotet[47] and Birch and Lahey[52] gave only an oil of camphoraceous odour. The latter authors similarly obtained an oil which did not readily crystallise until seed crystals became available. They found that the product was a new ketone α-apoaromadendrone (VI, R = O), readily converted by heat and alkali to apoaromadendrone, a stereoisomer which was the product always isolated by the earlier workers. Both products possessed camphoraceous odours.

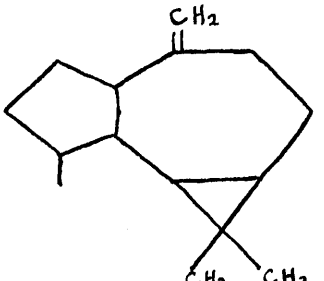
The oil from the ozonolysis of reticulene gave a small quantity of a crystalline substance, m.p. 200-201°C., with semicarbazide acetate but only an amorphous 2:4 dinitrophenylhydrazone. It is of interest that the semicarbazone of apoaromadendrone exists in two forms one of which melts at 201°C.

The only other product isolated from the ozonolysis of reticulene was formaldehyde, identified as its dimedone derivative, which established the presence of a vinylidene group in reticulene.

Dehydrogenation of dihydroreticulene with palladium on charcoal gave no products possessing typical aromatic absorption in the ultraviolet, but heating with selenium for 6 hours gave traces of a blue substance which showed ultra-

violet absorption characteristic of azulenes. Owing to the sensitivity of the method of detection however, this evidence is not conclusive, as the same result could be obtained from the presence of traces of impurities.

Dihydroreticulene gave a pale yellow colour with tetranitromethane but similar colours are also given by saturated substances containing a cyclopropane ring, e.g. cycloartanone. Both reticulene and dihydroreticulene showed low intensity end absorption in the ultraviolet region and it would be of interest to compare that of aromadendrene and dihydroaromadendrene under the same conditions, as cyclopropane rings have long been recognised to possess properties similar to those of an ethylenic bond (Walsh,[53]) particularly when in conjugation with other double bonds (Klotz,[54]) though the effects are not so intense in ultraviolet absorption spectra as those caused by ethylenic bonds themselves. If Birch and Lahey's revised formula (VII, tentative) for aromadendrene is correct, then its ultraviolet absorption spectrum would exhibit only very low intensity absorption as the authors give the figure of ϵ , 95 at 212 mμ for apoaromadendrone which would be expected to show the same absorption characteristics at 210-220 mμ as aromadendrene. However, as the isolation of the small amount of reticulene involved the use of solvents which were not spectroscopically pure, the end absorption



(VII)

observed could perhaps have no real significance.

In the infrared spectrum (Fig.3), reticulene showed the characteristic vinylidene bands at 882 and 1650 $cm.^{-1}$ with an overtone at 1790 $cm.^{-1}$, and a small peak at 1007 $cm.^{-1}$ (1009 $cm.^{-1}$ in dihydroreticulene, Fig.4), could be attributed to a cyclopropane ring (Derfer, Pickett and Boord,[55]) particularly as oxygen-containing substituents are absent (cf. Cole[56]).

The infrared spectra of terpenes and their hydrogenated products have proved of considerable use in the identification of skeletal structures of new terpenes, and a series of papers[57],[58], [59], [60] give a large number of absorption spectra of different types, but which unfortunately do not include that of aromadendrene.

The evidence thus points to the identity of reticulene and aromadendrene but complete confirmation or otherwise must await the arrival of authentic aromadendrene. Attempts to obtain this material have, so far, been unsuccessful.

Infrared absorption spectra

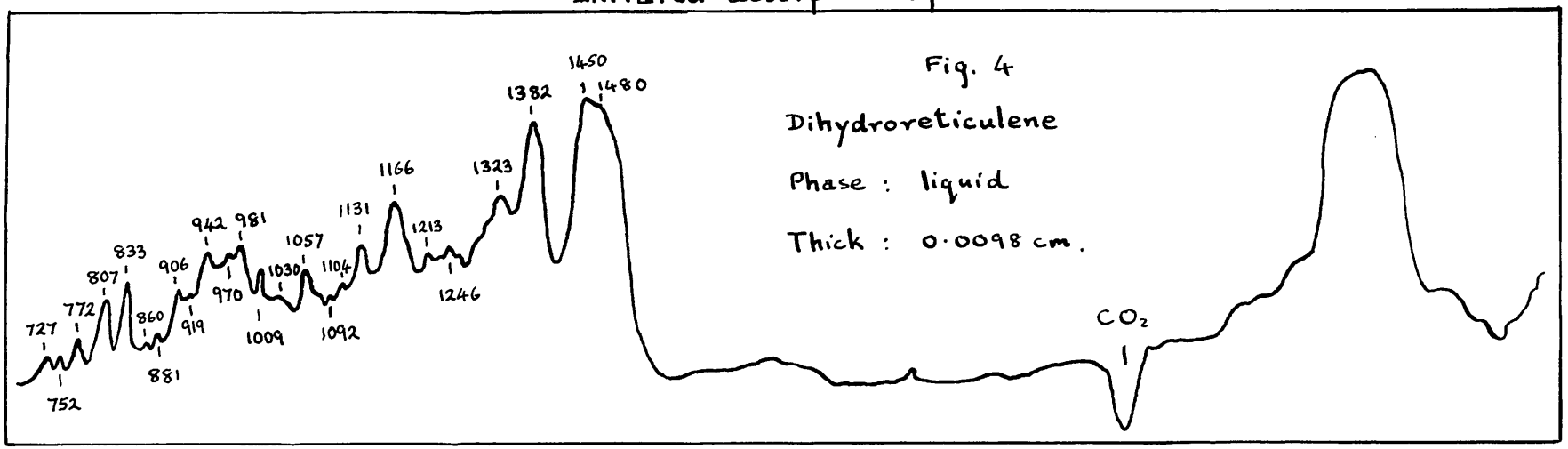


Fig. 4

Dihydroreticulene

Phase : liquid

Thick : 0·0098 cm.

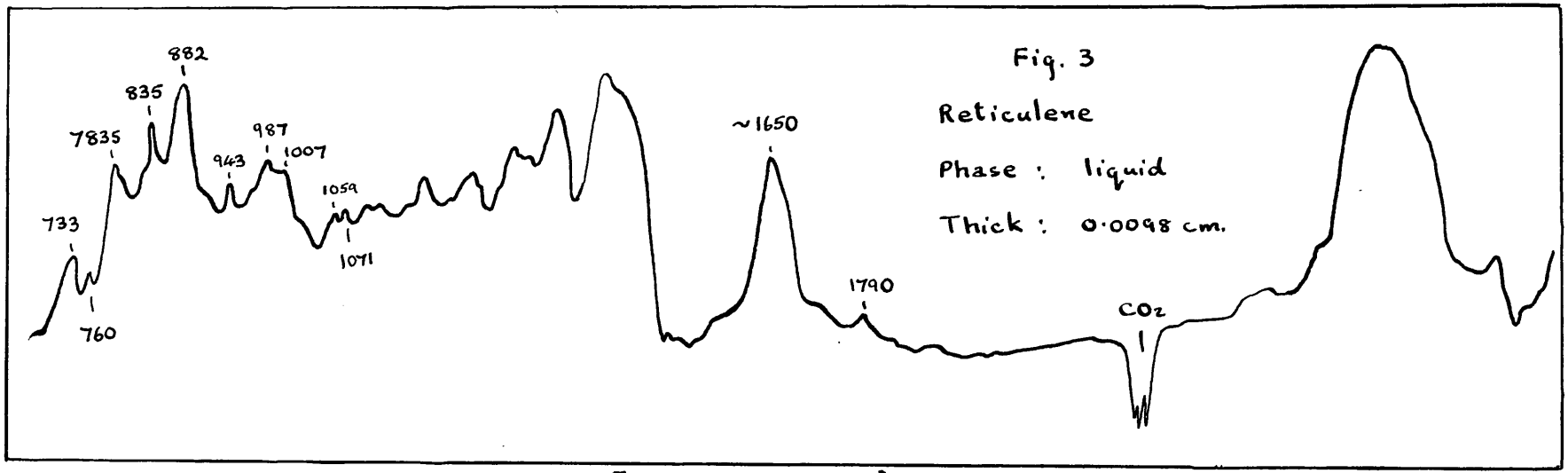


Fig. 3

Reticulene

Phase : liquid

Thick : 0·0098 cm.

Frequency ($cm.^{-1}$)

Examination of the unsaponifiable matter

Chromatography on a column of alumina of the light petroleum soluble unsaponifiable matter fraction 1A, (Table 17, page 84), gave several fractions which are summarised in Table 3.

Fraction A

This was a colourless oil which contained a trace of crystalline matter, and the possibility of it being a terpene (because of insufficient steam distillation) was disposed of by attempted distillation under reduced pressure. It was non-volatile and probably consisted of a mixture of hydrocarbons and possibly alcohols. It was not examined further.

Fraction B

This was clearly a mixture and as the quantity was small it was used to seed the larger quantity of unsaponifiable matter (fraction IB, Table 17). A pale brown soft mass was obtained on cooling, from which gleaming colourless crystals, $C_{26}H_{54}O$, m.p. 78-79°C. were isolated. This tentative identification of ceryl alcohol was confirmed by the preparation of the acetate and by oxidation of the alcohol to cerotic acid, m.p. 82°C. Oxidation of naturally occurring alcohols usually gives acids melting only two or three degrees higher than the melting point of the alcohols used,

and lower than the synthetic acids[61]. This is because the alcohols are usually mixtures and hence the term ceryl alcohol is used deliberately in this work, instead of the chemical name n-hexacosanol.

Table 3

Fraction	Volume of Eluant ml	Eluant	Weight of residue (g).	Remarks
A	1000	Petrol	0.677	Colourless oil, depositing a trace of crystals.
B	1200	Petrol 98 Benzene 2	0.217	Partial crystallisation, the crystals melting at 50-59°C.
C	900	Petrol 70 Benzene 30	0.163	Pale yellow oil which showed a slight tendency to crystallise.
D	1000	Benzene	0.361	Yellow viscous oil.
E	200	Benzene 99 Alcohol 1	0.073	Part of a pale yellow band just preceding the front of the main brown band. Yellow oil depositing a few needle crystals m.p.256-258°C.
F	300	Benzene 99 Alcohol 1	3.10	Dark yellow viscous oil
G	300	"	0.623	Pale yellow oil crystallising on cooling to give 0.14 g. of crystals melting at 132-137°C.
H	300	Alcohol	Trace	-

Fractions C, D and E

These were not examined, the only point of note being that the melting point of the crystals from fraction E recalls that of aristolin, an alcohol, m.p. 265°C.(decomp.) isolated by Hesse[17] from A. argentina.

Fraction F

Further chromatography on alumina gave no separation into bands and this fraction requires a different method of treatment, possibly by benzoylation and chromatography.

Fraction G

The crystals were recrystallised from a mixture of acetone and water to a constant melting point of 136-138°C., and although this was raised to 138-139°C. on admixture with authentic ß-sitosterol such mixed melting points of sterols must be accepted with caution (Fieser and Fieser,[62]). A more reliable guide (in conjunction with derivatives) was given by the optical activity $[\alpha]_D^{15°C.}$, -35°, (cf. Table 4). Identification of ß-sitosterol was completed by the preparation of the acetate, benzoate and by the colour change sequence in the Liebermann-Burchard reaction. As the crystals melted over a range (2 C°.) they were possibly still a mixture of sterols, and γ-sitosterol was the most likely contaminant as any other sitosterol present in appreciable amount would lower the specific rotation considerably (cf. Table 4).

Table 4

Sitosterol	$[\alpha]_D$	m.p.	Reference
α_1	-1.7	166	63
α_2	+3.5	156	63
α_3	+1.65	142	64
β	-36	140	65
γ	-43	148	66

Examination of the fatty acids

The acids were easily separated into two groups, one being soluble in light petroleum and the other (probably resin acids) in ether. The latter group was not examined further.

Fraction III A, (Table 17)

No pure crystalline material was isolated by crystallisation from acetone or by chromatography on a column of alumina, but the latter method indicated the presence of neutral material. The results of the experiment are summarised in Table 18 (page 91).

Fraction III B

As it appeared doubtful that the method adopted for fraction III A would yield pure acids, fraction III B was separated into saturated and unsaturated acids by Twitchell's method as modified by Hilditch[67].

Fractional distillation of the methyl esters of the saturated acids and saponification of certain fractions (Table 5) indicated the presence of C_{16} acids (fraction III B 1(a), III B 1(b), and III B 2) and acids with twenty-two or more carbon atoms (fraction III B 4).

Table 5

Fraction	Bath Temp. °C	Distillation Temp.	Pressure (m.m.Hg.)	Weight (g)	S.E.	I.V.	m.p. °C.
III B1	200-202	164-168	5	1.939	-	-	-
III B2	203-230	169-170	5	1.138	268.0	4	29-30
III B3	231-250	variable	5	0.344	-	-	25-29
III B4	250	190-198	3	0.596	364	6	40-48
III B5	Residue		-	0.220	-	-	
III B1(a)	180-187	148-150	3	1.551	268.4	4	27-28
III B1(b)	188-190	151-155	3	0.230	-	-	

Methyl palmitate was confirmed in fractions III B1(a) and III B2 by the melting point, equivalent weight and hydrolysis to palmitic acid of correct melting point, undepressed on admixture with authentic palmitic acid.

Fraction III B3 was not examined.

Saponification of fraction III B4 gave an acid of m.p. 74-76°C. and equivalent weight 372. It therefore consisted largely of lignoceric acid. The last runnings of fraction III B4 solidified in the condenser and repeated crystallisation from acetone yielded colourless crystals, m.p.61-63°C. It seemed probable that both these crystals and fraction III B5 would give an acid melting at a temperature higher than 74-76°C., otherwise it would be difficult to account

for the methyl ester of m.p. 61-63°C., as this is much higher than that for methyl behenate[68] and methyl lignocerate[69]. Saponification of fraction III B5 gave glistening micro-rosette crystals m.p. 78-79°C. undepressed on admixture with cerotic acid of m.p. 82°C. The melting points and mixed melting points of acids of a molecular weight greater than about 296 i.e. C_{20}, C_{22}, C_{24} etc. acids, are unreliable as a criterion of purity (Hilditch[70]), and it appeared advisable to check that some depression of melting point occurred with a mixture of two different acids. The following results provide a measure of support.

Acid	m.p.	Mixed with	Propor^{n}	Mixed m.p.	Remarks
Unknown*	73-75	n-eicosanoic	50:50	68-71	depressed
n-eicosanoic	75-76	cerotic	80:20	70.5-73	"
stearic	69-70	n-eicosanoic	50:50	63-68	"
stearic	69-70	unknown*	50:50	58-62	"

* Probably lignoceric acid.

Further support for the presence of lignoceric and cerotic acids is given by the isolation of these two acids from A. indica[4], for, as observed by Hilditch[71], plants of the same family tend to have common fatty acids. The terms

lignoceric and cerotic are used to represent the naturally occurring acids which are known to be mixtures, e.g. the melting point 74-76°C. could arise from a mixture of two or more acids. cf. Francis, Piper and Malkin[72], and Krishna Rao and Manjunath[73].

Chromatography of fraction III A had suggested that neutral material was present in the fatty acids, and extraction of the light petroleum solution of the unsaturated acids with sodium hydroxide solution left a neutral oil in the organic layer. The neutral oil yielded needle crystals, $C_{15}H_{20}O_2$, m.p. 134-136.5°C., $[\alpha]_D^{19°C.} = 0$, $\lambda_{max.}$ 209mμ ($\epsilon_{max.}$, 10,360), Legal test positive. The crystals were neutral but could be saponified, and acidification of the saponification liquors precipitated original material m.p. 136°C. The method of isolation of the crystals, and the chemical evidence supported a lactone structure and quantitative bromination of the lactone indicated the presence of two ethylenic bonds. Catalytic hydrogenation of the lactone, at a platinum catalyst in ethanol, was rapid, being complete in 90 minutes with the uptake of the equivalent of 4.15 mols. and the formation of an acid, $C_{15}H_{28}O_2$, m.p. 113.5-116°C.

These results indicated the presence of an α,β-unsaturated lactone containing three ethylenic bonds and hence a bicyclic structure including the lactone ring. Double bonds

which are α,β to carbonyl groups are not brominated under the conditions used so that only two ethylenic bonds would be indicated by this method, and the production of an acid by hydrogenation, with the uptake of four mols. of hydrogen, showed that hydrogenolysis had taken place. An allylic arrangement of one double with respect to the lactone oxygen could thus be postulated, cf. γ-santonin[74], the other possibility (Jacobs and Scott[75]) of a β,γ-unsaturated lactone being ruled out. Further experiments were restricted by lack of material, and as the melting points of the lactone and acid show a range of 2.5 C^{o}. the lactone has not been named as further work may show it to be a mixture of isomers.

The unsaturated acids were recovered from the sodium salts and converted to the methyl esters. From the results of the fractional distillation and analysis of the fractions (Table 6) an anomaly was apparent immediately. Fractions III B ii - III B iv were apparently unsaturated C_{16} acids with two ethylenic bonds (from saponification equivalents and iodine values), but this was extremely unlikely for naturally occurring fatty acids, so that the presence of other acids was indicated. The fractions were optically active and although hydnocarpic acid is also dextrorotatory and a C_{16} acid, it contains only one ethylenic bond. It cannot be assumed therefore for calculation purposes (as it

must be in the ester fractionation process) that only two adjacent homologous unsaturated members are present in each fraction, (Hilditch[76]) so that the usual component acid calculation could not be applied. From the specific rotations of the fractions it appeared that fractions III B viii and III B ix contained much less of the interfering substance, and the saponification equivalents and iodine values suggested the presence of a C_{18} di-ethenoid acid, probably linoleic acid as it is the most common of these acids and because it had already been isolated from A. indica[4]. Linoleic acid was confirmed by the preparation of the crystalline tetrabromo-derivative. The optically active fraction was not investigated further.

Glycerol was confirmed in the residue from fraction V A by the preparation of the crystalline tri-p-nitrobenzoate. From the small quantity of glycerol obtained it was clear that a high proportion of the fatty acids must exist as the sitosteryl and ceryl esters.

This concluded the general examination of the light petroleum-soluble fraction.

Table 6

Fraction	Bath Temp.	Distill^{n} Temp.	Press. m.m. Hg.	Weight (g.)	S.E.	I.V.	$[\alpha]_D^{21°C.}$	(C =
IIIBi	236	140-158	4	0.530	-	-	+50.0	1.002
IIIBii	237	159-164	4	1.924	267.5	186.2	+45.6	1.10
IIIBiii	242-246	165-170	4	2.944	266.7	176	+27.1	1.064
IIIBiv	247-256	171-172	4	2.925	274	164	+15.9	2.24
IIIBv	257-267	173-178 (falling)	4	2.560	279.6	157	+5.5	2.006
IIIBvi	268	Very	4	0.666	-	-	-	-
IIIBvii	280	variable			-	-	-	-
IIIBviii*	250	184	3	2.768	293.6	150.3	+8.3	1.928
IIIBix*	256	184 (falling)	3	1.623	301.3	150.4	+4.6	2.626
Residue	-	-	-	2.9	-	-	-	-

* From semi-micro apparatus.

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-dm. tube. Ultraviolet absorption spectra were determined in absolute ethanol on a Hilger Uvispek photoelectric spectrophotometer; infrared absorption spectra by using a recording double-beam infrared spectrometer built in this College by Dr.I.A.Brownlee (J.Sci.Instr., 1950, 27, 215). The author is indebted to Mr.A.Pajaczkowski and Dr.G.Houghton for these measurements, to Mr.W.McCorkindale and Dr.A.C.Syme for the micro-analyses, and to the staff of the Chemistry Department for the use of apparatus, and for samples of authentic ß-sitosterol and cycloartanone. The light petroleum or petrol used boiled over the range 40-60°C.

ISOLATION OF THE LIGHT PETROLEUM SOLUBLE FRACTION

Material

Three batches of drug were obtained at intervals of several months, so that the possibility of only one commercial drug lot being examined was regarded as unlikely. They all satisfied the pharmacognostical description of *A. reticulata* viz., small brown rhizomes bearing the remains of subaerial stems and numerous wiry roots, the latter forming the major proportion of the drug. One batch (No.3), contained about 4% of *A. serpentaria*, the two types being quite distinct and easily separated.

Preparation of drug

The roots and rhizomes were dried at 60°C. for two days and reduced in a disintegrator to an approximately 60 mesh powder.

Extraction

The powdered drug was packed into a copper percolator, covered with light petroleum and allowed to macerate overnight. It was then percolated at room temperature with light petroleum until the percolate was only pale yellow (three days). Evaporation of the extract on a water-bath yielded a dark green extract of pleasant odour. The yields of oil are recorded in Table 7. The oil-free drug was

dried at room temperature and reserved for future examination.

Table 7

Batch	Weight (lbs.)	Percolate (ml.)	Extract (g.)	Identification
1	6	15,000	106	A
2	28	30,000	700*	B
3	28	20,000	460*	C

* Evaporation of these extracts was not prolonged in order to avoid loss of volatile constituents.

ISOLATION OF ARISTOLACTONE

The oil (4g.) from which free acid and carbonyl compounds had been removed (see pages 58 and 59), was steam-distilled. A crystalline deposit (0.083g.) in the condenser was removed with ether and recrystallised from light petroleum, m.p. 108-110°C. As the main bulk of the oily extract gave no solid matter when kept at -15°C. for two days it was seeded with a crystal. A bulky mass of crystals separated within a few hours. The mixture was kept in a refrigerator (2 days), the crystals filtered off, washed free from adhering oil with ice-cold light petroleum and recrystallised from light petroleum to constant melting point, 110-111°C. The yields are recorded in Table 8.

Table 8

Batch	Crude Crystals (g.)	Pure crystals	Crystal form
1	2.5	2.0	plates*
2	16.0	13.0	needles
3	8.5	6.5	needles

* Recrystallised from acetone-water mixture.

Batch 3 was used for the isolation of aristolactone only and was not examined further.

SEPARATION OF FREE ACIDS

The oil which remained after separation of the lactone was diluted with light petroleum, extracted with a saturated solution of sodium bicarbonate, and the aqueous layer washed once with light petroleum. The acids were recovered by acidifying the aqueous layer, extracting with light petroleum, drying the extract with sodium sulphate and removal of the solvent. They formed a brown viscous oil, (0.26g. and 0.6g. from batches 1 and 2 respectively), which fluoresced blue in ultraviolet light. They were not examined further.

ISOLATION AND EXAMINATION OF CARBONYL COMPOUNDS

EXTRACT A

(i) The oil from this extract was shaken for 4 days with a saturated solution of sodium bisulphite and a small quantity of solid which collected at the interface was filtered off. The solid appeared to be unaffected by hot sodium carbonate solution (10%) and was not examined further.

(ii) Girard's method

The oil which was recovered from the bisulphite extraction was boiled gently under reflux for one hour with a solution of Girard's reagent T (10g.) and glacial acetic acid (10ml.) in ethanol (90ml.). The mixture was then diluted with iced water to reduce the alcohol content to not more than 10% $^{v}/_{v}$. Nine tenths of the acetic acid was neutralised by the addition of N sodium hydroxide solution (158ml.) and non-ketonic material was extracted with solvent ether (3 x 200 ml.). The clear aqueous solution containing ketonic material was made approximately normal with respect to sulphuric acid and the mixture allowed to stand for 1 hour. The now opalescent liquid was then extracted with solvent ether (3 x 200 ml.), the ether layers were bulked, dried with sodium sulphate and magnesium oxide (to remove acetic acid) and evaporated to yield a viscous oil of peculiar odour.

EXTRACT B

This extract was treated as described under Extract A, (ii) using proportionately larger quantities of reagents and solvents. The yields are recorded in Table 9.

Table 9

Extract	Yield (g.)	Appearance
A	3.14	yellow viscous oil $[\alpha]_D^{17°C.}$ +170°
B	21	dark orange oil, which was not examined further.

Attempted distillation under reduced pressure. There was no significant distillate up to a bath temperature of 240°C. and under a pressure of 2 m.m. of mercury. The ketone fraction gradually became darker in colour.

Attempted fractionation by means of light petroleum. The oil (0.2g.) was warmed with light petroleum (20ml.) and the insoluble matter (0.042g.) was filtered off. The latter was obtained as a brownish-yellow almost solid substance $[\alpha]_D^{13°C.}$ +62° (c = 0.428). The filtrate, after evaporation, yielded a pale yellow oil, (0.146g.), $[\alpha]_D^{13°C.}$ +179° (c = 2.07), $\lambda_{max.}$ 250 mμ., $E_{1cm.}^{1\%}$ 650.

Attempted fractionation by means of chromatography. The oil (0.7g.) was heated with light petroleum (50ml.), cooled

and filtered from insoluble matter (0.266g.). The filtrate was evaporated to about 5 ml. and placed on a column of aluminium oxide (45g.). The chromatogram was developed with light petroleum and two pale yellow bands were obtained. These were removed from the column by extruding the latter and separating them mechanically, followed by solution in acetone and evaporation of the solvent. The results are recorded in Table 10.

Table 10.

Band	Weight (g.)	$[\alpha]_D^{19°C.}$	Remarks
1	0.161	+121	Lower band on column
2	0.235	+ 22	

Attempted preparation of derivatives. (i) The light petroleum-soluble oil (0.1g.) was dissolved in ethanol (5ml.) and heated for 5 minutes with a mixture of 2:4-dinitrophenylhydrazine (0.2g.), sulphuric acid (0.5ml.) and ethanol (5ml.). The mixture was cooled and a deep red precipitate filtered off. Recrystallisation from ethanol-water mixture gave red floccules, m.p. 132-164°C.

(ii) The oil (0.15g.) in ethanol (1ml.) was treated with a solution of semicarbazide hydrochloride (0.5g.) and

sodium acetate (1g.) in water (3ml.). Sufficient ethanol was added to give a clear solution when hot. After cooling, a sticky precipitate was filtered off and recrystallised from ethanol-water as granules, m.p. 80-90°C. The granules were optically active (dextrorotatory).

ISOLATION AND EXAMINATION OF THE VOLATILE OIL

EXTRACT A

The oil, free from carbonyl compounds was steam-distilled, the flask being maintained at 120-130°C. by immersion in an oil-bath at 130°C. to keep the volume of liquid at about 250 ml. The distillate was collected in 27 fractions (Table 11), each fraction being extracted with light petroleum. The light petroleum extracts were dried with sodium sulphate, evaporated and the residue weighed. The residue in the steam distillation flask was reserved for saponification.

Crystals were deposited in most of the fractions after No.7, but not immediately, this taking place in some, only after several weeks. Constants were recorded for certain selected fractions in order to determine whether or not fractionation of constituents had taken place.

Table 11.

Fraction	Distillate (ml.)	Residue (g.)	$n_D^{17°C.}$	$[\alpha]_D^{18°C.}$	Appearance
1	25	1.67	1.4702	-10.8	mobile, colourless
2	385	11.74	1.4780	- 1.3	" "
3	365	2.24	1.5075	+43.6	pale yellow
4	345	1.33	1.5140	+60.8	yellow, viscous
5	380	0.92	1.5145	+92.6	" "
6	385	0.56	1.5145	+99.7	" "
7	535	0.59	1.5152	+108.6	" "
8	470	0.49	1.5170	-	" "
9	525	0.66	-	-	oil + crystals
10	525	0.44	-	-	" "
11	485	0.64	1.5192	+123	" "
12	425	0.38	-	-	" "
13	445	0.54	1.5204	-	" "
14	493	0.44	-	-	" "
15	428	0.40	-	-	pale yellow oil
16	450	0.42	-	-	" " "
17	550	0.32	-	-	" " "
18	425	0.27	-	-	" " "
19	405	0.25	-	-	" " "
20	448	0.25	-	-	" " "
21	430	0.29	-	-	" " "
22	363	0.18	-	-	" " "
23	365	0.20	1.5210	+134	" " "
24	440	0.16	-	-	" " "
25	450	0.23	-	-	" " "
26	450	0.12	-	-	" " "
27	470	0.10	-	-	" " "

EXTRACT B

This was transferred to a two litre flask and steam-distilled under the conditions described above. Fraction I (Table 12) came over so readily that it was collected, dried with sodium sulphate and reserved. The other fractions described in the table were obtained by allowing the oil to separate from the aqueous distillate, separating and drying as for fraction I. In this way the use of solvent was avoided in every case except fraction VI which represented the volatile oil extracted by means of ether after the above separation and before rejection of the aqueous liquors.

Table 12.

Fraction	Weight (g.)	Distillate (ml.)	Characteristics
I	200(ml.)	200	Colourless, mobile liquid, mainly light petroleum and ether, but containing traces of oil.
II	19.0	500	Colourless, mobile, mainly light petroleum.
III	22.4	1000	Almost colourless, fragrant oil.
IV	30.9	2000	Pale yellow oil; $d_{15°C.}^{15°C.}$ 0.960 $[\alpha]_D^{16°C.}$ -6.94^0
V	7.0	3000	Yellow oil; $d_{15°C.}^{17°C.}$ 0.962 $[\alpha]_D^{17°C.}$ +33.0
VI	19.0	6000	Yellow viscous oil.

FRACTION I

Fractionation of this liquid proved unsatisfactory as it contained traces of oil boiling over the range 60-240°C. The distillate (25ml.) of boiling range 60-68°C., was optically active (slightly laevorotatory) and was treated with a solution of bromine in ether until the reagent was no longer decolourised. Evaporation of the solvent gave a dark coloured oil (0.1g.) which did not crystallise even on long standing. When chromatographed on alumina, using light petroleum as eluant, the oil yielded three fractions, the first and last being colourless oils which were not identified, and the second a colourless crystalline solid, m.p. 87-88°C. when recrystallised from light petroleum. These crystals were also isolated from fractions II and III (see below) and details of chromatogram and constants are given there.

FRACTIONS II and III

These were combined and submitted to repeated fractional distillation under reduced pressure. The main fractions isolated are given in Table 13.

Table 13.

Fraction	B.p.°C./m.m.Hg.	$n_D^{20°C.}$	$d_{15°C.}^{15°C.}$	$[\alpha]_D^{13°C.}$	Weight(g.)
A	106-140 (atmos.)	1.4770	0.856	-30 (16°C.)	1.0
B	105-110 (bath)/75	1.4774	0.882	-50	1.43
C	114 (bath)/18	1.4765	1.000	-44.4	4.8
D	120-126 (bath)/18	1.4841	0.961	-23.3	3.8
E	130-132 (bath)/18	1.4910	0.937	-8.3	3.1
F	132-136 (bath)/18	1.4971	0.942	0	2.4
Minor and intermediate fractions					8.0
Residue					3.5

Fraction A

The oil (0.355g.) was dissolved in light petroleum (10ml.) and treated with a solution of bromine in light petroleum until the reagent was no longer rapidly decolourised. Evaporation of the solvent gave a colourless oil, which, when chromatographed on alumina (Table 14) gave three fractions. Fraction A 2 when submitted to further chromatography on alumina gave colourless crystals m.p.89°C. (from 70% ethanol), $[\alpha]_D^{17°C.}$ + 98° (c = 0.46).

Found C 40.65%, H 5.5%, Br 54.5%.

$C_{10}H_{16}Br_2$ requires C 40.6 %, H 5.5%, Br 53.9%.

Chromatogram = 10 cm. x 1 cm. Eluant : light petroleum.

Table 14.

Fraction	Eluant(ml.)	Residue	Remarks
A 1	20	0.152	Colourless,odourless oil. Not examined.
A 2	10	0.123	Colourless crystals and a trace of oil. Yield after further chromatography, 0.103 g.
A 3	light petroleum 1 ethanol 1	0.118	Light petroleum yielded only traces of oil so the column was stripped with the mixed solvent to give an oil of camphoraceous odour. Not examined.

Fraction B

The oil (0.93g.) was saponified with 0.5N alcoholic potassium hydroxide (10ml.) for 30 minutes, during which time the colour of the mixture deepened from yellow to reddish-brown. The equivalent weight of the oil was 1228. The non-acid fraction was extracted from the neutralised solution with light petroleum (2 x 30 ml.), the extract washed with brine (2 x 5 ml.), dried (sodium sulphate) and

evaporated to about 5 ml. Chromatography on a column of alumina using light petroleum as eluant gave the following fractions (Table 15)

Table 15.

Fraction	Eluant (ml.)	Residue (g.)	Remarks
B 1	25	0.417	$n_D^{15°C.}$ 1.4742; $d_{15°C.}^{15°C.}$ 0.859 $[\alpha]_D^{17°C}$ -59.0; $E_{1cm.}^{1\%}$ 313 at 210 mμ.
B 2	30	trace	-
B 3	30	"	-
B 4	40 (acetone)	0.097	Partly crystalline

Bromination of fraction B 1

The oil (0.20g.) when brominated and chromatographed as described under fraction A gave the following fractions:-

B 1a, colourless oily liquid (0.0876 g.); not examined:

B 1b, colourless liquid (0.0826 g.) which crystallised on standing:

B 1c, oil (0.0198 g.) of camphoraceous odour; not examined.

Fraction B 1b. Three recrystallisations from 80% ethanol gave crystals, m.p. 87.5-88°C., undepressed on admixture

with the dibromides obtained from fractions I and A. Seeding B 1a with the crystals failed to induce crystallisation.

Quantitative bromination of fraction B 1 (pyridine bromide method) gave bromine absorption equivalent to 1.05 ethylenic bonds.

Attempt at preparation of a nitrosite from fraction B 1.

The oil (0.040g.) was treated with glacial acetic acid (2 drops) and light petroleum (0.4ml.). A saturated solution of sodium nitrite (0.1ml.) was added and the mixture shaken gently. No crystalline precipitate formed in the light petroleum layer. (Absence of phellandrene).

Fraction C

The oil (4g.) was boiled under reflux for 30 minutes with ethanolic potassium hydroxide (0.66N; 50ml.), and neutralised by titration with 0.5N hydrochloric acid. Equivalent weight found, 276, equivalent to about 70% of bornyl formate. Extraction of the solution with light petroleum yielded an oily semi-crystalline mass, the oil from which was chromatographed from light petroleum on alumina. Two main fractions were obtained (fig.2) of which the first, reticulene (0.18g.) was a colourless oil. The second fraction, which was eluted by a mixture of

light petroleum and ethanol, was a colourless crystalline solid which was added to the remainder of the crystals, from which the oil had been pipetted, to give fraction C 1. The mixture, probably of borneol and reticulene, shown in fig.2 was not examined.

Fractions D, E and F

These were treated as described above for fraction C and the results are summarised in Table 16.

Table 16.

Fraction	Weight (g.)	Equiv^{t}. Weight	Reticulene (g.)	Crystals (g.)	Identification
C	4	276	0.18	2.7	C 1
D	3.714	370	0.409	2.25	D 1
E	3.075	715	1.794	0.7	E 1
F	1.897	1455	1.189	trace	-

Reticulene

Analysis of reticulene from fraction D

Found	C, 88.35;	H, 11.58
$(C_5H_8)_n$ requires	C, 88.2 ;	H, 11.8%

The constants of reticulene isolated from each fraction were:-

from fraction C : $[\alpha]_D^{15°C.}$ +1.6 (c,4.47); $d_{16°C.}^{16°C.}$ 0.913; $n_D^{16°C.}$ 1.4955

" " D : $[\alpha]_D^{15°C.}$ +1.5 (c,3.92); $d_{15°C.}^{15°C.}$ 0.912; $n_D^{15°C.}$ 1.4944

" " E : $[\alpha]_D^{15°C.}$ +1.6 (c,4.47); $d_{16°C.}^{16°C.}$ 0.913; $n_D^{16°C.}$ 1.4955

" " F : $[\alpha]_D^{15°C.}$ +1.1 (c,4.2) ; $d_{15°C.}^{15°C.}$ 0.914; $n_D^{14°C.}$ 1.4972

Quantitative bromination of reticulene Reticulene (0.0588g) was treated with pyridine bromide reagent (30ml.) and, after the addition of potassium iodide (2g.) the liberated iodine was titrated with 0.1N sodium thiosulphate. A blank was also carried out. Halogen uptake was equivalent to 2.2 mols.

Dihydroreticulene. The oil (1.08g.) was hydrogenated at a platinum catalyst in ethanol. Hydrogen uptake had almost ceased after 3 hours but the time of contact was extended to 18 hours, by which time the volume of hydrogen absorbed (123 ml. at 15°C.) was equivalent to 1.06 ethylenic bonds.

The ethanolic solution was filtered from catalyst and

the solvent removed by evaporation to give an oily residue which was distilled under reduced pressure to give a colourless oil (0.72g.) b.p. 130-135°C. (bath)/18 m.m., $d_{15°C.}^{15°C.}$ 0.900, $n_D^{17°C.}$ 1.4826, $[\alpha]_D^{17°C.}$ +4.0 (c = 5.21), λ 208mμ, ϵ, 1681.

Found	C, 86.64;	H, 12.66
$C_{15}H_{26}$ requires	C, 87.30;	H, 12.70 %

Found after redistillation over sodium:-

C, 87.55; H, 12.23 %

Dihydroreticulene gave a pale yellow colour with tetranitromethane, but further hydrogenation at a platinum catalyst in ethanol containing a trace of hydrochloric acid, and at a platinum catalyst in glacial acetic acid caused no further absorption of hydrogen, and the product still gave a slight yellow colour with tetranitromethane.

Quantitative bromination of dihydroreticulene. The oil (0.0393g.) absorbed bromine (pyridine bromide method) equivalent to 0.7 ethylenic bond.

Ozonolysis of reticulene. Method 1. The oil (0.388g.) was dissolved in chloroform (10ml.) which had been dried by azeotropic distillation. The mixture was cooled to -20°C. and a stream of ozonised oxygen passed through for

8 minutes. The reaction mixture was allowed to reach room temperature, glacial acetic acid (2ml.) and zinc dust (0.4g.) added, the latter being added in portions, with stirring, over a period of 15 minutes. The mixture was shaken frequently during 30 minutes and then filtered from zinc dust and zinc acetate. The residue was washed with chloroform and the washings added to the filtrate, which was poured into water (40ml.) and shaken thoroughly. The chloroform layer was extracted with further quantities of water (4 x 20ml.) and the aqueous layers bulked and reserved for examination for carbonyl compounds. The chloroform layer was dried (sodium sulphate) and evaporated to give an oil (0.39g.) of camphoraceous odour, $E^{1\%}_{1cm.}$ at 289 mμ, 2.56.

Examination of aqueous extract. This was filtered through a plug of cotton wool and the filtrate neutralised with sodium hydroxide solution (20%), and then made just acid with acetic acid. Dimedone (0.5g.) dissolved in ethanol (20ml.) was added and the mixture stirred thoroughly. After 1 hour, the precipitate which had formed was filtered off, washed with water and dried at 100°C. The product (small needle crystals) melted at 188-189°C. and this was undepressed on admixture with authentic dimedone formaldehyde derivative.

Examination of oily fraction. Attempted preparation of derivatives.

(i) 2:4-Dinitrophenylhydrazone. The oil (50mg.) was dissolved in ethanol (0.5ml) and a solution of 2:4-dinitrophenylhydrazine (0.1g.) in ethanol (5ml) and sulphuric acid (0.3ml) was added. The mixture was heated for 30 minutes, cooled and a flocculent precipitate, m.p. 105-150°C., filtered off. Recrystallisation of the precipitate from ethanol, benzene, carbon tetrachloride and ethyl acetate was unsuccessful, the material being deposited in floccules.

(ii) Semicarbazone. The oil (0.1g.) 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. After 1 hour the mixture was cooled, diluted with water to give an opalescence and set aside for 2 days. Micro-crystals were then filtered off and recrystallised from ethanol (70%), m.p. 200-201°C. Yield 5 mg.

Method 2. Reticulene (0.2g.) was dissolved in glacial acetic acid (5ml.) and ozonised oxygen passed through the solution at 0°C. for 20 minutes. The ozonide was decomposed by the addition of water and allowing the mixture to stand overnight. No crystals were obtained and the oil was recovered by neutralising the mixture with sodium hydroxide

solution (20%) and extracting with ether, as a viscous liquid of camphoraceous odour which failed to crystallise on standing, or after chromatography on a column of alumina.

Dehydrogenation of dihydroreticulene. (i) The oil (0.1g.) was heated with palladium (25%) on charcoal, as described under aristolactone Part II, page 174 . The product showed no ultraviolet absorption typical of aromatic compounds.

(ii) The oil (0.1g.) was heated with selenium (0.1g.) for 6 hours, the mixture being boiled gently. The mixture was diluted with cyclohexane (spectroscopically pure) and extracted with phosphoric acid (S.G. 1.75), the latter being washed once with cyclohexane. The acid was diluted with water and the mixture re-extracted with cyclohexane. An appropriate dilution of the organic layer was examined for ultraviolet absorption, which showed peaks at 244, 248, 294, 341-345 (flat), 357 and 367 mμ .

Water-insoluble acid from fractions C, D and E.

The aqueous layer remaining after the extraction of neutral fractions was acidified with dilute hydrochloric acid and extracted with light petroleum (3 x 25 ml.). The light petroleum layer was washed once with water (10ml.), dried (sodium sulphate) and evaporated to yield a colourless oil, optically inactive, and acid in reaction, $d^{15°C.}_{15°C.}$ 1.050; $n_D^{20°C.}$ 1.5019; $E^{1\%}_{1cm.}$ at 215 mμ, 177. Bromine absorption (pyridine bromide method) was equivalent to 0.9 ethylenic bonds (based upon $C_{10}H_{16}O_2$). Equivalent weight, (titration) 165. The acid (0.113g.) absorbed 18 ml. of hydrogen at 15°C. at a palladium (3%)-charcoal catalyst in ethanol (equivalent to 1.2 ethylenic bonds). The product was an oil $d^{15°C.}_{15°C.}$ 1.030.

The unsaturated acid partially crystallised on standing for several weeks and when chromatographed from light petroleum on a column of charcoal (1 part) and cellulose (3 parts) yielded a colourless crystalline acid, m.p.72-73°C., $\lambda_{max.}$ 206 mμ, ε, 9980 (based upon $C_{10}H_{16}O_2$).

The yields of oily acid from each fraction were:

0.29 g. (C); 0.353 g. (D); 0.272 g. (E).

S-Benzylthiuronium salt. The acid (0.080g.) was dissolved in ethanol (1ml.) and neutralised with 0.5N sodium hydroxide using phenolphthalein as indicator. A saturated aqueous

solution of S-benzylthiuronium chloride was added and the mixture cooled in ice to give a crystalline precipitate which was filtered off and recrystallised from ethanol (90%) to constant m.p. 136.5-137°C., and dried in vacuo over phosphorous pentoxide at 57°C.

Found	C, 64.9;	H, 7.44;	N, 8.46
$C_{18}H_{26}O_2N_2S$ requires	C, 64.7;	H, 7.84;	N, 8.37
$C_{18}H_{24}O_2N_2S$ "	C, 65.0;	H, 7.28;	N, 8.42%

Barium salt. An alcoholic solution of the acid was neutralised with 0.05N barium hydroxide and evaporated to dryness. The residue was recrystallised from ethanol as a micro-crystalline powder. Found (sulphated ash) Ba,49.26; $C_{10}H_{15}O_2Ba_{\frac{1}{2}}$ requires Ba, 49.43.

Ferric salt. This was formed as a buff precipitate on the addition of a solution of ferric chloride to an aqueous solution of the sodium salt. Decomposition of the precipitate with hydrochloric acid caused rapid resinification.

Silver salt. This was formed as a white gelatinous precipitate on the addition of silver nitrate to an aqueous solution of the sodium salt. Attempted recrystallisation from aqueous ethanol was unsuccessful.

Attempted preparation of amide. The acid (0.05g.) was treated with an ethereal solution of diazomethane until the yellow colour persisted. Evaporation of the ether gave an oil of camphoraceous odour. Ammonia solution (S.G. 0.88) was added to the oil and the mixture shaken occasionally during 1 week. No crystallisation occurred.

Water-soluble acid from fractions C and D

The aqueous liquid remaining after the removal of the water-insoluble acid, was steam-distilled and the distillate neutralised with 0.1N sodium hydroxide solution using phenolphthalein as indicator. Evaporation to dryness yielded a residue of sodium salt which possessed the following properties characteristic of formates.

(i) It reduced silver nitrate solution on warming the mixture.

(ii) It gave a red colour with ferric chloride solution and a buff precipitate when heated with the reagent.

(iii) When the salt was heated rapidly to 360°C. (by inserting into a sand bath at that temperature) the residue gave the reactions characteristic of oxalates.

(iv) The crystals (0.050g.) were refluxed for 1 hour with a solution of p-bromophenacyl bromide (0.2g.) in ethanol (2ml.). The mixture was cooled and allowed to stand for 3 days. A flocculent precipitate was filtered off and recrystallised from ethanol as long needles, m.p. 137-139°C., undepressed when mixed with a sample of authentic p-bromophenacyl formate.

(v) Sulphated ash: Found, Na, 32.8 .

Calculated for CHO_2Na: Na, 33.8%.

Alcohol from fractions C, D and E

The laevo-rotatory residues C 1, D 1 and E 1 (Table 16 page 71) were crystallised from light petroleum as colourless plates of (-)-borneol, m.p. 206°C. (with sublimation).

p-Nitrobenzoate The crystals (0.2g.) were dissolved in pyridine (2ml.) and boiled gently with p-nitrobenzoyl chloride (0.3g.) for 30 minutes. The mixture was cooled, diluted with water (10ml.) and the precipitate collected, washed with N sodium hydroxide solution (2 x 5 ml.), water (3 x 2 ml.) and dried over phosphorous pentoxide in vacuo. The product was recrystallised from light petroleum as needles of (-)-bornyl-p-nitrobenzoate (0.057g.), m.p. 134-135°C., $[\alpha]_D^{19°C.}$ -34.7° (c = 1.3 in chloroform). Hückel and Kaluba[77] give m.p. 136°C., $[\alpha]_D$ -34.2° (c = 4 in chloroform).

Found C, 67.9; 67.9; H,7.26;6.81; N,4.78

Calculated for $C_{17}H_{21}O_4N$: C, 67.3; H,7.0 ; N,4.64%

Oxidation to camphor A solution of potassium dichromate (4g.) in sulphuric acid (6g.) and water (9ml.) was added slowly to the crystals (0.4g.), and the mixture was heated on a boiling water-bath under a long air-condenser for 30 minutes. The white crystalline sublimate which collected

in the condenser was dried on filter paper and resublimed to give crystals of camphor m.p. 176°C. (with sublimation).

Camphor 2:4-dinitrophenylhydrazone The crystals (m.p. 176°C.)(0.05g.) were dissolved in ethanol (2ml.) and heated with a solution of 2:4-dinitrophenylhydrazine (0.2g.) in ethanol (10ml.) and sulphuric acid (0.5ml.) for 1 hour. The solution was diluted with dilute sulphuric acid (50ml.) and the precipitate filtered off. Recrystallisation of the product from ethanol gave needle crystals of camphor 2:4-dinitrophenylhydrazone, m.p. 174-175°C.

SAPONIFICATION OF RESIDUES FROM STEAM DISTILLATION

The residue from Extract A (page 63) was boiled gently under reflux for 4 hours with ethanolic potassium hydroxide, using potassium hydroxide (20g.) and ethanol (120ml.) for each 100g. of original extract. The ethanol was then removed by distillation under reduced pressure, the volume of the mixture being kept constant by addition of water. The mixture was cooled and extracted with light petroleum (3 x 300 ml.), the organic layer was dried (sodium sulphate) and the solvent evaporated to give fraction I A. The aqueous layer was re-extracted with ether (3 x 250 ml.), the extract was dried as above, and evaporated to yield fraction II A.

Acidification of the aqueous liquor with hydrochloric acid, followed by extraction with light petroleum (3 x 300ml), drying of the organic layers (sodium sulphate) and evaporation of the solvent gave the fatty acid fraction III A. The aqueous liquid still contained much viscous brown matter which was extracted by ether to give fraction IV A as a dark brown solution in ether. The aqueous acid liquor constituted fraction V A. In extracting the corresponding fractions from the residue from Extract B (page 65) the the volumes of solvent and reagents were increased in proportion to the weight of extract. The fractions are

identified by the letter B e.g. I B etc. and the results are recorded in Table 17.

Table 17.

Fraction	Nature	Appearance	Weight (g.) Extract A	Extract B
I	light petroleum-soluble unsaponifiable matter.	brown semi-solid	5.67	55
II	ether-soluble unsaponifiable matter	"	5.2	42
III	light petroleum-soluble acids	"	6.87	32
IV	ether-soluble acids	not isolated		
V	aqueous liquor	-	-	-

Fractions II A, II B, IV A, and IV B were not examined.

FRACTION I A. UNSAPONIFIABLE MATTER

Chromatography of fraction I A on a column of alumina (12" x 2") from light petroleum and appropriate solvents gave the results recorded in Table 3, (page 45).

Fraction A.

Attempted distillation under reduced pressure gave no volatile material, and this fraction was not examined further.

Fraction B.

The yield of crystals was small and they were used to seed fraction I B, (Table 17), after dissolving the fraction in light petroleum (100ml.) The mixture was cooled for 48 hours in a refrigerator and the pale brown mass which separated was filtered off, washed with ice-cold light petroleum and air-dried. Yield 4.1 g. The substance was dissolved in ethanol, decolourised with charcoal and recrystallised from ethanol (once) and ethyl acetate (3 times) to yield glistening colourless crystals (0.5g.), m.p. 78-79°C., unchanged on further recrystallisation. Very slow cooling of the solutions was found to be essential to obtain crystals of a size suitable for rapid filtration. The crystals were dried over phosphorous pentoxide *in vacuo* for 2 hours at 57°C.

Found		C, 81.51;	H, 14.34.
Calculated for	$C_{24}H_{50}O$	C, 81.4 ;	H, 14.1 .
"	" $C_{26}H_{54}O$	C, 81.6 ;	H, 14.2%.

The crystals gave no colour in the Liebermann-Burchard test, and were neutral in reaction.

Acetyl derivative The crystals (0.1g.) were added to acetic anhydride (3ml.) and the mixture was boiled gently for 1 hour. Ethanol (10ml.) was added, the solution boiled for a further 30 minutes, cooled and diluted with water. The precipitate was extracted with ether (50ml.), and the ether layer washed successively with brine (2 x 5ml), saturated sodium bicarbonate solution (2 x 10ml.), water (2 x 5ml.) and dried with sodium sulphate. Evaporation of the ether and recrystallisation of the residue from ethyl acetate gave small leaf crystals m.p. 61-63°C. (ceryl acetate m.p. 63, 64, 65°C. Beilstein)

Oxidation with chromic acid The crystals were dissolved in glacial acetic acid (1ml.) and chromium trioxide (15mg.) were added. The mixture was heated on a boiling water-bath for 1.5 hours, cooled, diluted with water and made alkaline with sodium hydroxide solution (20%). On shaking the mixture with ether a flocculent precipitate collected at the interface. The precipitate was washed with ether and

then made acid with dilute hydrochloric acid. Extraction of the acid liquid with ether, evaporation of the ether and recrystallisation of the residue from ethanol (70%) yielded micro-rosette crystals of an acid (6mg.) m.p. 82°C. Acidification of the alkaline aqueous liquors and extraction with ether gave a further small yield of acid.

Fractions C, D and E.

These were not examined.

Fraction G.

The crystals (0.14g.) from this fraction were recrystallised from a mixture of acetone and water to give the following fractions:-

G 1 (0.014g.) m.p. 127-132°C.
G 2 (0.069g.) m.p. 136-138°C. $[\alpha]_D^{15°C.}$ -35° (c = 0.99 in chloroform)
G 3 (0.031g.) m.p. 136-143°C.

Fractions G 1 and G 3 were not examined.

Fraction G 2 was recrystallised from a mixture of acetone and water but the melting point remained unchanged. Admixture with authentic ß-sitosterol of m.p. 139-140°C. raised the melting point to 137-139°C. The crystals gave the colour reactions characteristic of sterols viz. Salkowski reaction, orange-red; Liebermann-Burchard, mauve ⟶ blue ⟶ bottle-green. Different text-books give

different methods for carrying out the latter reaction cf. Gilman[78] and Fieser and Fieser[79] and for satisfactory results the following method was adopted. A few crystals (about 1mg.) were dissolved in chloroform (2 drops) and the mixture diluted with acetic anhydride (1ml.). Concentrated sulphuric acid was then added dfopwise until the colour was produced (2 or 3 drops sufficed).

Acetyl derivative. The crystals (5mg.) were heated with acetic anhydride (0.5ml.) for 3 hours on a boiling water-bath, the mixture diluted with ethanol (0.5ml.) and heated for a further 30 minutes under reflux. The solution was diluted with water, cooled, centrifuged and the supernatent liquid rejected. The white residue was recrystallised from ethanol (3 times) in a centrifuge tube to give long flat colourless needles m.p. 130-132°C. (Kofler block). A similar experiment using authentic ß-sitosterol (5mg.) gave crystals, m.p. 130-132°C. (Kofler block). A mixed m.p. was undepressed.

Benzoyl derivative. The crystals (0.025g.) were dissolved in pyridine (0.5ml.) containing benzoyl chloride (1 drop), and heated in a boiling water-bath for 1 hour. The mixture was cooled and diluted with ether, the ether layer washed with 2N hydrochloric acid (2 x 3 ml.),

potassium carbonate solution 10% (2 x 3 ml.) and water (3 x 3 ml.). After drying with sodium sulphate, the ether was evaporated and the slightly coloured residue recrystallised twice from benzene-ethanol mixture. The product melted at 143-145°C., (Kofler block), depressed to 118-120°C. on admixture with starting material.

FRACTION III A. FATTY ACIDS

Fraction III A, (6.87g.) was dissolved in acetone (50ml.) and set aside loosely covered, for several weeks. The semi-crystalline deposit (0.1g.) which formed, was filtered off and recrystallised from ethanol to a constant m.p. 73-75°C. A mixed melting point with n-eicosanoic acid was depressed to 68-71°C.

Preparation of amide of crystalline acid. The acid (5mg.) was heated gently for 10 minutes with thionyl chloride (3 drops). The excess of reagent was removed by evaporation and ammonia solution (S.G. 0.88; 5 drops) was added to the cooled residue. The solid which formed was dissolved by addition of ethanol and warming the mixture. On cooling, micro-crystals, m.p. 102-105°C., were obtained, but insufficient for recrystallisation.

Chromatography of acid mother liquors from fraction III A. The acetone was removed by evaporation and the residue (2.5g.) was chromatographed on a column of alumina 10" x 1½". Table 18 shows the course of the separation.

Fraction III A 3. This fraction slowly crystallised as long needles in a brown oil. Recrystallisation from ether

at -40°C. gave fine colourless needles, m.p. 128-130°C. (cloudy melt), becoming clear at 135°C. $E^{1\%}_{1cm}$ at 210mμ = 453. The crystals and oil were neutral in reaction and gave no colour in the Liebermann-Burchard test. The oil (0.4g.) was saponified with ethanolic potassium hydroxide 0.5N (5ml.) and neutralised 2.4ml. Isolation of the acidic fraction gave an oily product which was not characterised.

The remaining fractions in Table 18 were not examined.

Table 18.

Fraction	Volume of eluant (ml.)	Eluant	Weight of residue (g.)	Remarks
III A 1	500	light petroleum	-	-
III A 2	100	" " + ethanol 1%	0.041	Colourless, neutral oil
III A 3	100	"	0.563	brown viscous oil
III A 4	100	"	0.076	" " "
III A 5	100	"	0.017	" " "
III A 6	100	light petroleum + ethanol 5%	0.044	brown oil which did not solidify at -40°C.
III A 7	100	"	0.050	
III A 8	100	"	0.125	
III A 9	100	"	0.044	
III A 10	100	light petroleum + ethanol 50%	trace	
III A 11	300	light petroleum + glacial acetic acid 1%	1.427	deposited amorphous granules at -40°C.

FRACTION III B. FATTY ACIDS

The acids (32g.) were dissolved in 95% ethanol (150ml.) and the solution heated to boiling. A boiling solution of lead acetate (25g.) and glacial acetic acid (3ml.) in ethanol (150ml.) was added and the mixture allowed to cool overnight. The crystalline precipitate which had formed was filtered off, washed with 95% ethanol and recrystallised from 95% ethanol (200ml.) which contained glacial acetic acid (4ml.). M.p. of lead salt, 95-102°C. The combined filtrate and washings were reserved for the examination of the unsaturated acids.

Decomposition of the lead salts of the saturated acids. The salts were warmed with a mixture of equal parts of hydrochloric acid and water (40ml.) until a layer of fatty acids formed on the surface of the mixture, which was then cooled and transferred to a separator. The fatty acids were extracted with light petroleum (2 x 200 ml.) and the bulked light petroleum layers washed free from lead salts and mineral acid with water. After drying the fatty acids solution with sodium sulphate, the solvent was evaporated to leave a pale yellow solid (4.2g.) m.p. 47-55°C.

Conversion of saturated acids to methyl esters. The acids

were dissolved in methanol (25ml.) which contained sulphuric acid (0.5ml.), and boiled gently under reflux for 2.5 hours. The esters were isolated by diluting the solution with brine and extracting with light petroleum (150ml.) which was then washed successively with brine, water, saturated solution of sodium bicarbonate and water. The light petroleum layer was dried (sodium sulphate) and evaporated to give a pale yellow oil which solidified rapidly when cooled in ice.

Fractional distillation of the methyl esters of saturated acids. This was carried out using a short fractionating column and a 25 ml. flask. Fractions were collected at intervals and the saponification equivalent (S.E.) and iodine value (I.V.) determined on selected fractions. The results are summarised in Table 5 (page 49). In this table fractions III B 1(a) and III B 1(b) were obtained by redistilling fraction III B 1 from a semi-micro apparatus which was also used to distil fraction III B 4. The last runnings of this last fraction solidified in the condenser and were recrystallised from acetone-water to give leaf crystals, m.p. 61-63°C. The weight of fraction III B 4 included the weight of residue obtained from the mother liquors of the crystallisations.

General method for determination of the saponification equivalent. The ester (0.5-1.0g.), accurately weighed, was dissolved in ethanol (5ml.) previously neutralised to phenolphthalein, and boiled gently under reflux with 0.5N ethanolic potassium hydroxide (10ml.) for 1 hour. The mixture was cooled and the excess of alkali titrated with 0.1N hydrochloric acid using phenolphthalein indicator (a ml.). A blank determination was carried out (b ml.). The saponification equivalent was calculated from the formula:-

$$\frac{\text{Weight of ester} \times 10{,}000}{(b - a)}$$

General method for determination of the iodine value. The ester (0.05-0.06g.) accurately weighed, was dissolved in carbon tetrachloride (5ml.) in a glass stoppered flask, and solution of iodine monochloride (British Pharmacopoeia; 20ml.) added. After 30 minutes, potassium iodide (1g.) and water (50ml.) were added, and the liberated iodine titrated with 0.1N sodium thiosulphate (a ml.). A blank determination was carried out (b ml.). The iodine value was calculated from the formula:-

$$\frac{(b - a) \times 0.01269 \times 100}{\text{Weight of ester}}$$

Fraction III B 1(a). Identification of palmitic acid.

Isolation of the acid from fraction III B 1(a) after its saponification gave a crystalline solid readily divided into two fractions by means of acetone. The acetone-insoluble fraction, a white crystalline acid (0.12g.) m.p. 104-150°C., was not examined further.

The acetone-soluble acid was deposited as small colourless crystals, m.p. 61-62.5°C., on the careful addition of water to the acetone solution. The melting point was undepressed on mixing with authentic palmitic acid of m.p. 60-62.5°C.

Fraction III B 2. Palmitic acid was identified as described above.

Fraction III B 3. The melting point suggested it was a mixture and it was not examined further.

Fraction III B 4. The acid isolated from the saponified ester melted at 74-76°C. after recrystallisation from acetone. Equivalent weight found = 372.

Fraction III B 5. This was dissolved in hot ethanol (10ml.) and activated charcoal (0.5g.) added. The mixture was filtered whilst hot and the filtrate boiled with 0.5N

ethanolic potassium hydroxide (10ml.) for 30 minutes. The alcohol was removed by distillation and the residue diluted with water (40ml.). The slightly opalescent solution was extracted with ether (50ml.) to remove any unchanged ester. The acid isolated from the aqueous layer by acidification and extraction with ether was recrystallised from acetone as glistening micro-rosette crystals, m.p. 78-79°C. undepressed on admixture with cerotic acid, m.p. 82°C. Yield, 15mg.

Unsaturated acids. The reserved filtrates from the separation of the lead salts of the saturated acids were distilled under reduced pressure to remove ethanol, and the residue transferred to a separator with the aid of light petroleum (300ml.). The light petroleum layer was washed with water and then with dilute hydrochloric acid (to decompose any lead salts still present), and the aqueous and acid washings rejected.

Extraction of neutral material. The light petroleum layer was extracted with an excess of sodium hydroxide solution, separation into two phases being assisted by addition of sodium chloride. The light petroleum layer was washed with brine and then with water, the washings being added to the alkaline extract, dried (sodium sulphate) and

evaporated to yield a clear brownish-red oil (3.1g.) which deposited needle crystals (0.3g.) m.p. 118-125°C. Repeated crystallisation from a mixture of equal parts of acetone and water gave needle crystals (0.05g.) m.p. 134-136.5°C., $[\alpha]_D^{19°C.}$ 0 (c = 2.11), $E^{1\%}_{1cm.}$ at 209 mμ, 447.

Found	C, 77.59;	H, 8.93.
$C_{15}H_{20}O_2$ requires:	C, 77.6;	H, 8.68%

The crystals were neutral, but saponification of 0.022g. gave the equivalent weight as 253 ($C_{15}H_{20}O_2$ requires equivalent weight 232), and acidification of the saponification liquors precipitated original material, m.p. 136°C. Bromine absorption (pyridine bromide method) was equivalent to 2.04 ethylenic bonds.
The crystals gave a positive Legal test.

Hydrogenation of crystals. The substance, m.p. 125-130°C., (0.211g.) was hydrogenated at a platinum catalyst (50mg.) in ethanol (7ml.) Uptake of hydrogen was complete in 90 minutes at 24°C. with the absorption of 92 ml. of hydrogen (after deduction of the volume absorbed by the catalyst). Equivalent uptake: 4.15 mols. Filtration of the reaction mixture and evaporation of the filtrate yielded a colourless oil which solidified on cooling. Repeated crystallisation from light petroleum yielded

colourless needles of an acid, m.p. 113.5-116°C.

Found	C, 75.69;	H, 12.05.
Calculated for $C_{15}H_{28}O_2$	C, 75.02;	H, 11.75%

Isolation of unsaturated acids. The alkaline aqueous liquor remaining after the extraction of neutral material was acidified with dilute hydrochloric acid and the acids recovered by solvent extraction (light petroleum) and evaporation.

Conversion to methyl esters. This was carried out as described under the saturated acids but using methanol (100ml.) which contained sulphuric acid (2ml.).

Fractional distillation of the methyl esters. The flask (250ml.) was used with a vacuum-jacketed Widmer column, and distillation of the esters was continued as far as possible with this apparatus. The distillation was completed in a semi-micro apparatus with a short fractionating column. The results of the distillation and examination of the fractions are recorded in Table 6 (page 54).

Identification of linoleic acid. The acid was extracted from the saponified ester of fraction III B viii in the

normal manner and was obtained as a brown oil. This was dissolved in light petroleum and treated with a solution of bromine in light petroleum until bromine was in excess. Some flocculent red matter was filtered off and the filtrate was cooled in a refrigerator for 1 hour. The almost colourless solid which separated was collected, washed with light petroleum and recrystallised from a mixture of ether (1 part) and light petroleum (5 parts) as small colourless needles, m.p. 115-116°C., after sintering at 113°C. The crystals contained bromine and the melting point was undepressed when they were mixed with authentic tetrabromostearic acid prepared from linoleic acid.

FRACTION V A

This fraction was neutralised with dilute sulphuric acid using phenolphthalein as indicator, and evaporated on a boiling water-bath, filtering off a small quantity of brown flocculent matter when the volume had been reduced. Evaporation to dryness was completed and the residue mixed with sodium sulphate (30g.). The mixture was extracted continuously with acetone for 4 hours, the acetone extract decanted from a small amount of insoluble salt, and evaporated to dryness. The residue was dissolved in water, decolourised with activated charcoal, the solution filtered and the filtrate evaporated to dryness. The residue was dissolved in ethanol, traces of salt removed by filtration, the filtrate evaporated, and the residue dried at 100°C. for 15 hours. The residue (0.22g.) was transparent, semi-solid and tasted of glycerol.

Preparation of glyceryl tri-p-nitrobenzoate. The above residue (0.11g.) was dissolved in pyridine (2ml.) and p-nitrobenzoyl chloride (0.4g.) dissolved in pyridine (5ml.) added. The mixture was heated on a boiling water bath for 30 minutes, cooled and diluted with water (15ml.). An excess of sodium hydroxide solution was added and the

mixture set aside with occasional shaking. The oily precipitate solidified on standing overnight and recrystallisation from acetone containing a trace of water yielded small needle crystals, m.p. 193-195°C., undepressed on admixture with authentic glyceryl tri-p-nitrobenzoate m.p. 194-196°C. Nef,[80] and Jaquemain and Muskovitz[81] give m.p. 192°C.

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§§§§§§§§§§§§§§§§§§§§

PART II

ARISTOLACTONE

THEORETICAL

PRELIMINARY EXAMINATION OF ARISTOLACTONE

Elementary analysis and molecular weight determination of aristolactone, isolated as described in Part I, gave the molecular formula as $C_{15}H_{20}O_2$. Aristolactone exhibited a light-absorption maximum at 211 mμ (ϵ, 11,500) which was attributed to an α,β -unsaturated ester or lactone function. Hydrolysis gave an acidic fraction in quantitative yield and failure to isolate a separate alcohol fragment was taken to indicate a lactone rather than an ester structure. A small portion only of the acid crystallised and this gave a crystalline but unstable S-benzylthiuronium salt. The bulk of the acid was unstable, changing rapidly into a neutral oil which could not be characterised.

Determination of the iodine value of aristolactone under varying conditions, gave fractional values in all cases, the uptake being the equivalent of between 2.3 and 2.7 ethylenic bonds, according to the reaction times. The non-reactive nature of double bonds α,β to groups such as carbonyl or carboxyl has long been recognised, and a check on readily available materials viz., santonin, ethyl acrylate and methyl methacrylate confirmed that under the experimental conditions used, little or no absorption of halogen took place. The fractional value is attributed

to some substitution or to slow addition to the α,β - double bond. Two double bonds are thus definite, with possibly a third.

Hydrogenation of Aristolactone.

The presence of a third double bond was confirmed by micro-hydrogenation. On a larger scale considerable variation was observed in the rate of hydrogen uptake with the catalysts and solvents used. With palladium on charcoal, and ethanol as solvent, only one mol. was absorbed, the product being dihydroaristolactone, $C_{15}H_{22}O_2$, m.p. 79-80°C., $[\alpha]_D^{17°C.}$ -77°. Using platinum oxide in ethanol, two mols. of hydrogen were rapidly absorbed but isolation of the product at this stage gave only an oil which was not fully characterised. When allowed to continue, the hydrogenation was completed by the absorption of a further mol., the rate being determined by the efficiency of the catalyst - for example, different batches of platinum oxide were noticeably different, one requiring the addition of a trace of hydrochloric acid to complete the hydrogenation. The product was the crystalline hexahydroaristolactone, $C_{15}H_{26}O_2$, m.p. 103.5-104°C., $[\alpha]_D^{17°C.}$ +3°, which was obtained in two different forms from subsequent hydrogenations - needles m.p. 99-100°C., and plates m.p. 100-102°C., both giving identical infrared absorption spectra, analysis,

optical rotation and hydroxy-acid, though a mixed m.p. was 101-104°C. From the marked changes in optical activity upon hydrogenation at least one of the double bonds must be associated with a centre of optical activity (Chanley and Polgar[1]). Hexahydroaristolactone, unlike aristolactone, was stable to cold alkali, but was readily saponified on heating with alkali (equivalent weight 239, theoretical 238). The resulting hydroxy-acid, $C_{15}H_{28}O_3$ m.p. 86-87°C., $[\alpha]_D^{16°C.}$ +16°, obtained from <u>both forms</u> of hexahydroaristolactone was readily converted to the parent lactone when heated just above its melting point in a sealed tube. Trace amounts of a second acid, m.p. 121-122°C., were also obtained from the hydrolysis products of hexahydroaristolactone. This acid failed to relactonise when heated, but instead, underwent a slow resinification to give a dark coloured product of indefinite melting point. The acid was completely saturated to tetranitromethane and this excluded the possibility of it arising from traces of unreduced aristolactone.

<u>Infrared absorption spectra of aristolactone and hexahydroaristolactone</u>.

These confirmed the presence of a lactone ring in both substances. In carbon tetrachloride, aristolactone showed a strong band at 1770 cm.$^{-1}$ (1736 cm.$^{-1}$ in paraffin mull) characteristic of an α,β-unsaturated γ-lactone, with the expected shift to 1780 cm.$^{-1}$ (1750 cm.$^{-1}$ in paraffin mull)

in hexahydroaristolactone. The difference in position of the band when the substance is examined in solution, compared with its position when examined in the form of a mull with paraffin, is attributed to solvent effect.

Marked changes in the properties of the lactone ring following hydrogenation, are also reflected in the ester-group absorptions of the two lactones in the region 1000-1250 $cm.^{-1}$, the two bands at 1034 and 1064 $cm.^{-1}$ of aristolactone being replaced by a single band at 1167 $cm.^{-1}$ in hexahydroaristolactone. A strong band at 890 $cm.^{-1}$ and a medium one at 1650 $cm.^{-1}$ which are present in aristolactone (in carbon disulphide), and absent from the spectrum of hexahydroaristolactone are attributed to a vinylidene group, which was confirmed by the identification of formaldehyde as one of the products of ozonolysis. Medium bands at 782 and 840 $cm.^{-1}$, and a weak band at 800 $cm.^{-1}$ in the spectrum of aristolactone, bands which are absent from that of the saturated compound, suggest that of the two remaining double bonds, one at least is tetrasubstituted.

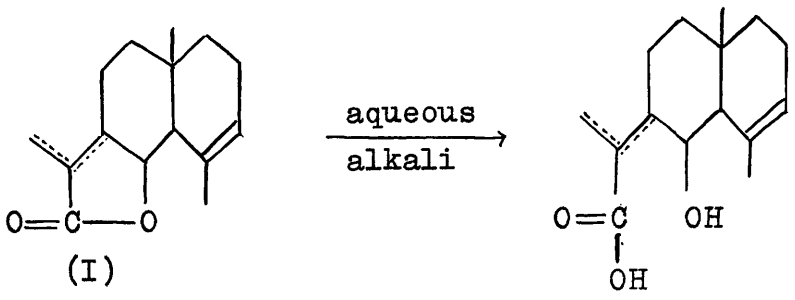
As a model compound for comparison purposes tetrahydroalantolactone was obtained by hydrogenation of helenin, a mixture of lactones, consisting of alantolactone, isoalantolactone and dihydroisoalantolactone[2,3], all of which give the same tetrahydrolactone $C_{15}H_{24}O_2$ on hydrogenation. Comparison of its infrared absorption

spectrum with that of hexahydroaristolactone (needles) (both substances as a paraffin mull) showed a marked similarity (appendix 2), particularly with regard to the bands at 960 and 1013 cm.$^{-1}$ compared with 951 and 1008 cm.$^{-1}$ in hexahydroaristolactone, attributed to the presence of a cyclohexane ring. (Marrison,[4]). Differences do exist to show that the two compounds are not identical, notably in the doublet nature of some of the peaks in the tetrahydroalantolactone absorption spectrum, for example at 704 and 726 cm.$^{-1}$, 851 and 858 cm.$^{-1}$, and 984 and 991 cm.$^{-1}$ regions. The bands at 885, 925 and 1675 cm.$^{-1}$ are probably caused by the presence of traces of unsaturated compounds in the tetrahydroalantolactone used.

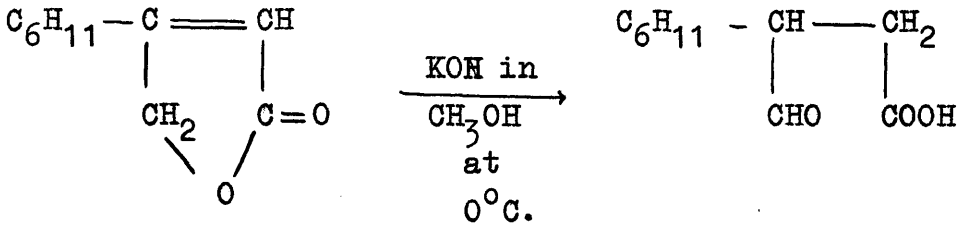
THE ACTION OF ALKALI UPON ARISTOLACTONE

The action of alkali upon unsaturated lactones

The actual products isolated from alkaline hydrolysis of unsaturated lactones depend to a large extent upon the conditions used and the position of the double bond or bonds in the molecule. Attention is here confined to a few examples of α,β -unsaturated lactones and the simplest case is the formation of the corresponding hydroxy-acid by the action of aqueous alkalion for example alantolactone (I)

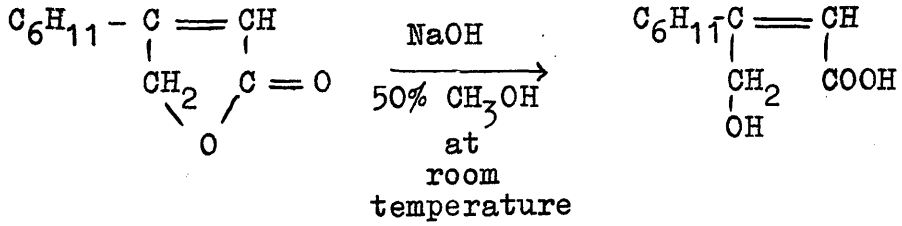


With the cardiac glycosides, two modes of action of alkali were observed[5] and demonstrated by means of the model compound ß-cyclohexyl- $\Delta^{\alpha,\beta}$ -butenolide (II) (among others). With potassium hydroxide in absolute methanol at 0°C., ß-cyclohexyl-ß-formylpropionic acid (III) was isolated from the reaction mixture in quantitative yield.



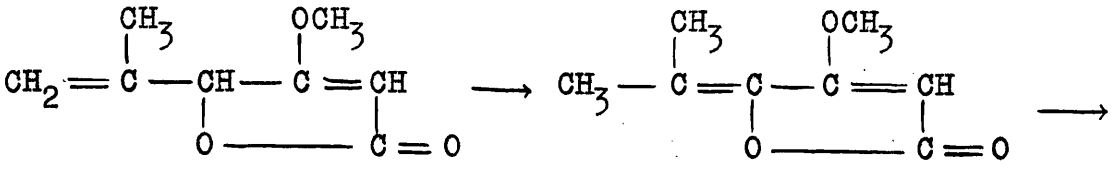
(II) (III)

With sodium hydroxide in 50% methanol at room temperature the sodium salt of the hydroxy-acid (IV) was obtained together with traces of the aldehydo-acid, the proportion of the latter increasing with temperature until at the boiling point the ratio was 2:1.

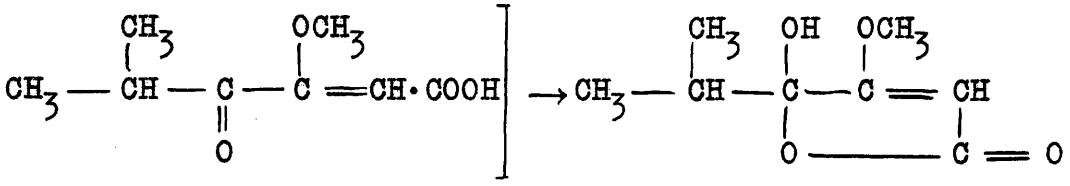


(IV)

Raphael[6] in his synthesis of dihydropenicillic acid (VII) obtained the following change on treating the lactone af 3-hydroxy,2-methoxy-4-methyl-hexa-2:5-dienoic acid (V) with a mixture of N sodium hydroxide solution and methanol.

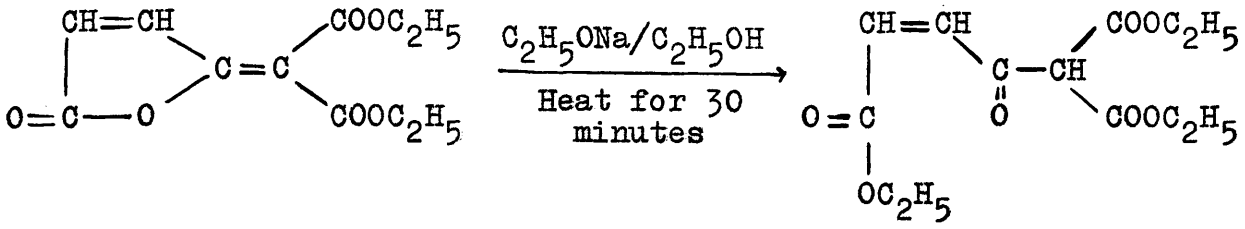


(V) (VI)



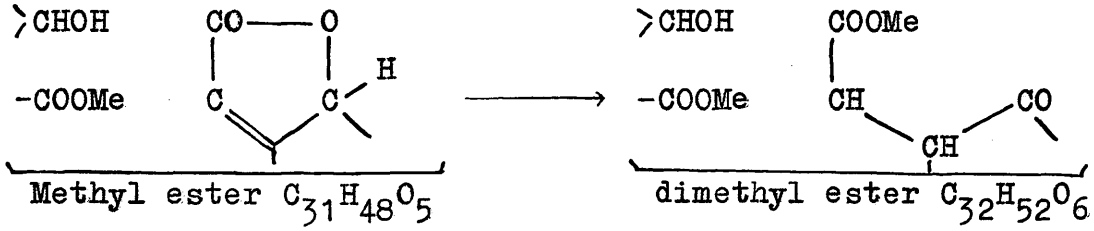
(VII)

The mechanism suggested was a prototropic change followed by fission of the lactone ring. Using weaker alkali, viz., ammonia, (VI) was actually isolated. Eisner, Elvidge and Linstead[7] have recorded that the action of ethanolic sodium ethoxide on (VIII) gives the keto-ester, ethyl cis-ß-carbethoxyacrylylmalonate (IX) whereas with aqueous alkali, fission to maleic acid and ethyl malonate occurs.



(VIII) (IX)

Finally McKean and Spring[8] have noted the formation of a keto-ester (X) by the alkaline rearrangement of the α,β-unsaturated lactone (XI).



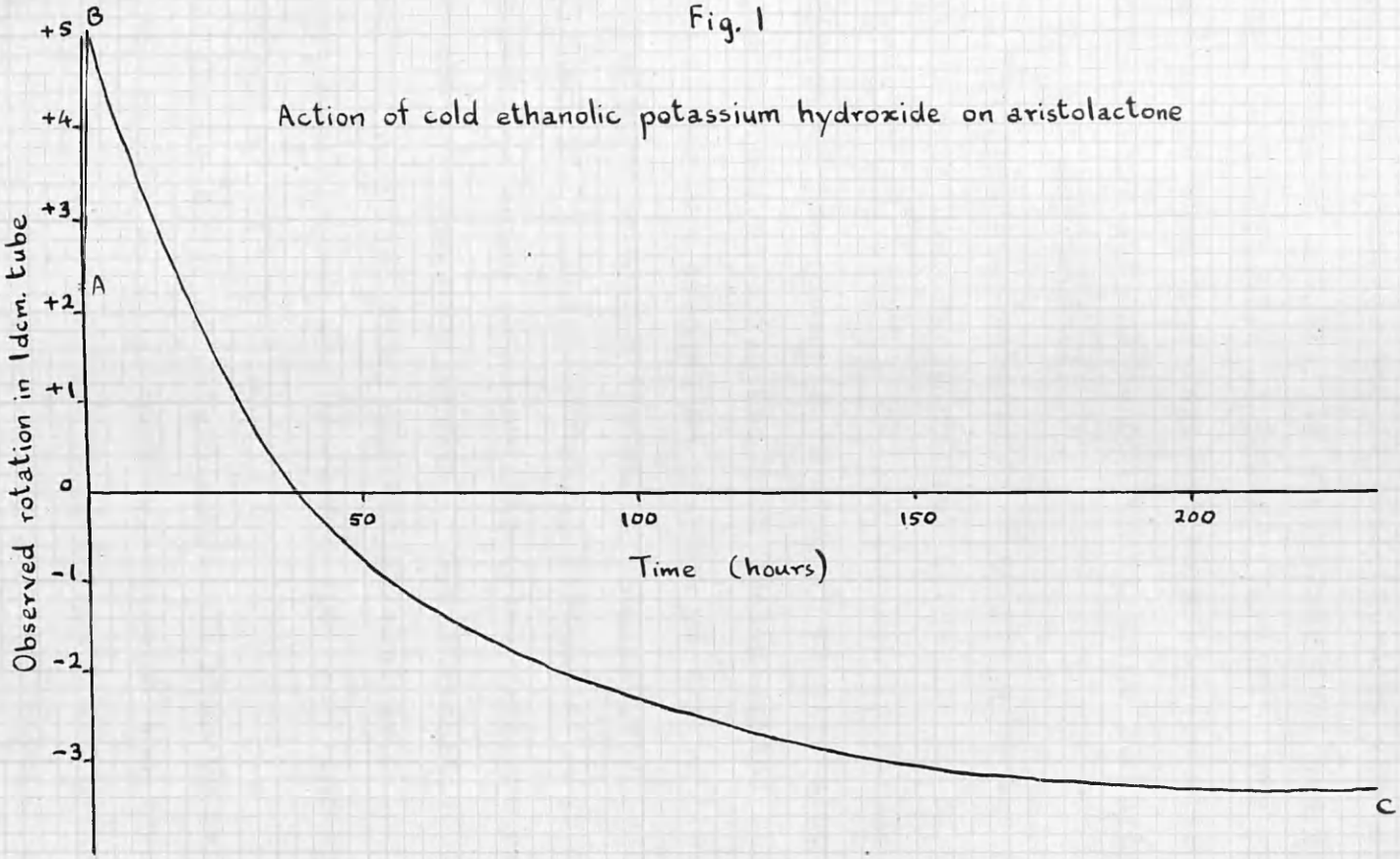
(XI) (X)

Alkaline rearrangement of aristolactone

The optical changes caused by the hydrolysis of aristolactone indicated structural alterations more complex than the opening of the lactone ring. (Fig.1) Treatment with cold ethanolic potassium hydroxide caused an initial positive shift up to the maximum at B, the shift being relatively rapid and complete in 50 minutes. The succeeding fall in rotation from B to C was very much slower, reaching a constant negative value after about 10 days. Titration of

Fig. 1

Action of cold ethanolic potassium hydroxide on aristolactone

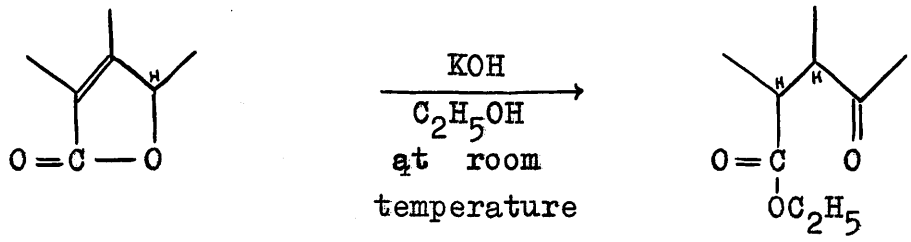


a series of aliquot portions withdrawn during the reaction showed that, whereas the initial increase did not involve utilisation of alkali, the succeeding fall was accompanied by the neutralisation of one equivalent. The first reaction is therefore regarded as a base-catalysed rearrangement and the second reaction as the hydrolysis of the primary product.

The product, isolated in high yield at the point of maximum rotation, was a neutral substance $C_{17}H_{26}O_3$ m.p. 56-57°C., $[\alpha]_D^{19°C.}$ +317°. It was apparently formed by the addition of the elements of ethanol to aristolactone, but repeated recrystallisation from acetone-water showed that ethanol was not present as solvent of crystallisation. The infrared spectrum (paraffin mull) showed peaks at 1726 and 1186 $cm.^{-1}$ (ester carbonyl); peaks at higher frequencies which could be associated with the original lactone function were absent. The presence of an isolated carbonyl group was established by a low intensity absorption maximum at 291 mμ (ε, 250) and confirmed by a shoulder at 1704 $cm.^{-1}$

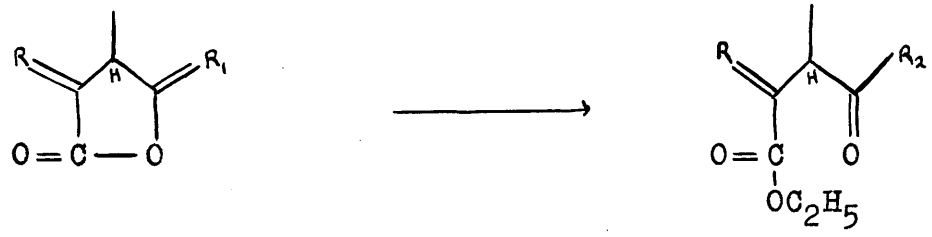
The reaction of aristolactone is seen to be analogous to the formation of a carbonyl-ester of the type described by Paist, Blout, Uhle and Elderfield[5] and by McKean and Spring[8]. This is in accord with the partial structures (XII) and (XIII) for aristolactone and the rearranged

product, termed ethyl oxoaristate, respectively.

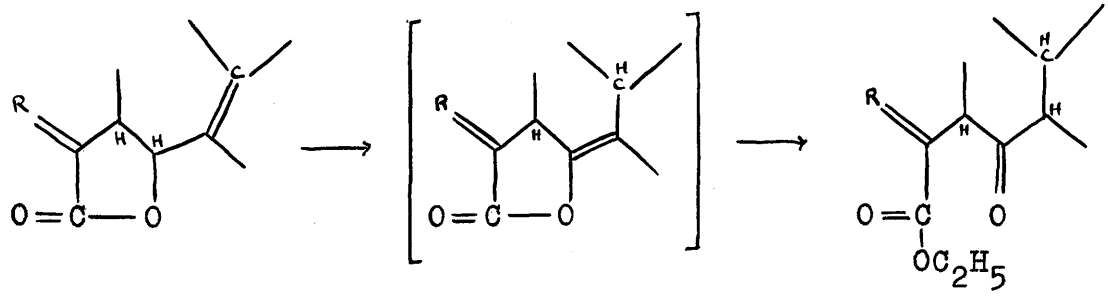


(XII) (XIII)

The following partial structures and reactions could also be postulated; (XIV) to (XV) and (XVI) to (XVII)



(XIV) (XV)



(XVI) (XVII)

The loss of an ethylenic bond in the formation of ethyl

oxoaristate was confirmed by micro-hydrogenation and by determination of iodine value which showed the equivalent of 1.95 and 2.5 ethylenic bonds respectively. The high value of the latter determination suggested that the fractional values obtained in the halogenation of aristolactone could be due to substitution rather than to slow addition to the α, β -double bond.

The high iodine value also eliminated the partial structures (XIV) and (XVI), because if the rearranged product still contained an α, β -double bond the result would be an apparent total absorption of halogen equivalent to only 1-1.5 double bonds for the ester. It thus confirmed that it is the α, β -double bond which takes part in the conversion to the keto-ester. Like aristolactone, ethyl oxoaristate also showed different rates of hydrogenation for its double bonds, one being readily hydrogenated to give ethyl dihydro-oxoaristate $C_{17}H_{28}O_3$, m.p. 65-66°C., $[\alpha]_D^{17°C.}$ +131°, which was still unsaturated to tetranitromethane. Complete hydrogenation (2 mols.) gave a saturated ester as an oil of equivalent weight 281 (theoretical 282) which on hydrolysis yielded an acid, also as an oil. The oil showed an absorption maximum at 287 mμ (ϵ, 51) consistent with the carbonyl group beigg resistant to hydrogenation under the conditions used.

Ethyl oxoaristate was first obtained by the catalysed

reaction of ethanolic ammonia on the lactone. Ammonia is known to form adducts with certain α,β -unsaturated lactones (Hansen[2]; Ruzicka and Pieth[3]) and although aristolactone failed to form such an adduct, a catalysed reaction with ethanolic ammonia in the presence of zinc dust gave ethyl oxoaristate. The reaction which was followed polarimetrically, was complete in about 72 hours, the rotation then having reached a maximum. If permitted, this reaction also continued with the same slow fall in rotation observed when ethanolic potassium hydroxide had been used.

Methyl oxoaristate $C_{16}H_{24}O_3$, m.p. 68-69°C., $[\alpha]_D^{14°C.}$ +342°, was similarly obtained by the action of methanolic potassium hydroxide on aristolactone. Recrystallisation of the product from ethanol showed that the elements of methyl alcohol were not present as solvent of crystallisation. Hydrolysis of methyl oxoaristate with hot ethanolic potassium hydroxide confirmed that it was an ester of equivalent weight 266 (theoretical 264) in agreement with the above formula, and methyl alcohol was identified as one of the products of hydrolysis, the other being an oily acid which was not characterised.

The infrared absorption spectrum (appendix 2) of methyl oxoaristate in carbon disulphide showed peaks at 1735 and 1150-1200 $cm.^{-1}$ (broad band : carboxylic ester) and

at 1720 cm.$^{-1}$ (ketone). Peaks at 890 and 1650 cm.$^{-1}$ (vinylidene) were identical in position and intensity with those of the parent lactone, indicating that the vinylidene group was apparently unaffected by the alkaline rearrangement. Peaks at 782, 800 and 840 cm.$^{-1}$ observed in the spectrum of aristolactone were also present in that of methyl oxoaristate, though much reduced in intensity. This demonstrates the tetrasubstitution of the second double bond of methyl oxoaristate, and suggests that the third double bond, which is eliminated in the alkaline rearrangement of aristolactone, may possibly also be tetrasubstituted. On the other hand, a distinctive peak at 813 cm.$^{-1}$, of medium intensity is usually attributed to a trisubstituted double bond. This peak is absent from the spectrum of aristolactone and the possibility arises of a double bond shift in addition to that involved in the formation of the keto-ester. The conclusions concerning the tetrasubstituted double bond are supported by the ultraviolet absorption spectra, which showed a small shift in the maximum and a reduction in its intensity from 211 mμ (ϵ, 11,500) in aristolactone to 209 mμ (ϵ, 6360) in methyl oxoaristate, and 209 mμ (ϵ, 7000) in ethyl oxoaristate. The position and intensity of the maxima for ethyl and methyl oxoaristate are characteristic of a tetrasubstituted ethylenic bond[9].

Further evidence for the rearrangement postulated comes from the observation that the bands in the spectrum of hexahydroaristolactone at 953 and 1010 cm.$^{-1}$ attributed by Marrison[4] to a cyclohexane ring, were replaced in the spectrum of methyl oxoaristate by one at 1100 cm.$^{-1}$ (cyclohexanone derivatives, cf. Lecompte[10]). These bands also provide evidence for the presence of a second ring in aristolactone, and support the thesis (based upon its formulation as $C_{15}H_{20}O_2$ with three double bonds) that the parent lactone is bicyclic, so that the second ring can be tentatively assigned as six-membered.

ACID ISOMERISATION OF ARISTOLACTONE

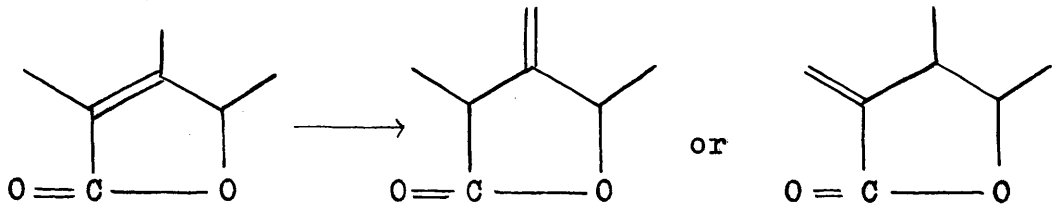
Aristolactone was isomerised by boiling glacial acetic acid to a new lactone, isoaristolactone, m.p. 90-91°C., $[\alpha]_D^{17°C.}$ -44°. Much resinification occurred and some material was recovered as an oil of similar rotation to that of isoaristolactone, but which was apparently unchanged on further treatment with glacial acetic acid. Chromatography of the oil on cellulose-charcoal mixture gave a further yield of crystalline lactone m.p. 90-91°C., and two oils, one partially crystalline $[\alpha]_D^{17°C.}$ -38°, indicating possible contamination with unchanged aristolactone. Further boiling with glacial acetic acid with the addition of a trace of sulphuric acid to catalyse the reaction caused the immediate formation of a deep brown colour in the mixture, from which was isolated a deep yellow oil.

Dry hydrochloric acid in ethanol caused only a very slow change in aristolactone at room temperature, but this change was accelerated on boiling the solution gently for two hours. Much darkening in colour ensued, and the product was considerably less pure than that isolated in the glacial acetic acid experiment. Even so, the melting point was raised on admixture with isoaristolactone and there can be little doubt that the two products are identical.

The ultraviolet absorption spectrum of isoaristolactone was similar to that of aristolactone but also showed a low intensity maximum at 272 mμ(ε , 640). isoAristolactone was non-aromatic, giving a yellow colour with tetranitromethane. It was relatively stable to alkali, being saponified only very slowly by boiling ethanolic potassium hydroxide, about 44% of the lactone being recovered unchanged after 30 minutes. The seat of the isomerisation is therefore in or near the lactone ring.

The marked change in specific rotation (+156° to -44°) suggested either a change in configuration of the molecule or a rearrangement of an ethylenic bond in close association with an asymmetric centre[1]. The former hypothesis was rendered unlikely on the basis of hydrogenation experiments. Complete hydrogenation of isoaristolactone at a platinum catalyst in ethanol, confirmed the presence of three ethylenic bonds and gave hexahydroaristolactone as sole product, indicating that the isomerisation was not a simple stereoisomeric change. The hydrogenation showed a definite break in rate of hydrogen uptake after the rapid absorption of one mol. and then gave dihydroaristolactone in excellent yield. This difference from aristolactone, which showed a break in uptake after the slow absorption of 2 mols., supported the hypothesis that a double-bond shift had occurred in the isomerisation.

The α,β-double bond in partial structures (XIV) and (XVI) is already in a stable position exocyclic to a five-membered ring[11], whereas (XII) with the double bond endocyclic to the five-membered ring represents a less stable structure which would tend to rearrange to (XVIII) or (XIX).



(XII) (XVIII) (XIX)

Spectroscopic evidence supports the view that the same double bond is involved in both acid and alkaline rearrangements. In carbon disulphide isoaristolactone showed typical vinylidene bands at 892 and 1656 cm.$^{-1}$ of the same intensity as those in the parent lactone (appendix 2). Bands at 787 and 842 cm.$^{-1}$ (tetrasubstituted ethylene), however, were of similar intensity to those recorded for methyl oxoaristate (reduced intensity compared with aristolactone). Bands at 815 and 833 cm.$^{-1}$ are associated with a trisubstituted double bond. Dihydroaristolactone (appendix 2) showed similar bands and, moreover, exhibited a maximum at 209 mμ (ϵ, 7800) (isolated tetrasubstituted ethylene). Further evidence for the double-bond shift is adduced from a comparison of the ultraviolet absorption

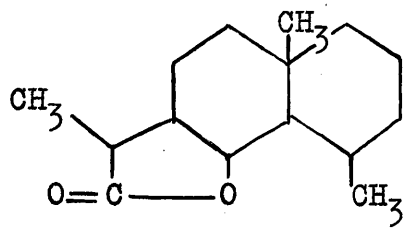
spectra of aristolactone and isoaristolactone, (appendix 2). The broader maximum shown by aristolactone at 211 mμ is in accord with that expected for the summation of the band due to the isolated tetrasubstituted ethylene at 209 mμ with that at a longer wavelength due to the α,β -unsaturated lactone function (cf. Barton and de Mayo[12]). The much more sharply defined maximum at 209 mμ in isoaristolactone is regarded as the summation of two similar peaks, both at the same wavelength, one being due to the isolated tetrasubstituted double bond as in dihydroaristolactone. Comparison of the molecular extinction coefficients of dihydroaristolactone and isoaristolactone suggests that the new double bond in isoaristolactone may be trisubstituted.

DEHYDROGENATION OF ARISTOLACTONE

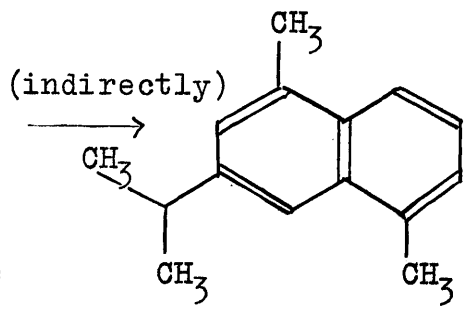
The molecular formula, $C_{15}H_{20}O_2$, for aristolactone and the presence of three ethylenic bonds indicated a bicyclic structure, including the lactone ring. The basic skeleton must therefore be monocyclic, and dehydrogenation experiments, necessarily on a small scale, were likely to prove inconclusive. Linstead, Michaelis and Thomas[13] applied palladium on charcoal, and platinum on charcoal catalytic dehydrogenation to various monoterpenes and sesquiterpenes at relatively low temperatures and achieved satisfactory yields. The method was cleaner than that using selenium and gave satisfactory results with small quantities of material.

The method adopted therefore, to supply qualitative evidence of a six-membered ring system in aristolactone was to heat the hexahydro-compound (0.05-0.1g.) at its boiling point in the presence of palladium (25%) on charcoal for 20 minutes. Aromatic systems were detected in the decomposition products by spectrophotometric means. The disadvantage of sublimation during the heating process was entirely overcome in the apparatus shown. (Fig.2, page 174). It was essential to start with the fully saturated lactone as isomerisation of aristolactone to <u>iso</u>aristolactone was known to give what could be interpreted as benzenoid

absorption in the ultraviolet spectrum. The catalyst used is capable of dehydrogenating fully saturated substances[14] but to test the method control compounds which were known to dehydrogenate to naphthalenic and benzenoid systems were used viz.

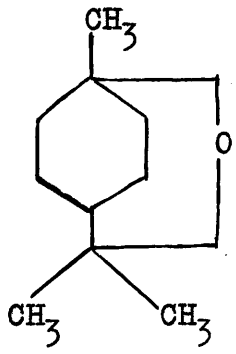


tetrahydroalantolactone

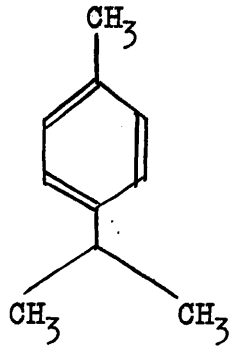


(XX)

and



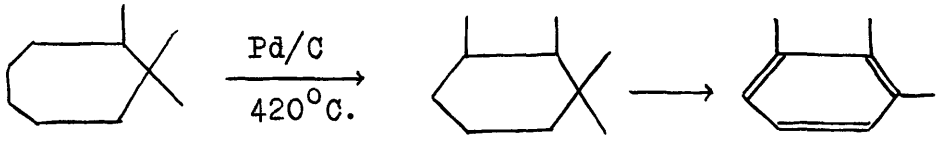
cineole



(XXI)

The ultraviolet absorption curves obtained (appendix 2) provided evidence of a naphthalenic skeleton in (XX) and a benzenoid system in (XXI). Hexahydroaristolactone gave rise to products similar in absorption characteristics

to those obtained from cineole. This evidence alone, however, cannot be regarded as conclusive, as ring contraction is not unknown in dehydrogenation experiments. For example Ruzicka and Seidel[15] observed the formation of 1:2:3-trimethylbenzene (XXII) from 1:1:2-trimethylcyclo-heptane (XXIII). The results must be considered together



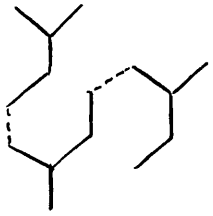
(XXIII) (XXII)

with those of the infrared absorption curves.

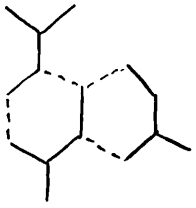
Dehydrogenation of aristolactone itself under the same conditions confirmed the presence of benzenoid constituents in the product, but the bulk of the material reverted to a discoloured resinous mass. No crystalline material (other than starting substance) was isolated from the dehydrogenation experiments.

CONSIDERATION OF POSSIBLE STRUCTURES FOR ARISTOLACTONE

Ruzicka[16] in discussing the application of the isoprene rule to the sesquiterpenes has pointed out, that with the single exception of eremophilone, all sesquiterpenes conform to the isoprene rule. It is usual in the sesquiterpene group for the isoprene units to be arranged in a regular head to tail manner as in the farnesol type (XXIV). The only known exception to the



(XXIV)



(XXV)

farnesol rule is the carotol type (XXV) in which the three isoprene units are arranged in an irregular manner.

As a guiding principle the view that the basic skeleton of aristolactone consisted of three isoprene units was adopted. Consideration has been given only to the possibility that the structure is basically either of the farnesol type or of the carotol type.

The foregoing evidence has established that aristolactone is a bicyclic sesquiterpene lactone, triply unsaturated, one ring being six-membered and the other

having an α,β -unsaturated γ -lactone structure. As the discussion of possible structures is based upon the presence of three ethylenic bonds, a summary of the evidence for their presence is given.

1. Analysis of the fully saturated lactone

Found:	Needles plates	C	75.2 % 75.65% ,	H	11.4 % 11.1 %
$C_{15}H_{26}O_2$	requires	C	75.6 % ,	H	11.0 %
$C_{15}H_{24}O_2$	"	C	76.2 % ,	H	10.24%

The nature of this evidence is satisfactory.

2. Analysis of the hydroxy-acid derived from the saturated lactone

Found:		C	70.3 % ,	H	11.2 %
$C_{15}H_{28}O_3$	requires	C	70.3 % ,	H	11.0 %
$C_{15}H_{26}O_3$	"	C	70.83% ,	H	10.3 %

Evidence satisfactory.

3. Quantitative bromination of aristolactone and <u>iso</u>aristolactone indicated over 2 double bonds (2.3 - 2.7). This evidence is not conclusive as substitution might have occurred.

4. The uptake of hydrogen on complete hydrogenation of aristolactone indicated the presence of three ethylenic bonds. This evidence is not conclusive as leakage is

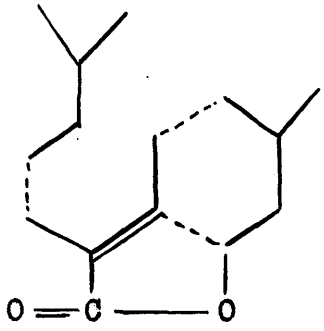
a possible source of error, though this is unlikely to occur to the same extent with varying quantities of material.

5. Ethyl dihydro-oxoaristate was still unsaturated (tetranitromethane). This evidence is satisfactory as one double bond was lost in conversion to the keto-ester and another in the partial hydrogenation of the ester. The third double bond was shown by tetranitromethane.

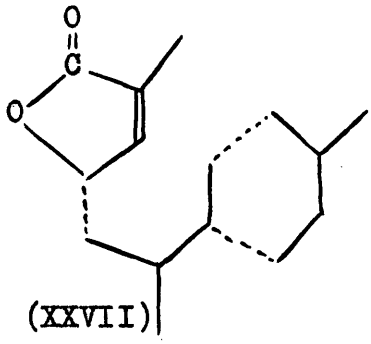
6. Oxidation of aristolactone with potassium permanganate in acetone gave a compound tentatively assigned a glycol structure formed by the addition of two hydroxyl groups across one double bond. Bromination of this product indicated the presence of two ethylenic bonds. This evidence is not conclusive as the structure was not definitely proved and the remarks under 3. above also apply.

Many of the above points taken individually, are not conclusive, but when considered collectively they provide strong evidence in favour of three ethylenic bonds in aristolactone.

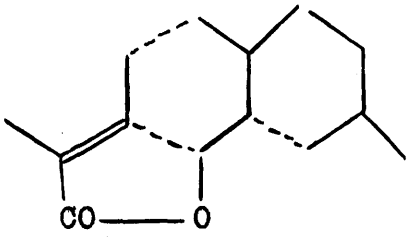
Application of the isoprene rule limits the possible basic skeletons to the following, in which only the double bond of the lactone ring is shown.



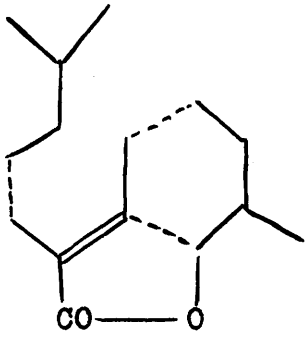
(XXVI)



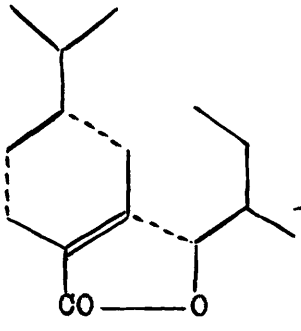
(XXVII)



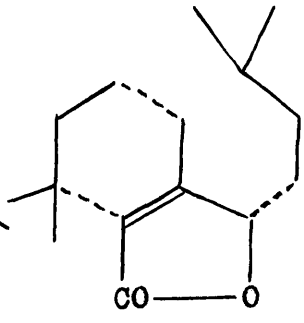
(XXVIII)



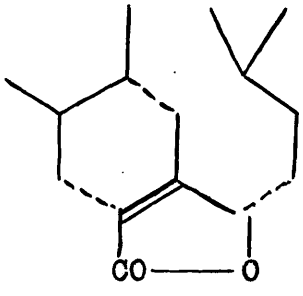
(XXIX)



(XXX)



(XXXI)



(XXXII)

Structures (XXVI) and (XXVII) only, conform to the farnesol rule, although (XXVII) represents a type of which there is no known representation. The remaining structures do not conform to the farnesol rule but are based on that of

carotol in which only two of the three isoprene units are arranged in a regular manner. Certain of these structures can be eliminated from consideration on the basis of degradation evidence which is now considered.

Oxidation with potassium permanganate

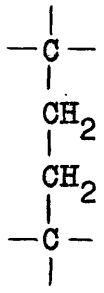
Oxidation of aristolactone with potassium permanganate in acetone gave rise to two products, the first, a crystalline neutral substance, m.p. 158.5-160°C., $[\alpha]_D^{17°C.}$ +128°, $\lambda_{max.}$ 209 mμ (ϵ, 3400). Bromination of this material indicated the presence of two ethylenic bonds. Hydrolysis with ethanolic potassium hydroxide demonstrated the presence of potential acidic groups, (equivalent 246), whilst acidification of the hydrolysis liquor caused precipitation of original substance. These facts are readily explained by a glycol structure formed by the addition of hydroxyl groups to the α,β -ethylenic bond, and this is supported by the analysis which indicated the molecular formula $C_{15}H_{22}O_4$ or $(C_8H_{12}O_2)_n$. The latter may be rejected since more deep-seated degradation would give rise to acids and ketones, but the substance was neutral and exhibited no ultraviolet absorption characteristic of aldehydes or ketones. A further objection is the relatively small change in specific rotation (+156 to + 128°). The presence of potential acidic groups in a neutral substance and the

ease of regeneration by the addition of acid after saponification, clearly indicated a lactone structure. The second product was an acidic oil which separated on paper into at least two components one of which contained a carbonyl group(s). Further oxidation of this oil with alkaline permanganate gave succinic acid, identified by paper chromatography.

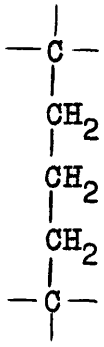
Oxidation with chromic acid

Vigorous oxidation with potassium dichromate and sulphuric acid gave rise to simple acids which were separated into volatile and non-volatile acids by steam distillation. Ether extraction of the non-volatile residue gave an acidic semi-crystalline oil. The volatile acids (equivalent to 1.6 mols.) gave a crystalline sodium salt which possessed slight reducing properties, probably because of the presence of formic acid. The bulk of the salt was sodium acetate, identified by the preparation of the crystalline S-benzylthiuronium salt m.p. 141-142°C. undepressed on admixture with an authentic derivative prepared from sodium acetate. The melting point of this derivative varies in the literature[17],[18],[19] and although variations in the rate of heating undoubtedly contribute to this[20] they do not appear to supply an entirely satisfactory explanation.

Recrystallisation of the non-volatile acids gave succinic acid which was confirmed by the preparation of the S-benzylthiuronium salt. The presence of succinic acid indicated the partial structure (XXXIII) and possibly (XXXIV).



(XXXIII)



(XXXIV)

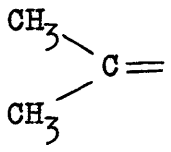
A search was therefore made for glutaric acid (which could arise from (XXXIV), in the mother liquors from the succinic acid crystallisation. Paper chromatography using the solvent system liquified phenol containing 1% of formic acid, gave well defined spots of succinic acid and a carbonyl-containing acid, but not a trace of glutaric acid.

Oxidation with chromic acid in pyridine-water gave unchanged aristolactone, but chromic acid in acetic acid-water mixture at 50°C. caused some oxidation, and trace amounts of a neutral substance m.p. 186-187°C. were isolated. They were too small for characterisation, and the only other product - an acidic oil - rapidly resinified when warmed with thionyl chloride, which was used in an

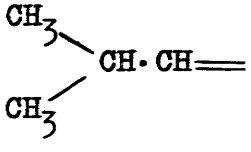
attempt to prepare an amide.

Ozonolysis

Ozonolysis of aristolactone gave formaldehyde as the sole volatile product isolated as its dimedone derivative in a yield corresponding to 59% of the theoretical required for one vinylidene group. Ethyl oxoaristate similarly yielded formaldehyde corresponding to 37% of the theoretical amount for a single vinylidene group. This confirms the earlier conclusion, based on infrared evidence that the vinylidene is not involved in the alkaline rearrangement of aristolactone and hence is remote from the lactone ring. The isolation of formaldehyde in relatively high yield and the high state of purity of the crude derivative in both the above reactions made the presence of other volatile products unlikely. A careful search revealed no evidence of the presence of either acetone or other volatile substances so that terminal groups of the type (XXXV) and (XXXVI) are absent.



(XXXV)



(XXXVI)

Citral was used as a model compound to ensure that acetone

could be detected in the ozonolysis products by the technique used.

Decomposition of the ozonide by three different methods, (a) water[21], (b) zinc dust and glacial acetic acid[22] and (c) palladium on charcoal catalytic hydrogenation[23] all failed to yield crystalline products. The oils which were always formed were almost completely soluble in dilute alkali with the formation of deep brown solutions as soon as heat was applied. Solutions in cold alkali also showed discolouration. The presence of readily oxidisable groups was confirmed by the strong reducing action on ammoniacal silver nitrate, whilst the formation of a red colour with alkaline sodium nitroprusside and strongly positive iodoform reaction indicated the presence of one or more $CH_3 \cdot CO-$ groups.

Two of the possible skeletons (XXVII) and (XXVIII) considered above would be expected to yield pyruvic acid on ozonolysis and a careful search was therefore made for this acid in the subsequent examination of the products. The ozonide was decomposed with water and the acidic products before and after hydrolysis examined chromatographically on paper using the solvent systems (i) butanol-pyridine-water-ethanol[24] and (ii) pyridine-ammonia-water[25]. The acid spots were detected with the spray reagents bromocresol green, Tollen's reagent and the special system of reagents

devised by Martin for the production of coloured spots with certain acids[25]. The solvent system (i) gave satisfactory results, but with the pyridine-ammonia-water system there was a tendency for the spots to spread. Two acids of widely differing R_F values were shown to be present (Table 1) and of these two, one, (A) of R_F 0.62 (solvent (i)) is almost certainly laevulinic acid. The slight reduction with Tollen's reagent is possibly due to traces of impurity. The acid (B) has not been identified but its lack of reduction with Tollen's reagent demonstrated clearly that it was not pyruvic acid. Skeleton structures (XXVII) and (XXVIII) may therefore be eliminated from further consideration.

Table 1.

Substance ozonised	Control Acid		R_F Solvent(i)	R_F Solvent(ii)	Sprayreagent (ii)	Sprayreagent (iii)	Ref.
Aristolactone	-	(A)	0.62	0.80	slight reduction	no colour	
"	-	(B)	0.31	0.45	no reduction	"	
Citral	-		0.62	0.75	"	"	
-	Laevulinic		0.62	0.76	"	"	24
Methyl Methacrylate	-		0.29	-	strong reduction	Mauve	
-	Pyruvic		0.28	-	"	"	

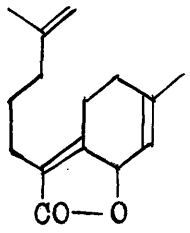
Confirmation of laevulinic acid was obtained when the 2:4-dinitrophenylhydrazone mixture prepared from the oily ozonolysis product was examined chromatographically on paper using solvent (i). Two spots were obtained one of which (R_F 0.66) was identical with that of the derivative from laevulinic acid. The other (R_F 0.9) was not identified, but was not the pyruvic acid derivative which had an R_F value of 0.39 .

No crystalline substance or derivative could be isolated from the oily ozonolysis products.

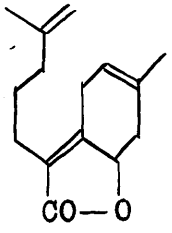
Consideration of the remaining skeletal structures

In considering the possible structures it must be remembered that one of the double bonds is represented by a vinylidene group and that no conjugation of ethylenic bonds is present. The following structures based on (XXVI), (XXIX) and (XXX) will therefore be considered first.

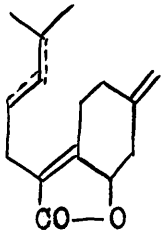
(XXVI)



A

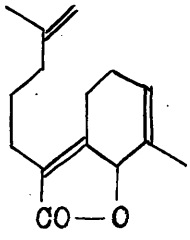


B



C

(XXIX)



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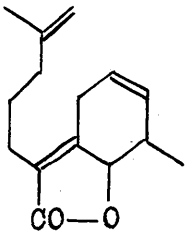

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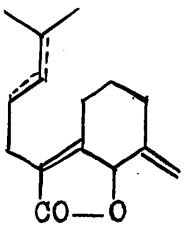
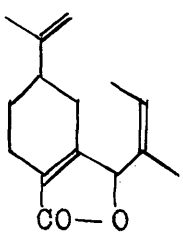
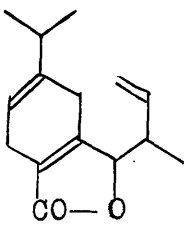


F

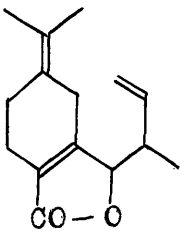
(XXX)



G



H



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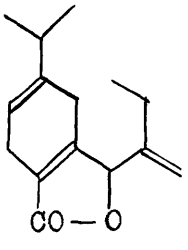

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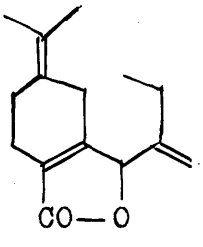
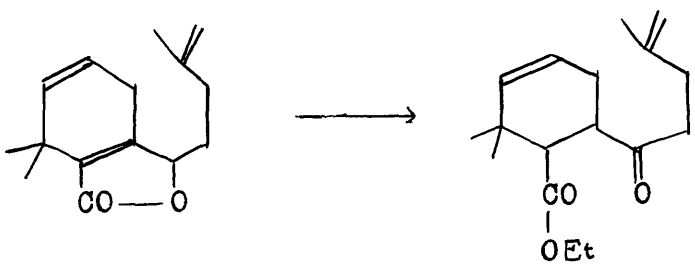


K

Ultraviolet absorption spectra (aristolactone and ethyl and methyl oxoaristate) indicate a second tetra-substituted double bond so that structures A, B, C, D, E, F, G, H, J are considered unlikely. Ethyl oxoaristate does not contain a double bond in conjugation with a carbonyl group so that structures A, D, F, G, J, K which would give this can be rejected. Those structures which would give glutaric acid on chromic acid oxidation, (A, B, D, E), are similarly ruled out. Ozonolysis of compounds such as C, F, G, I, K, would give volatile aldehydes or ketones other than formaldehyde, but none were found. Finally, two of the structures, H and J, could not give rise to succinic acid on chromic acid oxidation. All the given structures are thus shown to be untenable even if no allowance is made for the least conclusive evidence, that of the tetrasubstitution of the second double bond.

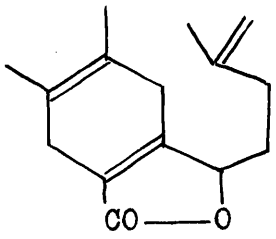
Skeletal structure (XXXI) gives rise to only one compound (XXXI)A and this is rendered unlikely on the basis of ultraviolet absorption measurements on ethyl oxoaristate



(XXXI)A

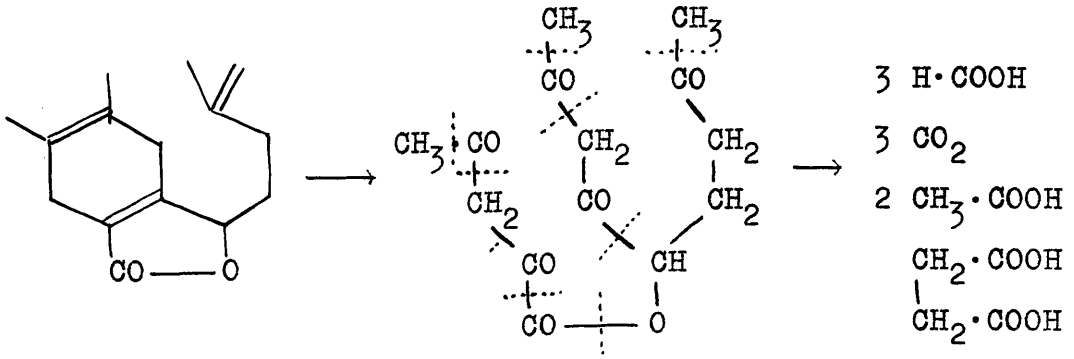
(ϵ, 6000-7000) at 209 mμ. Calc. $\lambda_{max.}$ < 200 mμ, "$\epsilon_{max.}$" 300-600[9], for the product shown.

Structure (XXXVII) based on skeleton structure (XXXII) however provides a satisfactory explanation of many of the observed facts, accommodating the α,β - unsaturated lactone fused in a bicyclic structure to a six-membered ring, the tetrasubstituted double bond and a vinylidene group.



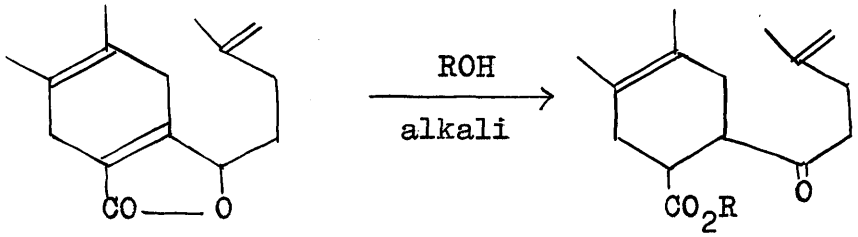
(XXXVII)

Chromic acid oxidation of aristolactone is a complex process but if it is assumed to proceed according to the following scheme then the proposed structure readily accounts for the production of formic (traces), acetic and succinic acids.



The actual isolation of formic acid in large yield would be unlikely under the strong oxidising conditions.

Ethyl oxoaristate, the product of alkaline rearrangement in ethanol would then be represented by (XXXVIII), the vinylidene group and the isolated tetrasubstituted double bond taking no part in the reaction as shown by the formation of formaldehyde on ozonolysis and by the ultraviolet and infrared spectra.

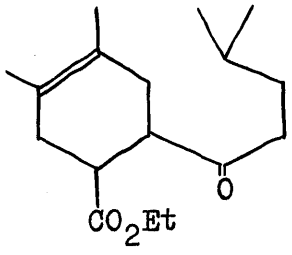


(XXXVIII)

The loss of an asymmetric centre and the creation of two new ones in ethyl oxoaristate would account for the large increase in optical rotation which accompanies the rearrangement. The instability of ethyl oxoaristate which slowly liquified over a period of months even when carefully stored, a process which, as already shown, was greatly accelerated in the presence of alkali, may be accounted for by inversion at one or both optical centres.

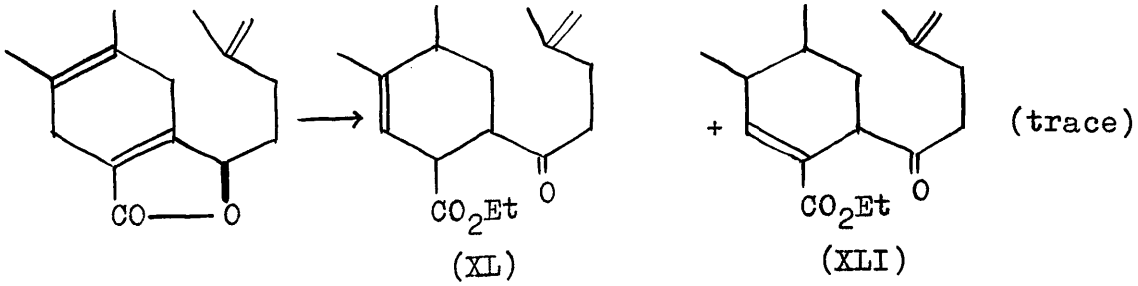
Catalytic hydrogenation of ethyl oxoaristate gave ethyl dihydro-oxoaristate which could be assigned structure (XXXIX) since ozonolysis no longer gave formaldehyde, whilst the ultraviolet absorption spectrum

showed maxima at 208 mμ(ε, 3570) and 287mμ(ε , 52) due to the isolated double bond and the carbonyl group respectively. It is interesting to note the decrease in the maximum at 208 mμ with the saturation of the vinylidene group, a result which is at variance with the proposed structures.



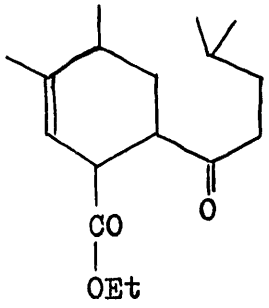
(XXXIX)

It had earlier been observed that the absorption spectrum of ethyl oxoaristate possessed a peak at 815 $cm.^{-1}$ attributed to a trisubstituted double bond. This fact, together with the complete ultraviolet absorption curve of ethyl oxoaristate (appendix 2) offers a reasonable explanation of the apparent anomaly. As is well known in terpene chemistry, the homogeneity of products is frequently open to doubt because of the presence of closely related isomers. In this case the reaction of alkali on aristolactone may well proceed as follows



(XL) (XLI) (trace)

Structures (XL) and (XLI) explain the ultraviolet absorption spectrum since the presence of (XLI) would be indicated by the inflexion at 217 mμ. Hydrogenation of the vinylidene group and the subsequent process of purification could give ethyl dihydro-oxoaristate (XLII).



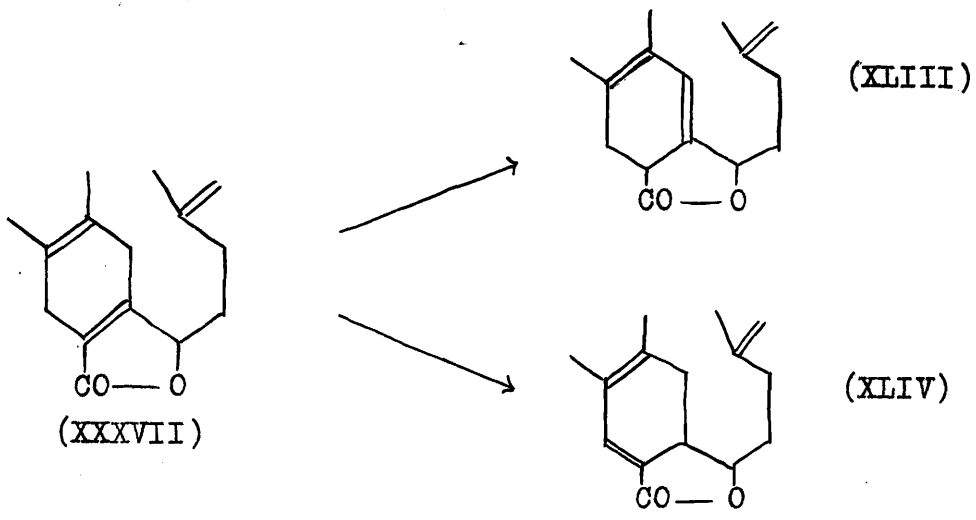
(XLII)

The decrease in the absorption maximum could therefore be caused by the elimination of the α,β -unsaturated component, and the trisubstituted double bond is present to account for the infrared absorption spectrum.

It has already been established that the rearrangement of aristolactone to isoaristolactone in glacial acetic acid involved a shift of the lactone α,β -double bond out of conjugation, with the concomitant increase in the stability of the lactone ring. The presence of the vinylidene absorption band at 890 $cm.^{-1}$ in isoaristolactone confirmed that the vinylidene group was not associated with the lactone ring of either substance.

The assumption of structure (XXXVII) for aristolactone would therefore appear to lead to either of the structures

(XLIII) or (XLIV) for isoaristolactone.

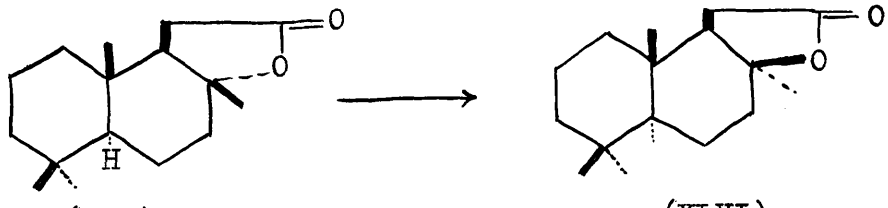


Structure (XLIV) is rejected on the grounds that the ultraviolet absorption spectrum of isoaristolactone showed no evidence of triple conjugation which would be expected to give a high intensity absorption maximum in the region 270-280 mμ[26]. The low intensity absorption (ε , 640) could well arise however from traces of structure (XLIV) present as impurity, as a small plateau at about 273 mμ has been noted in other products and explained in a similar manner[27]. This is not entirely satisfactory in this case as dihydroaristolactone, which was obtained by two different routes, still absorbs to the same extent at 272 mμ as does isoaristolactone, and it appears unlikely that the same amount of impurity would arise in both

processes.

Structure (XLIII) also appears to be ruled out by the low intensity and position of the absorption at 272 mμ , as application of Woodward's rules (Fieser and Fieser[28]) to the homoannular diene leads to a theoretical maximum at 283 mμ (5 substituents, lexocyclic bond) the normal intensity for such a system being of the order of ε, 6000. The full explanation of the spectral anomalies however, must await the synthesis of suitable model compounds.

The very large change of optical rotation from +156° in aristolactone to -44° in <u>iso</u>aristolactone is of the same order as that observed (+117 to -30°) for the <u>trans</u> (XLV) ⟶ <u>cis</u> (XLVI) isomerisation of the lactone from sclareol (Klyne[29]). Klyne has deduced in agreement with Linstead[30] that <u>cis</u> fusion of a five-membered and a six-membered ring is more stable than <u>trans</u> fusion, and it may well be that the acid isomerisation of aristolactone is capable of explanation on similar terms as a result of a double bond shift.



(XLV) (XLVI)

It has already been shown that hydrogenation of

isoaristolactone with platinum in ethanol occurs rapidly (10 minutes) with the formation of dihydroaristolactone. Ozonolysis of dihydroaristolactone gave no formaldehyde indicating that the vinylidene group had been reduced. Further hydrogenation to hexahydroaristolactone took place only very slowly (48 hours) and this is consistent with the presence of hindered tri- or tetra-substituted ethylenic bonds. The relatively small rotation difference between isoaristolactone ($[\alpha]_D$ -44°) and dihydroaristolactone ($[\alpha]_D$ -77°) is in agreement with the placing of the vinylidene group in a position remote from any centre of optical asymmetry.

The formation of dihydroaristolactone from both aristolactone and isoaristolactone is anomalous since both hydrogenations clearly involve reduction of the vinylidene group as shown by the evidence of ozonolysis and infrared spectra. Moreover it is also established that this same vinylidene group is remote from the lactone ring and other optical centres in both aristolactone and isoaristolactone so that mere reduction of this group in aristolactone could not possibly account for the very large change in optical rotation observed (+156 to -77°). It must be concluded therefore that this reduction which is a relatively slow process, taking 24 hours with 3% palladium on charcoal, is accompanied by a rearrangement of the α,β-double bond

similar to that observed in the formation of isoaristolactone. Similar double bond shifts have been observed in the hydrogenation of sterols of the ergosterol type[31,32]. Shaking with the catalyst in the absence of hydrogen however did not cause any rearrangement (cf. Stavely and Bollenback[33]). The product of these hydrogenations is therefore renamed dihydroisoaristolactone.

Tetrahydroaristolactone which was obtained as an oil when aristolactone was partly hydrogenated with platinum in glacial acetic acid showed absorption characteristics completely in agreement with those expected for an α,β-unsaturated lactone, having a maximum at 218 mμ (ϵ, 7000) (appendix 2). This product, being an oil, was probably not completely homogeneous. Its formation with the retention of the α,β-double bond is interesting since it is established that the same double bond undergoes a shift in the formation of dihydroisoaristolactone. The explanation probably lies in the much more rapid reduction which takes place with platinum in glacial acetic acid. Under these conditions the vinylidene group and the isolated double bond no doubt undergo more or less simultaneous reduction.

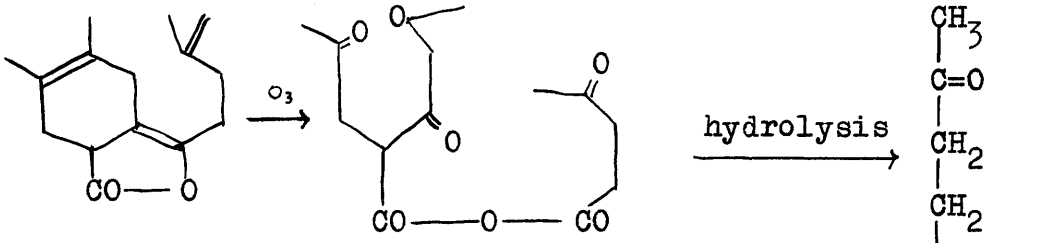
CONCLUSION

In concluding the account of the preliminary investigation into the structure of aristolactone it seemed desirable to draw attention to several unexplained points.

The infrared spectra of aristolactone, isoaristolactone and dihydroisoaristolactone showed a strong absorption band at 927 cm.$^{-1}$ which was absent from the spectra of the keto-ester and the fully saturated lactone. It therefore appeared to be associated with unsaturation and with the lactone ring. In carbon tetrachloride, aristolactone showed two peaks (1370 and 1390 cm.$^{-1}$) which are normally associated with a gem-dimethyl group but in the infrared spectrum of hexahydro-aristolactone there is only one peak at 1383 cm.$^{-1}$ and a very much smaller one at 1350 cm.$^{-1}$ That there should be an increase in one of the peaks is to be expected ($=CH_2 \xrightarrow{Pt/H_2} -CH_3$) but the apparent shift of one peak is unusual. It should be pointed out however that examples of splitting of the $-CH_3$ band at about 1380 cm.$^{-1}$ do occur in the absence of a gem-dimethyl group, particularly in the 3- and 17-steroidal acetates[34].

The ultraviolet absorption spectrum of aristolactone could be interpreted as being due to summation of a

tetrasubstituted double bond in the β,γ -position of the lactone and another tri- or tetra-substituted double bond elsewhere in the molecule (XLVII) and although it would explain the reactions of the ozonolysis products - the formation of laevulinic acid, the slow but definite titration of the product in the cold, and the apparent

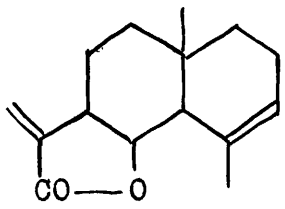


(XLVII) primary product (anhydride) laevulinic acid

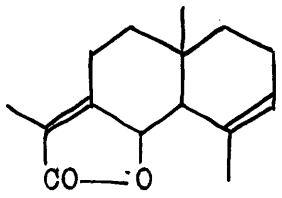
absence of an α -hydroxy ketone, the following aspects make this structure unlikely. Firstly, such a lactone would have the characteristic infrared absorption band at about 1800 cm.$^{-1}$ (β,γ -unsaturated γ -lactone). Secondly, the oil obtained on partial hydrogenation of aristolactone (probably the tetrahydro-compound) showed maximum absorption at 218 mμ (ϵ, 7000) attributed to α,β-unsaturation. Thirdly, hydrogenation of (XLVII) would most likely be accompanied by hydrogenolysis and would give the desoxy-acid as main product (Jacobs and Scott[35]) but aristolactone gave mainly the saturated lactone.

It is interesting to speculate on the possibility of aristolactone being a mixture of isomers (XXXVII) and

(XLVII), (cf. alantolactone which is thought to be a mixture of (XLVIII) and (XLIX)), but until the actual



(XLVIII)



(XLIX)

interpretation of the results of degradation of aristolactone was attempted, aristolactone had every appearance of being a single substance. It is clear that the complete explanation of all the observed facts must await the result of further degradation experiments.

EXPERIMENTAL

ARISTOLACTONE

Isolation

Aristolactone was isolated from the light petroleum-soluble extract as described in Part I and formed needles (from light petroleum), m.p. 110.5-111°C., $[\alpha]_D^{14°C.}$ +156.4° (c, 1), $\lambda_{max.}$ 211 mμ (ε, 11,500).

Found	C, 77.5;	H, 8.8 .
Calc. for $C_{15}H_{20}O_2$	C, 77.6;	H, 8.7%.

Equivalent (by hydrolysis), 233; M.(Rast) 227 ± 13. Calculated for $C_{15}H_{20}O_2$, equivalent, 232; M.232. Infrared spectrum: (a) 0.5M in carbon tetrachloride - peaks at 1770 (α,β -unsaturated γ -lactone); 1034 and 1060 (ester function); (b) in paraffin mull - peak at 1736 (α,β -unsaturated γ -lactone); (c) 0.5M in carbon disulphide - peaks at 1650 (m) and 890 (s) (vinylidene), 840 (m), 800 (w), and 782 (m) $cm.^{-1}$ (tetrasubstituted ethylene).

Bromination of aristolactone

The method adopted was that of the British Pharmacopoeia for the iodine value of oils using pyridine bromide reagent and varying the standard time (10 minutes) to determine the rapidity of absorption. Several model

compounds were tested by the standard method and the results recorded in Table 2.

Table 2.

Substance	Weight (mg.)	Time (min.)	Absorption as ml. N/50 soln.	Equivalent double bonds
Aristolactone	4.5	1	4.57	2.3
"	6.5	30	7.35	2.6
"	9.3	10	10.90	2.7
Santonin	9.5	10	0.02	-
Ethyl Acrylate	45	10	0.10	-
Methyl Methacrylate	46.5	10	0.70	0.07

Hydrolysis

(i) Aristolactone (0.216g.) was refluxed for 30 minutes with ethanolic potassium hydroxide (0.66N, 3ml.) and the mixture neutralised with 0.1N sulphuric acid, a blank determination being carried out. Equivalent weight found, 233. The neutral solution was extracted with light petroleum (2 x 20 ml.), the bulked light petroleum layers washed with water (2 x 5 ml.), dried (sodium sulphate) and evaporated to yield an acid residue (0.01g.)

The aqueous layer was acidified with dilute hydrochloric acid and extracted with light petroleum (2 x 25 ml.). The combined extracts were washed with

water (2 x 5 ml.), dried (sodium sulphate) and evaporated to yield a viscous oil (0.207g.), which partly crystallised to give an acid, m.p. 118°C., in small amount. The residual oil (0.140g.) was neutralised with 0.1N sodium hydroxide (equivalent weight found, 363) and neutral and acidic fractions isolated from the solution as described above to give:-

Neutral fraction - 0.052g.

acidic " - 0.087g. both as oils.

The S-benzylthiuronium salt was crystalline but gave a gum on recrystallisation from ethanol (70%).

(ii) Aristolactone (0.0728g.) was dissolved in cold ethanolic potassium hydroxide (0.66N, 5ml.) and the solution examined polarimetrically in a 1 dcm. tube at 13°C. The rotations observed are plotted in Fig.1.

(iii) Aristolactone (0.261g.) was dissolved in ethanol (90%) which had been freed from carbon dioxide, freshly prepared N ethanolic potassium hydroxide (3ml.) was added, and the solution made up to 50 ml. with ethanol (90%). At intervals aliquot parts (5ml.) were titrated with 0.05N hydrochloric acid (factor, 1.051), the rotation of the alkaline solution being determined at the same time. The results are shown in Table 3.

Table 3.

Number	Time (min.)	$\alpha_D^{15°C.}$	Titre ml.	Alkali neutralised (0.05N, ml.)
1	0	+1.05	5.72	-
2	5	+1.38	5.70	0.02
3	10	+1.53	-	-
4	15	+1.65	5.60	0.13
5	25	+1.74	5.50	0.23
6	35	+1.78	5.52	0.22
7	55	+1.81	5.43	0.31
8	75	+1.79	5.43	0.31
9	30 (days)	-0.30	3.60	2.22
10	38 "	-0.48	3.50	2.34

Neutralisation of fractions 7 and 8 yielded ethyl oxoaristate m.p. 55°C. The product isolated from fraction 10 after acidification was an oily acid which failed to crystallise.

Ethyl oxoaristate

Aristolactone (0.1565g.) was dissolved in a mixture of N ethanolic potassium hydroxide (2ml.) and ethanol (90% ; 25ml.). The solution was neutralised with 0.1N hydrochloric acid as soon as the optical rotation had reached a

maximum. Dilution of the mixture with water yielded ethyl oxoaristate (0.126g.) in needles, m.p. 56-57°C. (from acetone-water), $[\alpha]_D^{19°C.}$ +317° (c, 1), $\lambda_{max.}$ 209 mμ (ε, 7000) and 291 mμ (ε, 250).

Found		C, 73.14;	H, 9.42 .
$C_{17}H_{26}O_3$	requires	C, 73.3 ;	H, 9.31%.

Infrared spectrum in paraffin mull : peaks at 1726 and 1186 (ester carbonyl), 1704 (sh.) cm.$^{-1}$ (ketone). Bromine absorption as previously described for aristolactone was equivalent to 2.5 ethylenic bonds.

Methyl oxoaristate

Aristolactone (0.252g.) was dissolved in N methanolic potassium hydroxide (2ml.) and methanol (90%; 25ml.). The product, isolated as above, yielded methyl oxoaristate (0.250g.) in needles, m.p. 68-69°C. (from aqueous ethanol), $[\alpha]_D^{14°C.}$ +342° (c, 1.23), $\lambda_{max.}$ at 209 mμ (ε, 6360) and 290 mμ (ε, 264).

Found		C, 72.1;	H, 9.1 .
$C_{16}H_{24}O_3$	requires	C, 72.7;	H, 9.1%.

Infrared spectrum : peaks at 1650 (m) and 890 (s) (vinylidene), 840(w), 800(w) and 781 (w) (tetrasubstituted ethylene), 1735 and 1150-1200 (broad band) (ester carbonyl), 1720 (ketone), and 1100 cm.$^{-1}$ (cyclohexanone derivative).

Bromine absorption was equivalent to 2.15 double bonds.

Hydrolysis of methyl oxoaristate

The ester (0.1625g.) was refluxed for 30 minutes with ethanolic potassium hydroxide (0.66N, 3ml.) which had been prepared from ethanol (methanol free). The solution was neutralised with 0.1N sulphuric acid. (Found, equivalent weight 266, $C_{16}H_{24}O_3$ requires 264).

The alcohol was removed from the neutral solution by distillation and the distillate tested for methyl alcohol by the following method of the British Pharmacopoeia.

The distillate (5ml.) was treated with a solution of potassium permanganate (3%) in phosphoric acid (2ml.), and allowed to stand for 10 minutes. The mixture was decolourised with a solution of oxalic and sulphuric acids in water (2ml.), decolourised solution of magenta (5ml.) was added, and the mixture allowed to stand for 30 minutes. A pale blue colour developed of about the same tint as that developed by a control containing methanol (8mg.) in ethanol (10%, 5ml.).

The aqueous liquor remaining in the distillation flask was acidified with dilute sulphuric acid and saturated with sodium chloride. The oil was extracted with light petroleum (2 x 30 ml.), washed with water (2 x 5 ml.), dried (sodium sulphate) and the solvent

evaporated to yield an extremely viscous oil which did not crystallise and failed to yield crystalline fractions when chromatographed from petrol on a column of a mixture of cellulose (3 parts) and charcoal (1 part).

Attempted preparation of p-nitrobenzyl oxoaristate

The crude acid (0.075g.) from the hydrolysis of methyl oxoaristate was neutralised with 0.5N sodium hydroxide and treated with p-nitrobenzyl bromide (0.15g.) dissolved in ethanol (3ml.). The mixture was boiled for 1 hour and cooled. An oil, which resisted attempts to crystallise, was precipitated.

Reactions of Aristolactone with alcoholic ammonia

(i) Aristolactone (0.050g.) was dissolved in ethanol (95%; 5ml.) and the solution was saturated with ammonia and set aside overnight. The solution was concentrated to remove most of the alcohol, and diluted with water. The precipitate was recrystallised from light petroleum to give unchanged aristolactone m.p. 107-109°C.

(ii) Aristolactone (0.074g.) was dissolved in ethanol (95%; 5ml.), zinc dust (0.030g.) added and the mixture saturated with ammonia. The product isolated (after the solution had reached a maximum optical rotation), by removal of ethanol and dilution with water, was

recrystallised from ethanol (70%) to yield needles of ethyl oxoaristate (0.035g.) m.p. 56-57°C.

Found		C, 73.8;	H, 9.5 .
$C_{17}H_{26}O_3$	requires	C, 73.3;	H, 9.3%.

(iii) Aristolactone (0.020g.) treated as in (ii) but omitting the ammonia was recovered unchanged. Table 4 records the optical rotations observed in experiments (i), (ii) and (iii).

Table 4

Time (hours)	Experiment (i)	(ii)	(iii)
0	+1.47	+1.70	+0.64
1	+1.47	+1.79	+0.66
2	+1.47	+1.84	+0.66
18	+1.45	+2.72	+0.66
42	+1.44	+3.33	+0.65
66	-	+3.54	
210	-	+3.18	

(iv) Aristolactone (0.070g.) when treated with methanolic ammonia and zinc dust as in (ii) yielded methyl oxoaristate (0.026g.) m.p. 68-69°C. (from 70% ethanol), undepressed on admixture with a sample prepared from methanolic potassium hydroxide and aristolactone.

(v) Aristolactone (0.030g.) when treated with isopropanol and zinc dust as in (ii) was recovered unchanged.

(vi) Aristolactone (0.030g.) after treatment with triethylamine (0.5ml.) in ethanol in the presence of zinc dust as in (ii) was recovered unchanged.

Reaction of Aristolactone with sodium ethoxide

Aristolactone (0.54g.) was dissolved in ethanol (20ml.) and a solution of sodium ethoxide (3ml.) added. A rapid increase in optical rotation took place and when a maximum was reached (63 minutes) an aliquot part was neutralised with N hydrochloric acid, diluted with water and the crystalline precipitate recrystallised from ethanol to give needles m.p. 56°C. undepressed on admixture with ethyl oxoaristate.

The optical rotation of the remaining solution was allowed to reach a negative value and hydrolysis was completed by gently refluxing. Isolation of the acid in the normal manner gave an oil which yielded a small quantity of a crystalline acid, m.p. 139-140°C. $[\alpha]_D^{17°C.}$ -1.8°, insufficient for further characterisation.

ISOMERISATION OF ARISTOLACTONE

Action of glacial acetic acid

Aristolactone (0.0168g.) was dissolved in glacial acetic acid and the solution made up to 5.0ml. with more acid. The solution (0.75ml; equivalent to 2.52mg. of lactone) was introduced into each of 5 boiling tubes which were calibrated at 1.00ml and were of the following dimensions:- bulb : 0.75ml. capacity approximately

stem : 10 cm. (to function as condenser).

They were prepared from Pyrex glass tubing. The bulbs of the tubes were immersed in an oil bath at 130°C. and at intervals one was removed, cooled and the solution made up to 1.00ml. with glacial acetic acid. The optical rotation of the solution was measured in a micro-polarimeter tube (1 dcm.). Table 5 records the results.

Table 5.

Tube No.	Boiling time (hours)	$\alpha_D^{17°C.}$	$[\alpha]_D^{17°C.}$
-	0	+0.31	+153
1	0.5	+0.25	+ 99
2	1.66	+0.09	+ 36
3	3.33	+0.03	+ 12
4	5	0.0	0
5	11	-0.04	- 15

The material recovered by neutralisation of the acetic acid with sodium hydroxide (20%) and extraction with light petroleum was an oil. On the basis of these results, aristolactone (1.0g.) was dissolved in glacial acetic acid (10ml.) and the solution boiled gently under reflux, in an atmosphere of carbon dioxide, for 12 hours. The fall in optical rotation was followed until, after 7 hours the solution became too dark for further observations. The solvent was removed by distillation under reduced pressure and the residue taken up in light petroleum, filtered from brown resinous material (0.1g.) and the organic layer washed with sodium bicarbonate solution (2 x 10 ml.) and water (2 x 10 ml.). Evaporation of the dried solution (sodium sulphate) gave a pale yellow oil (0.92g.) which solidified on standing overnight. Repeated recrystallisation from light petroleum yielded needle crystals (0.15g.) m.p. 90-91°C., $[\alpha]_D^{17°C.}$ -44° (c, 1.4), $\lambda_{max.}$ 209 mμ (ε, 11,200) and 272 mμ (ε, 640).

Found	C, 77.55;	H, 8.7 .
$C_{15}H_{20}O_2$ requires	C, 77.6 ;	H, 8.7%.

Infrared spectrum of a 0.5M solution in carbon disulphide : peaks at 892 (s) and 1655 (vinylidene), and 787 (m) and 842 $cm.^{-1}$ (m) (tetrasubstituted ethylene). Bromine absorption by the standard method was equivalent to 2.35

double bonds. A second crop of crystals (0.2g.) m.p. 83-87°C. and an oil (0.48g.) $[\alpha]_D^{17°C.}$ -38.1° were also obtained.

Chromatography of the oil obtained by isomerisation

The oil (0.48g.) was boiled gently under reflux with glacial acetic acid (5ml.) for a further 3 hours, and recovered as an oil as described above. This was dissolved in light petroleum (5ml.) and chromatographed on a column (20cm. x 2.5cm.) of a mixture of equal parts cellulose powder and charcoal. The result is recorded in Table 6.

Table 6.

Fraction	Eluant	Volume (ml.)	Residue (g.)	Remarks
1	light petroleum	65	trace	-
2	" petrol,80 ether ,20	15	0.128	crystals
3	"	15	0.317	semi-crystalline $[\alpha]_D^{17°C.}$ -38°
4	"	20	0.127	oily, $[\alpha]_D^{17°C.}$ +10°
5	ethanol	20	0.025	amorphous, brown

Recrystallisation of fraction 2 from petrol yielded rectangular plates ($[\alpha]_D^{17°C.}$ -47°,) m.p. 90-91°C., undepressed on admixture with isoaristolactone.

Action of ethanolic hydrochloric acid

Aristolactone (0.1g.) was dissolved in ethanol (95%, 5ml.) and the solution was saturated with hydrochloric acid. The optical rotation fell during one week from +2.18° to +1.78°. The solution was refluxed for 2 hours to accelerate the change, and the reaction mixture diluted with water. The precipitate was crystallised from ethanol (70%) after decolourisation with charcoal, and then from light petroleum to yield needle crystals m.p. 82-87°C. raised to 85-88°C. on admixture with isoaristolactone.

Attempted hydrolysis of isoaristolactone

isoAristolactone (0.106g.) was refluxed with ethanolic potassium hydroxide (0.66N, 2ml.) for 40 minutes. The solution was diluted and neutralised with 0.05N sulphuric acid, giving isoaristolactone (0.046g.) m.p. and mixed m.p. 90-91°C. The mother liquors were extracted with light petroleum, acidified, then re-extracted with light petroleum and dried (sodium sulphate) to yield an acidic oil after removal of the solvent. The oil failed to crystallise and was not examined further.

HYDROGENATION OF ARISTOLACTONE

(i) Hexahydroaristolactone

Aristolactone (1.3g.) was hydrogenated in ethanol at a platinum catalyst. Hydrogen uptake was rapid for the first 60 minutes and then slowed down, but appeared to be complete after 1140 minutes with the absorption of 377 ml. (at N.T.P.). Equivalent ethylenic bonds, 2.99. The mixture was filtered and the ethanol removed under reduced pressure to yield a crystalline solid, which after recrystallisation from light petroleum gave colourless needles of hexahydroaristolactone (0.46g.), m.p. 103.5-104°C., $[\alpha]_D^{17°C.}$ +3° (c, 1.2).

Found		C, 75.65;	H, 11.1 .
$C_{15}H_{26}O_2$	requires	C, 75.6 ;	H, 11.0%.

Equivalent weight (hydrolysis) 233; $C_{15}H_{26}O_2$ requires 238.
Infrared spectrum : (i) 0.5M in carbon tetrachloride - peaks at 1780 (saturated γ-lactone), 1167 (ester function); (ii) in paraffin mull - 1750 cm.$^{-1}$ (saturated γ-lactone).
Less pure crystals (0.59g.) were also obtained from the reaction. Subsequent hydrogenations yielded hexahydroaristolactone first in needles, m.p. 99-100°C., and then in plates, m.p. 100-102°C., $[\alpha]_D^{17°C.}$ +3°

Found C, 75.2; H, 11.4%

The infrared spectra were identical with that described above. A mixture of the needles and the plates had m.p. 101-104°C.

Hydrolysis of hexahydroaristolactone

(i) Hexahydroaristolactone (needles; m.p. 103.5-104°C; 0.121g.) was refluxed with ethanolic potassium hydroxide (0.66N, 5ml.) for 30 minutes and the excess of alkali titrated with 0.1N hydrochloric acid . (Equivalent, 233). The neutral solution was acidified with acetic acid and extracted with light petroleum to yield the acid as plates of hexahydrohydroxyaristic acid, m.p. 86-87°C. (from light petroleum), $[\alpha]_D^{16°C.}$ +16° (c, 1.6)

Found		C, 70.3;	H, 11.2 .
$C_{15}H_{28}O_3$	requires	C, 70.3;	H, 11.0%.

Fractional crystallisation of the mother liquors yielded a small quantity (5mg.) of a second acid, in short needles, m.p. 121-122°C., insufficient for further characterisation. The acid gave no colour with tetranitromethane.

When mineral acids were used to precipitate the acid from the neutralised solution, it was obtained as an oil which slowly crystallised to the original lactone and then dissolved in light petroleum leaving trace amounts of the second acid undissolved.

(ii) Hexahydroaristolactone (plates; m.p. 100-102°C; 0.3g.) was refluxed with ethanolic potassium hydroxide (0.66N, 5ml.) for 30 minutes. The product (found: equivalent, 239. $C_{15}H_{28}O_3$ requires equivalent 238) isolated as described in (i), formed plates m.p. 86-87°C. undepressed on admixture with the acid obtained from (i).

Re-lactonisation of hexahydrohydroxyaristic acid

The acid (m.p. 86-87°C., 0.032g.) was heated in a sealed tube at 100°C. for 3 hours over phosphoric oxide. After sublimation under reduced pressure, hexahydroaristolactone was isolated as feathery needles, m.p. and mixed m.p. 100-103°C.

The acid (m.p. 121-122°C.) when treated in a similar manner, gradually reverted to a red resin, m.p. 112-140°C.

(ii) Dihydroisoaristolactone

Aristolactone (0.276g.) was hydrogenated in ethanol in the presence of palladium-charcoal, the reaction being stopped after the absorption of 32.5 ml. of hydrogen at 17°C. (equivalent to about 1 mol.). The mixture was filtered and the filtrate evaporated to give dihydroisoaristolactone m.p. 79-80.5°C., $[\alpha]_D^{17°C.}$ -77° (c,0.87),

(from light petroleum), $\lambda_{max.}$ 209 mμ (ε, 7725) and 271 mμ (ε, 585).

(iii) Attempted preparation of tetrahydroaristolactone

Aristolactone (0.234g.) was hydrogenated in glacial acetic acid, which contained a trace of hydrochloric acid, in the presence of platinum, the reaction being stopped after the absorption of 47 ml. at 15°C., (equivalent to 1.97 double bonds). The mixture was filtered, the filtrate diluted with water and extracted with ether (2 x 50 ml.). The bulked ether layers were washed with brine (2 x 10 ml.) and then with a saturated solution of sodium bicarbonate until free from acid. The ether layer was dried (sodium sulphate) and evaporated to yield an oil which failed to crystallise. $[\alpha]_D^{20°C.}$ +4° (c, 1), $\lambda_{max.}$ 218 mμ $E^{1\%}_{1cm.}$ 300.

Action of palladium/charcoal catalyst on aristolactone

(i) Aristolactone (0.1g.) was dissolved in ethanol (5ml.) and shaken for 24 hours with palladium (3%) on charcoal. Isolation of the product in the normal manner yielded unchanged aristolactone m.p. and mixed m.p. 108-110°C.

(ii) Aristolactone (0.1g.) was treated as in (i) but the catalyst was saturated with hydrogen. The product was isolated as crystals m.p. 90-105°C., $[\alpha]_D^{17°C.}$ +123°. Further

shaking in the presence of hydrogen resulted in the formation of dihydroisoaristolactone m.p. 79-81°C.

Reduction with Lithium Aluminium Hydride.

Aristolactone (0.12g.) was added portionwise during a period of 10 minutes to lithium aluminium hydride (0.039g.) in dry ether (5ml.), and the mixture warmed gently for 5 minutes. The excess of reagent was destroyed by the cautious addition of water, and dilute hydrochloric acid added to dissolve the complex and aluminium hydroxide. The solution was extracted with ether (2 x 30 ml.), the extract dried (sodium sulphate) and evaporated to yield a colourless odourless oil which, on dissolving in acetone and cooling in a refrigerator deposited needles (0.010g.) m.p. 245-246°C.

Found		C, 76.45;	H, 9.98
$C_{15}H_{24}O_2$	requires	C, 76.25;	H, 10.24%

HYDROGENATION OF isoARISTOLACTONE

(i) Hexahydroaristolactone

isoAristolactone (0.140g.) was hydrogenated at a platinum catalyst for 48 hours. The hydrogen uptake (46.5ml.) at 17°C. corresponded to 3.2 mols., and isolation of the product as described under aristolactone gave needles of hexahydroaristolactone, m.p. and mixed m.p. 102-104°C. Found: equivalent by hydrolysis 242; $C_{15}H_{26}O_2$ requires 238.

(ii) Dihydroisoaristolactone

isoAristolactone (0.111g.) was hydrogenated for 10 minutes in ethanol in the presence of platinum. Absorption (11ml. at 19°C.) was equivalent to 0.9mol. Filtration and evaporation of the filtrate gave needles, and subsequently, plates of dihydroisoaristolactone m.p. 79.5-80°C. $\lambda_{max.}$ 209 mμ (ϵ, 7800) and 271 mμ (ϵ, 606), $[\alpha]_D^{17°C.}$ -75°

Found	C, 77.0 ;	H, 9.5 .
$C_{15}H_{22}O_2$ requires	C, 76.85;	H, 9.5%.

Infrared spectrum in carbon disulphide (0.5M) : peaks at 894 (ms) and 1673 (w)(vinylidene ?), 1775 (s) (saturated γ-lactone), and 782 (m) and 840 (m) $cm.^{-1}$(tetrasubstituted ethylene). Bromine absorption by the standard method was equivalent to 1.6 ethylenic bonds. A mixed melt with authentic dihydroisoaristolactone was 78-80°C.

HYDROGENATION OF ETHYL OXOARISTATE

(i) Ethyl oxoaristate (0.0032g.) when hydrogenated in acetic acid in the presence of platinum absorbed the equivalent of 1.95 mols. of hydrogen.

Ethyl tetrahydro-oxoaristate

(ii) Ethyl oxoaristate (0.117g.) was hydrogenated in ethanol in the presence of platinum. Absorption (24.4 ml. at N.T.P.) was equivalent to 2.3 ethylenic bonds. Filtration and evaporation of the solvent yielded an almost colourless oil (0.115g.) (probably ethyl tetrahydro-oxoaristate). Found : equivalent by hydrolysis, 281. $C_{17}H_{30}O_3$ requires equivalent, 282. The hydrolysis liquors were acidified with dilute hydrochloric acid, the oily acid extracted with light petroleum and dried (sodium sulphate). Evaporation of the solvent yielded an acidic oil (0.075g.), $\lambda_{max.}$ 287 mμ(ϵ , 51), which only partially crystallised after several weeks.

Ethyl dihydro-oxoaristate

(iii) Ethyl oxoaristate (0.281g.) was hydrogenated for 14 minutes at a platinum catalyst in ethanol, the reaction being stopped after the absorption of the equivalent of 1 mol. The mixture was diluted with light petroleum, filtered, and the filtrate evaporated to yield needles

of ethyl dihydro-oxoaristate m.p. 65-66°C. (from ethanol 90%, and after sublimation), $[\alpha]_D^{17°C.}$ +131° (c, 1.21).

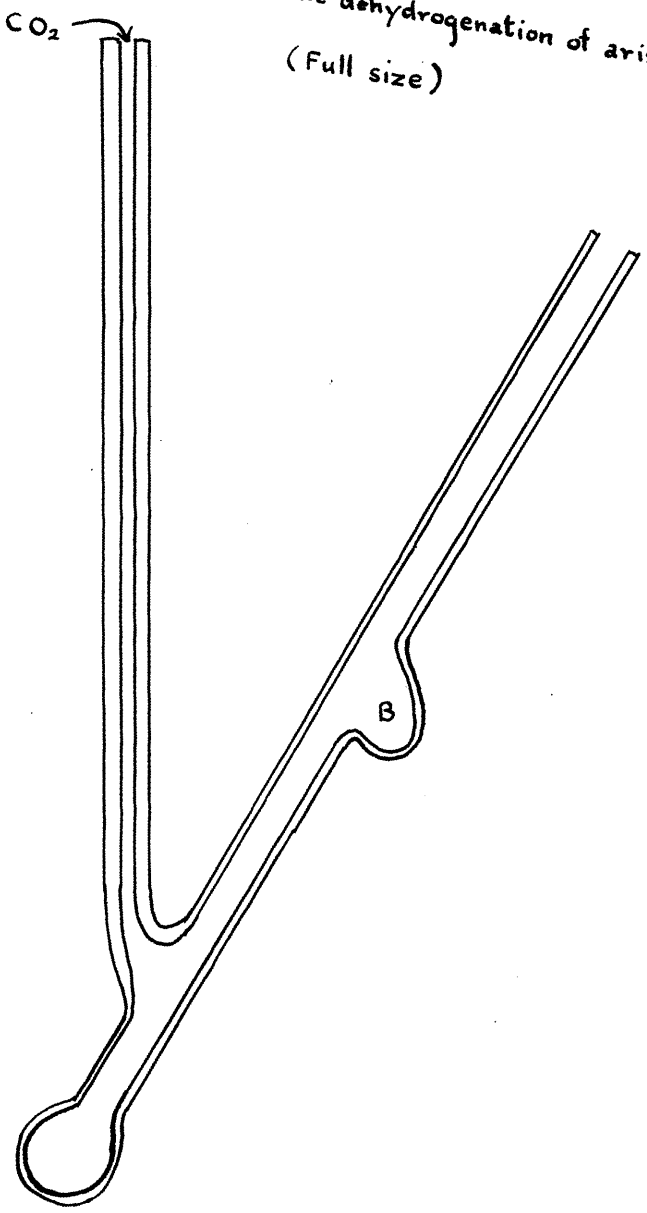
Found	C, 72.81; H, 10.06 .
$C_{17}H_{28}O_3$ requires	C, 73.17; H, 10.18%.

$\lambda_{max.}$ 208 mμ (ε, 3570) and 287 mμ (ε, 52). The product gave a yellow colour with tetranitromethane.

Fig. 2

Apparatus used in the dehydrogenation of aristolactone

(Full size)



DEHYDROGENATION

General method

The fully saturated substance (0.05-0.1g.) was heated for 20 minutes with palladium (25%) on charcoal (20mg.), gentle boiling being maintained by means of a micro-burner. A slow stream of carbon dioxide was passed through the apparatus (Fig.2) during the experiment. A small quantity of the product was distilled into the pocket B, which was kept at a low temperature by means of small pieces of solid carbon dioxide, and diluted with cyclohexane. About half the mixture was withdrawn, diluted to 10 ml. with more cyclohexane, and an appropriate dilution examined spectrophotometrically for the presence of aromatic constituents.

The absorption curves of the products obtained from hexahydroaristolactone, tetrahydroalantolactone and cineole are shown in Appendix 2.

OXIDATION OF ARISTOLACTONE

(a) Chromic acid

Experiment 1

Aristolactone (0.288g.) was heated for 2 hours with chromium trioxide (0.5g.) and a solvent (6ml.) consisting of pyridine (7 parts) and water (3 parts). The mixture was cooled, diluted with water and extracted with ether (1 x 50 ml.) which was then washed with water (2 x 10 ml.), dried (sodium sulphate) and evaporated to yield crystals (0.21g.) which after recrystallisation from petrol melted at 102-106°C., raised to 104-106°C. when mixed with aristolactone.

Experiment 2

Aristolactone (0.476g.) was treated with a mixture (14ml.) of potassium dichromate (4.5g.), sulphuric acid (4ml.) and water (10ml.) and cautiously warmed. A vigorous reaction occurred and cooling was necessary. When the reaction had ceased the mixture was refluxed gently for two hours and then steam-distilled. The distillate was neutralised with 0.5N sodium hydroxide (5.8ml., equivalent to 1.6 mols.) and evaporated to dryness to yield a crystalline sodium salt, which possessed a slight reducing action (neutral silver nitrate and mercuric

chloride solutions), and gave a red colour with ferric chloride solution. The distillate, (recovered whilst evaporating to dryness) was neutral and gave no reactions for carbonyl compounds. It was rejected.

S-Benzylthiuronium acetate

The salt (0.02g.) was dissolved in water (1ml.) and a saturated solution of S-benzylthiuronium chloride (2ml.) added. The precipitate was recrystallised from aqueous ethanol as needles m.p. 141-142°C. undepressed by the derivative (m.p. 143-144°C.) obtained from authentic sodium acetate, but depressed to 137°C. with the corresponding derivative from sodium formate.

Residue from steam-distillation

This was dark green and contained specks of a semi-resinous substance. Extraction with ether (6 x 50 ml.), drying (sodium sulphate) and evaporation yielded a slightly coloured, semi-crystalline residue (0.106g.). Recrystallisation from light petroleum-ethanol mixture gave slightly impure crystals which were further purified by dissolving in ether and filtering off flocculent insoluble matter. Evaporation of the filtrate yielded acid crystals m.p. 183-185°C. (with preliminary softening). A mixed m.p. with authentic succinic acid was 183-186°C. (with preliminary softening).

S-Benzylthiuronium succinate

The acid (0.01g.) was almost neutralised with 0.1N sodium hydroxide and a solution of S-benzylthiuronium chloride (0.02g.) in water (0.5ml.) added. The mixture was cooled in ice and the crystalline precipitate recrystallised from water to give crystals melting at 153-154°C. A mixed melting point with authentic succinic acid derivative (of m.p. 154-155°C.) was 153-154°C.[19]

Mother liquors from the crystallisation of succinic acid

These were evaporated, and the semicrystalline residue dissolved in ethanol (3ml.). The solution (about 0.01ml.) was chromatographed on Whatman paper (No.1) by the ascending technique using as solvent[cf.36] 1% of formic acid in liquified phenol of the British Pharmacoepoeia. The chromatogram was dried at 100°C. (15 minutes) and sprayed with a solution of bromocresol green. Careful exposure to ammonia vapour gave the acids as bright yellow spots on a blue background. Comparison with spots of authentic succinic (R_F 0.71) and glutaric acids (R_F 0.85) showed the presence of the former (R_F 0.71) and the absence of the latter. A second unidentified acid running almost with the solvent front was shown to possess a carbonyl group by spraying with an acid solution of 2:4-dinitrophenylhydrazine.

Experiment 3

Aristolactone (0.5g.) was heated at 50°C. for five hours with chromium trioxide (0.5g.) dissolved in glacial acetic acid (10ml.) and water (4ml.). The mixture was cooled, diluted with water (50ml.) and extracted with ether (3 x 250 ml.) to yield 0.503g. of viscous oil which was neutralised with 0.5N sodium hydroxide (2.65ml.). Extraction of the neutral solution with ether yielded a neutral oil (0.196g.) and acidification of the aqueous liquors and re-extraction with ether gave an oily acid which deposited traces of needle crystals, m.p. 186-187°C. (from a mixture of ethanol and ether at -30°C.), which were neutral in reaction and depressed the melting point of succinic acid. They did not absorb in the ultraviolet and the quantity was too small for further investigation. The oily acid was rapidly converted to a red-brown resin when warmed with thionyl chloride.

(b) Potassium permanganate

Aristolactone (0.54g.) was dissolved in acetone (50ml.) and the mixture cooled in an ice-bath. Potassium permanganate (0.9g.) was added in portions over a period of 75 minutes. The dark brown precipitate was filtered off and the pink filtrate just decolourised by the addition of a trace of aristolactone. Evaporation of the acetone

yielded a semi-crystalline residue (0.048g.). Extraction of the brown precipitate with boiling water (4 x 20 ml.) gave a further yield of crystals (0.047g.) on cooling the extracts in a refrigerator. The mother liquors were extracted with ether (3 x 50 ml.) and the ether extracts bulked, washed with water (5ml.), dried (sodium sulphate) and evaporated to give a neutral crystalline residue (0.120g.). Recrystallisation of these fractions gave needles m.p. 158.5-160°C. (from aqueous ethanol), $[\alpha]_D^{17°C.}$ +128° (c, 0.89). $E^{1\%}_{1cm.}$ at λ 208 mμ, 138.

Found		C, 68.01;	H, 8.55 .
$C_{15}H_{22}O_4$	requires	C, 67.67;	H, 8.34 .
$(C_8H_{12}O_2)_n$	"	C, 68.52;	H, 8.64%.

Bromine absorption by the standard method was equivalent to 2.03 double bonds (based on $C_{15}H_{22}O_4$ which requires 2 double bonds).

Hydrolysis of the potassium permanganate oxidation product

The product (0.0098g.) was heated for 2 hours with ethanolic potassium hydroxide (0.66N, 2ml.), and the mixture neutralised with 0.02N hydrochloric acid. Equivalent, 246, $C_{15}H_{22}O_4$ requires 266. Acidification of the neutral solution with dilute hydrochloric acid precipitated crystals m.p. 159-160°C. (sintered 147°C.)

undepressed on admixture with original material.

The aqueous residue remaining after the ether extraction was steam-distilled to give a neutral distillate which gave only a slight opalescence with an acid solution of 2:4-dinitrophenylhydrazine. The distillate was not examined further. The non-volatile residue was concentrated to small bulk and acidified with dilute sulphuric acid. The oil, which was precipitated, was extracted with ether (2 x 50 ml.), washed with water (2 x 5 ml.), dried (sodium sulphate) and evaporated to yield a yellow viscous oil (0.321g.) of glue-like odour. It gave a positive reaction for a methyl ketone when tested with alkaline nitroprusside, but only traces of a flocculent precipitate with 2:4-dinitrophenylhydrazine. Equivalent weight (neutralisation) 232. The sodium salt (50mg.) failed to yield a S-benzylthiuronium salt.

Further oxidation of the oil with alkaline permanganate and extraction of acidic fragments with ether, gave a semicrystalline oil in which succinic acid was identified by paper chromatography as described under chromic acid oxidation, Expt. 2.

OZONOLYSIS OF ARISTOLACTONE

Experiment 1

Aristolactone (0.415g.) was dissolved in chloroform (15 ml.) and cooled to -20°C. A stream of ozonised oxygen was passed through the solution for 27 minutes and the mixture treated as described under "Ozonolysis of reticulene" Method 1, (page 73) to obtain an aqueous extract and a water-insoluble viscous oil (0.466g.)

Examination of the aqueous extract

The extract was neutralised with sodium hydroxide (20%) solution and made faintly acid with acetic acid. A solution of dimedone (0.5g.) in ethanol (50%; 20ml.) was added, the mixture stirred well and set aside for 1 hour in a refrigerator. The precipitate was filtered off quantitatively; yield, 0.308g. m.p. 187-189°C. (softened at 186°C.), raised to 190-191°C. (from aqueous ethanol) undepressed on admixture with authentic formaldehyde dimedone derivative.

The filtrate from the above was distilled into a solution of 2:4-dinitrophenylhydrazine (0.1g.) in sulphuric acid (1ml.) freshly diluted to 30ml. with water. Only a slight opalescence was obtained.

Examination of the water-insoluble oil

(i) The oil (0.099g.) was hydrolysed with ethanolic potassium hydroxide (0.66N, 3ml.) for 30 minutes, the solution rapidly becoming dark red as soon as heat was applied. Equivalent weight found, 227. The neutral solution was distilled into 2:4-dinitrophenylhydrazine (0.1g.) dissolved in sulphuric acid (2ml.) and water (30ml.). There was no precipitate. The aqueous residue in the distillation flask was acidified with dilute sulphuric acid and a brown amorphous precipitate filtered off. The filtrate yielded to ether only a trace of oil, which was not examined, and gave positive reactions with Schiff's reagent, alkaline nitroprusside solution, ammoniacal silver nitrate and in the iodoform reaction.

(ii) The oil (0.1g.) was treated with a solution of hydrogen peroxide (30%, 0.5ml.) and sodium hydroxide solution added gradually to keep the solution neutral to phenolphthalein. The solution still gave a positive reaction with Schiff's reagent, and an orange precipitate with an acid solution of 2:4-dinitrophenylhydrazine. Attempted recrystallisation of the precipitate gave amorphous products m.p. 70-100°C.

(iii) The water-insoluble oil was acid in reaction, but attempted preparation of an amide by means of thionyl

chloride followed by ammonia resulted in the formation of a dark brown semi-solid mass from which no crystalline product was obtained.

Experiment 2

Aristolactone (0.895g.) was dissolved in ethyl acetate (20ml.) and treated with ozonised oxygen at -15°C. until the solution did not decolourise bromine solution. The solution was allowed to reach room temperature and was hydrogenated at a palladium (25%) on charcoal catalyst for 5 hours (absorption, 360 ml. at 15°C.). The filtered solution was extracted with water (6 x 15 ml.) and the aqueous extracts bulked. The ethyl acetate layer was dried (sodium sulphate) and evaporated to yield a viscous oil (0.387g.).

Examination of aqueous extract

(i) The extract was steam-distilled until 250 ml. of distillate was obtained. The distillate was neutralised with 0.5N sodium hydroxide (3.6ml.) and redistilled into an acid solution of 2:4-dinitrophenylhydrazine. The precipitate was filtered off (m.p. 157-160°C.) and recrystallised from ethanol to give needles, m.p. 163-165°C. undepressed on admixture with authentic formaldehyde 2:4-dinitrophenylhydrazone m.p. 164-166°C. The aqueous liquors in the distillation flask were reserved for future

examination.

(ii) (a) The aqueous solution of non-volatile matter was neutralised with 0.5N sodium hydroxide (2.7ml.) and evaporated to dryness at room temperature to yield a dark brown viscous oil, (0.58g.) which gave positive reactions in the iodoform, alkaline nitroprusside and ammoniacal silver nitrate tests. Rigby's test for acyloins was negative.

(b) The oil (0.093g.) was treated with periodate solution (100ml.) and 2N-sulphuric acid (10ml.) for 24 hours. Oxidation corresponded to 2.1ml. 0.1N sodium thiosulphate.

(c) Attempted preparation of a 2:4-dinitrophenyl-hydrazone yielded amorphous precipitates (m.p. 60-90°C.) which could not be crystallised.

(d) The oil (0.08g.) was dissolved in water (2ml.) and 2N sulphuric acid (2ml.) and titrated with 0.1N potassium permanganate (8.2ml.). The solution yielded only a trace of oil to ether, and gave an amorphous 2:4-dinitrophenylhydrazone m.p. 105-130°C. which could not be recrystallised.

Experiment 3

Aristolactone (0.387g.) was treated with ozone as described in experiment 1. The chloroform was removed at 35-40°C. under reduced pressure and the glassy residue was shaken continuously with water (3ml.) for 15 minutes, then frequently during half an hour whilst warming the flask in a water bath at 50°C. Decomposition was completed by heating in a boiling water-bath for 1 hour. The mixture was cooled and small volumes of the almost clear solution (0.01-0.02ml.) were placed on Whatman paper (No.1) and chromatographed by the ascending technique using the following solvents

(i) Butanol (66), pyridine (12), water (18) and ethanol(4)

(ii) Pyridine (6), ammonia solution (10% NH_3, 2) and water (1).

The chromatograms were dried at 100 C. for 10-15 minutes and the spots detected by the following reagents:-

(i) Bromocresol green indicator

(ii) Tollen's reagent

(iii) Potassium ferricyanide (10%) and drying at 100°C; ferric alum (1%) in ethanol (70%) and air drying; finally ammonia (10%) and air drying. Any colours at the various stages when using this reagent were noted. Comparison spots using pyruvic and laevulinic acids and the ozonolysis products of citral and methyl methacrylate were also

prepared (Table 1). Solvent (i) was most satisfactory, the spots being compact whereas with solvent (ii) they were diffuse.

The aqueous liquid gave an amorphous precipitate with an acid solution of 2:4-dinitrophenylhydrazine, but of wide melting range (90-140°C.). The precipitate, when chromatographed on Whatman paper (No.1) gave two spots (R_F 0.66 and 0.90). Solvent (i) and ascending technique were used and no development of spots by means of reagents was necessary as they were self-indicating. Spots for comparison were obtained by using the 2:4-dinitrophenyl-hydrazones of pyruvic acid (R_F 0.39) and laevulinic acid (R_F 0.68) separately and in admixture, complete separation being obtained in the latter case.

OZONOLYSIS OF ARISTOLACTONE DERIVATIVES

Ethyl oxoaristate

Ethyl oxoaristate (0.29g.) dissolved in chloroform (10ml.) was treated with ozonised oxygen at 0°C. for 20 minutes. The glassy ozonide obtained after evaporation of the solvent was decomposed by the addition of water (15ml.) and allowed to stand overnight. The bulk of the water was distilled into an ice-cooled flask. The oily residue (0.259g.) gave positive reactions with alkaline nitroprusside and ammoniacal silver nitrate.

Examination of the aqueous distillate

Dimedone (0.5g.), dissolved in ethanol (30%, 20ml.) was added to the distillate and the mixture was set aside for 2 hours. The crystalline precipitate which settled out (0.112g.) was recrystallised from ethanol/water mixture to give needles m.p. 188-190°C. undepressed on admixture with authentic formaldehyde derivative. The filtrate from the dimedone derivative was distilled into an acid solution of 2:4-dinitrophenylhydrazine and a trace of precipitate was obtained. The m.p. 118-129°C. was raised to 130-134°C. after one recrystallisation from alcohol, but there was insufficient for further examination.

Examination of the oil. $\lambda_{max.}$ 280 mμ ($E^{1\%}_{1cm.}$ 14.2). Neutralisation equivalent, 228. Oxidation with alkaline permanganate and isolation of the acid gave an oil which showed no signs of crystallisation.

Dihydroisoaristolactone

Dihydroisoaristolactone (0.176g.) was dissolved in chloroform (5ml.) and treated with ozonised oxygen for 20 minutes, the issuing gases being passed through a wash-bottle containing water (10ml.) before passing into the potassium iodide trap. The solution of ozonide was decomposed using the glacial acetic acid and zinc dust method as described for aristolactone (experiment 1). The aqueous extract was treated with dimedone (0.2g.) in solution in 20% ethanol made from the water used in the trap. No precipitate was obtained on standing for 3 days. The solution was distilled into an acid solution of 2:4-dinitrophenylhydrazine but only an opalescence was produced.

The oil gave a positive reaction with alkaline nitroprusside.

Ethyl dihydro-oxoaristate

Ethyl dihydro-oxoaristate (0.1g.) was ozonised as described for aristolactone,(Experiment 1).

The aqueous extract gave no colour with Schiff's reagent and failed to give a precipitate with dimedone solution. The oil gave a positive reaction with alkaline nitroprusside.

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§§§§§§§§§§

A P P E N D I X 1.

Helenin

The substance was obtained from Messrs. Lights as a colourless crystalline solid, m.p. 71-81°C., with a slight odour.

Tetrahydroalantolactone

Helenin (1.5g.) was dissolved in ethanol (20ml.) and hydrogenated at a platinum catalyst (0.1g.). Hydrogen uptake (342ml. at 19°C.) was equivalent to 2.4 ethylenic bonds. The catalyst was filtered off, and the solution concentrated to crystallisation. The crystals were recrystallised from ethanol as long needles, m.p. 145.5 - 146.5°C., $[\alpha]_D^{17°C.}$ +11.7° (c = 3.24)

Lit. values for tetrahydroalantolactone: m.p. 140 - 141°C. (3) 147.5°C. (37), $[\alpha]_D$ +11° (c = 2.5); $[\alpha]_D$ +15° (c = 5), (3) .

Dehydrogenation

Tetrahydroalantolactone (0.07g.) was treated as described under aristolactone, page 174. The absorption curve of the product is shown in appendix 2.

Methyl Methacrylate

The ester, b.p. 101-102°C., was prepared by the dry distillation of Pyrex sheet as described by Vogel (38).

Ozonolysis

Methyl methacrylate (0.5g.) in chloroform (20ml.) was treated with ozonised oxygen for 40 minutes. The solvent was removed under reduced pressure and the residue allowed to stand overnight with water (20ml.). Distillation of the mixture yielded formaldehyde (identified by means of its dimedone derivative) and methyl pyruvate identified by means of a positive nitroprusside reaction and by giving pyruvic acid after hydrolysis. The acid was identified by paper chromatography. R_F ozonolysis acid, 0.29. R_F pyruvic acid, 0.28 .

Bromination

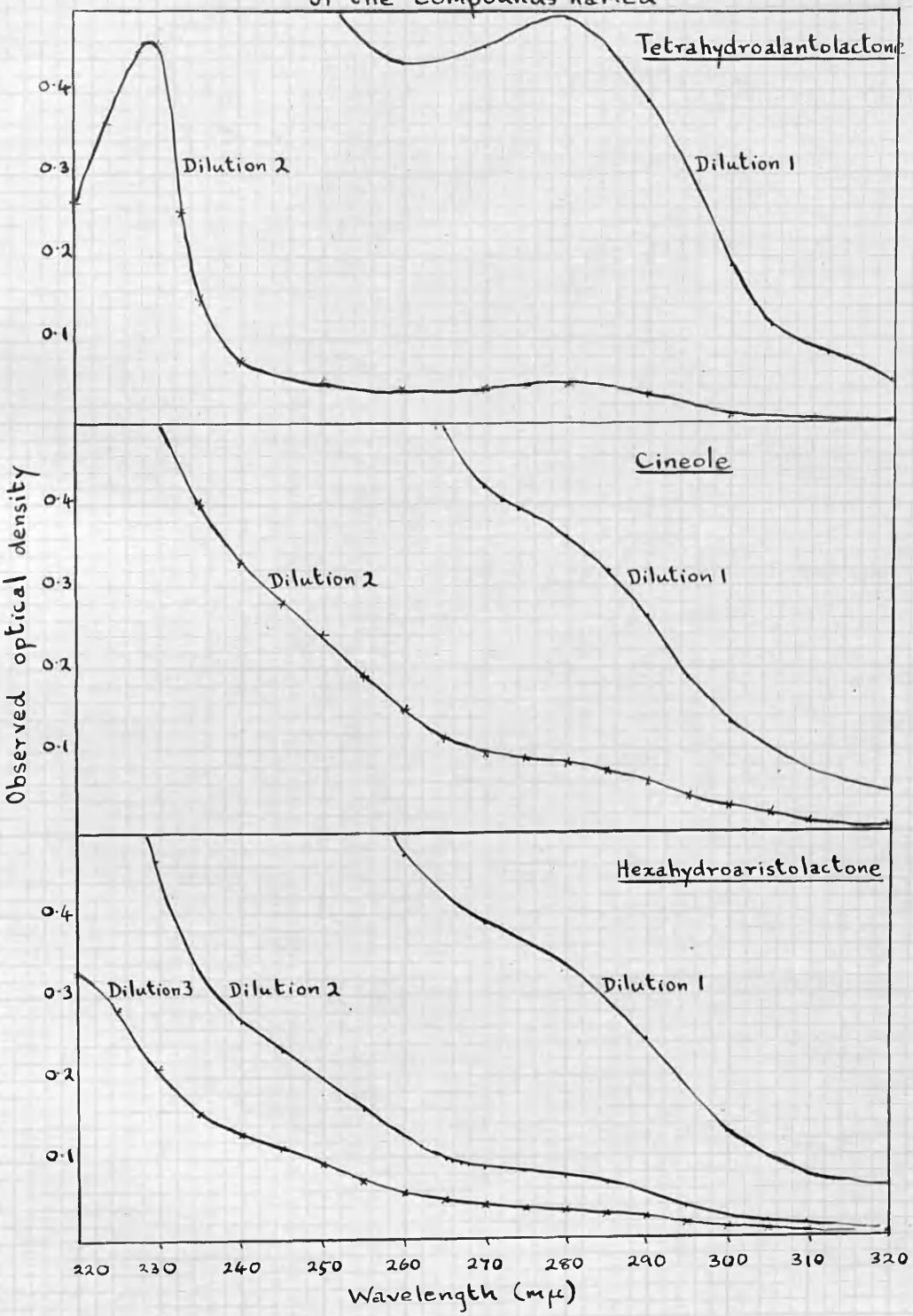
0.0465g. absorbed bromine equivalent to 0.7ml. 0.1N sodium thiosulphate.

Cineole

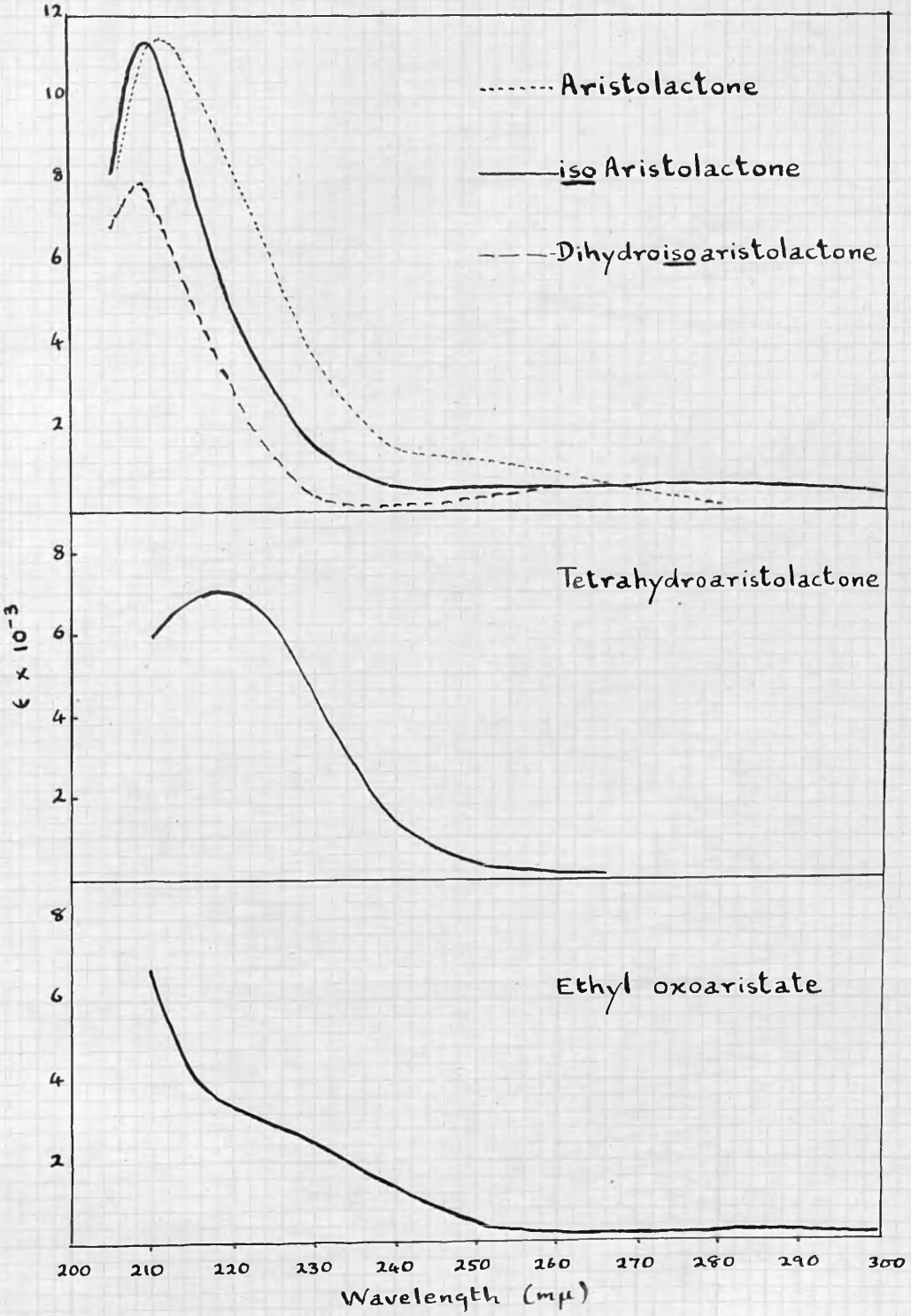
0.08g. was dehydrogenated by the method used for aristolactone (page 174) and aromatic constituents detected in the product.

APPENDIX 2.

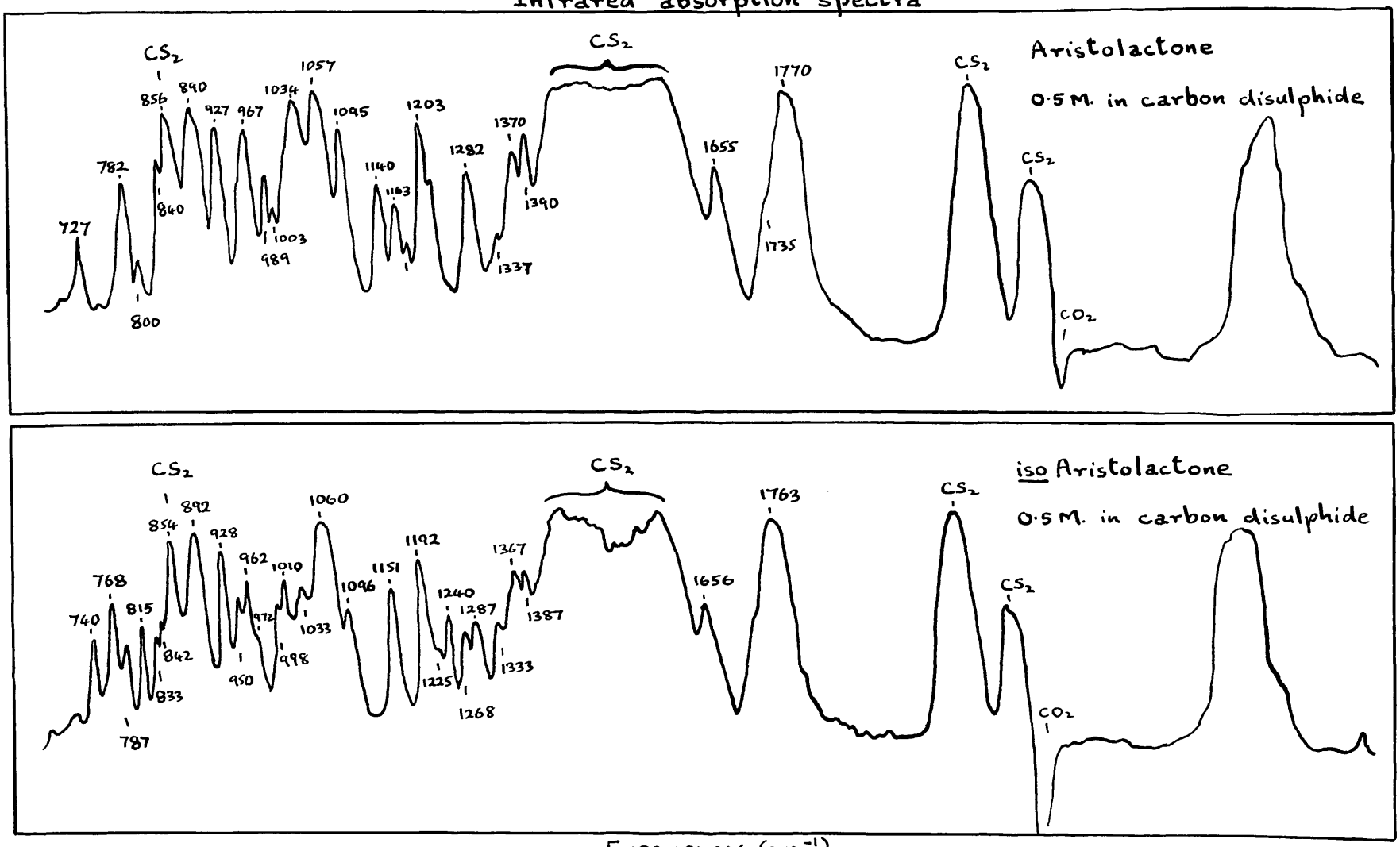
Ultraviolet absorption curves of the products of dehydrogenation of the compounds named



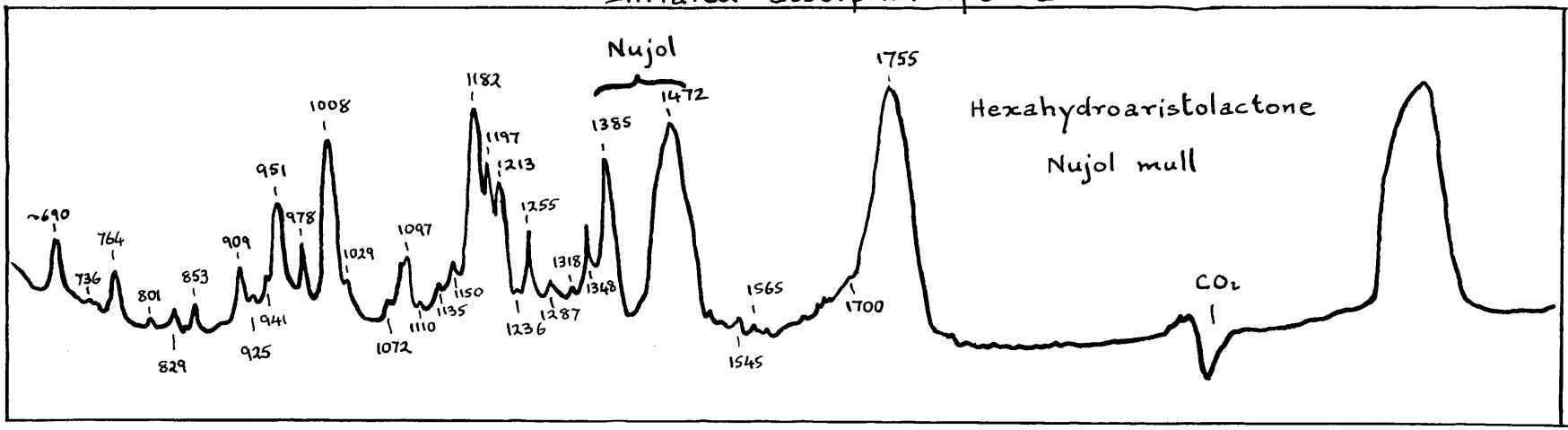
Ultraviolet absorption curves

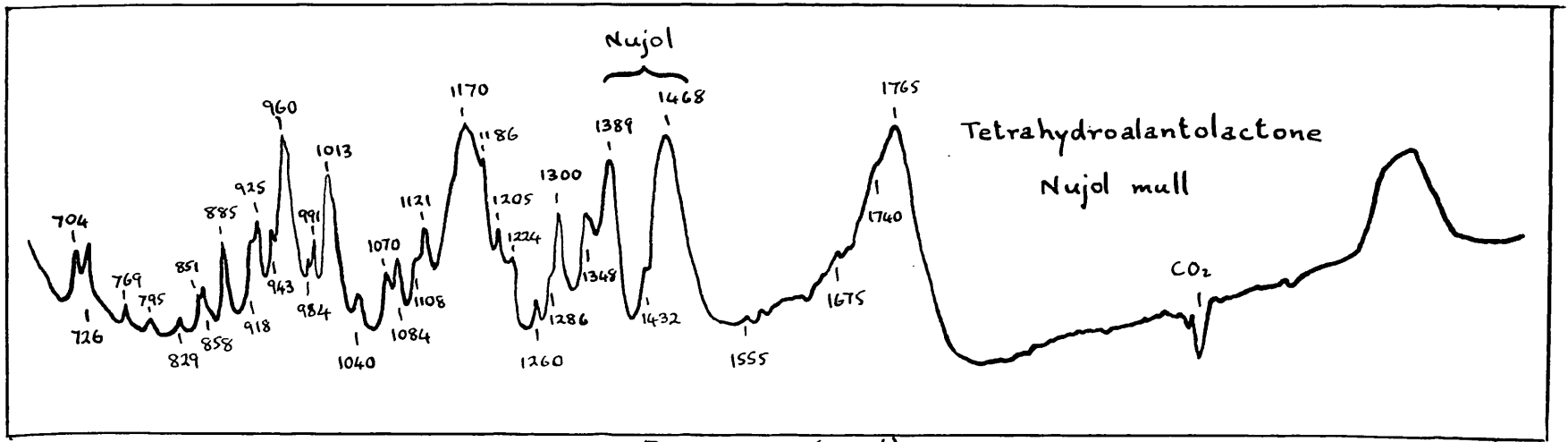


Infrared absorption spectra



Infrared absorption spectra





Frequency (cm^{-1})

Infrared absorption spectra