THE CHEMISTRY OF *ARISTOLOCHIA* Spp.

PART I. THE PETROL-SOLUBLE FRACTION FROM *Aristolochia reticulata*

BY J. B. STENLAKE and W. D. WILLIAMS

From The School of Pharmacy, The Royal Technical College, Glasgow

SPECIES of *Aristolochia* have been widely used in medicine since Greek and Roman times, though they have now largely fallen into disuse. Numerous observations have been recorded on the action and chemical constituents of *Aristolochia* of many different species (for references *see* Rosenmund and Reichstein[1]), but for the most part these studies are incomplete. At least one bitter principle, aristolochic acid, from which the *Aristolochia* are thought to derive their action, appears to be common to most species. The constitution of this acid is unknown, though Rosenmund and Reichstein[1] and others[2,3,4,5,6,7,8] have examined some of its reactions. The presence of an ethereal oil in *A. serpentaria* was mentioned first by Bucholz[9] and again, later by Spica[10], who recorded the presence of borneol. Peacock[11] examined the ethereal oil from *A. reticulata* and found it to consist mainly of an oily substance, $C_{15}H_{25}O_2$ which gave borneol and an acid (probably $C_5H_9O_2$) on saponification. A monoterpene, b.pt. 157° C., a fraction $C_{18}H_{29}O$, b.pt. 240° C., and a blue fluorescent oil were also obtained. More recently, 3 sesquiterpene fractions, designated ishwarene, ishwarol and ishwarone respectively have been found in the oil from the roots of *A. indica*[8]; the chemical structures of these substances have not yet been elucidated.

The present studies are concerned with the petrol-soluble fraction isolated from a batch of *A. reticulata* (serpentary root). The oil (3·9 per cent.), obtained by cold percolation of dried powdered serpentary root with light petroleum, was dark brown in colour, and of pleasant odour, similar to that of the root itself. Acidic substances were first removed by extraction of the oil in light petroleum, with saturated aqueous sodium bicarbonate, as a glassy resinous solid (0·25 per cent. of the oil), of high equivalent weight (*ca.* 1400), which exhibited a violet fluorescence under ultra-violet light.

Steam distillation of a small portion of the residual oil yielded, in the later runnings, a small quantity of a colourless crystalline solid. A much larger quantity of this same material was slowly deposited from the main bulk of the oil when it was cooled and seeded with crystals of the solid. This substance, a hitherto unknown lactone $C_{15}H_{20}O_2$, designated *aristolactone*, was crystallised in colourless platelets, m.p.t. 110·5° to 111° C. and will be described in greater detail in later communications.

Ketonic material present in the oil did not form a sodium bisulphite addition complex, but was readily extracted with Girard's reagent-T as a brownish oil (3 per cent.), which was strongly dextrorotatory, ($[\alpha]_D^{17° C.} + 170°$). A small portion of this ketonic 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 various terpene fractions cannot be discounted entirely. None of the usual crystalline derivatives could be isolated from either of these two ketone fractions, which therefore appear to require further fractionation.

Steam distillation of the oily residue yielded a number of volatile fractions (Table I).

TABLE I

Fraction	Weight, g.	Characteristics
I	200·0	Colourless mobile liquid (mainly light petroleum and ether) but containing traces of oil.
II	19·0	Colourless mobile liquid (mainly light petroleum).
III	22·4	Almost colourless fragrant oil.
IV	30·9	Pale yellow oil, $d_{15° \text{ C.}}^{15° \text{ C.}}$ 0·960, $[\alpha]_D^{16° \text{ C.}} - 6·94°$.
V	7·0	Yellow oil, $d_{15° \text{ C.}}^{17° \text{ C.}}$ 0·962, $[\alpha]_D^{17° \text{ C.}} + 33·0°$.

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.pt. 87° to 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 in the sequel. Repeated refractionation of fractions II and III gave further fractions as shown in Table II.

Unfortunately, fractionation on this small scale was not complete and most of the fractions were found to be mixtures. We have, however, succeeded in identifying a number of the components. Analysis of the physical data recorded for the various fractions suggested the presence of at least 3 components, a monoterpene (A and B), an ester (C and D) and a sesquiterpene (E and F).

Few monoterpenes have densities as high as those recorded for fractions A and B ($d_{15° \text{ C.}}^{15° \text{ C.}}$ 0·856 and 0·882 respectively) and contamination with denser ester fractions present in C and D was suspected. However, saponification revealed the presence of only traces of esters and chromatography of the neutral material gave an oil, $d_{15° \text{ C.}}^{15° \text{ C.}}$ 0·859, $n_D^{15·5° \text{ C.}}$ 1·4742, $[\alpha]_D^{17° \text{ C.}} -59°$, $[R_L]_D 44·59$. These constants are substantially in agreement with those for (+)-$\triangle^4$-carene (I), which are given[12] as $d_{30° \text{ C.}}^{30° \text{ C.}}$ 0·8552, $n_D^{30° \text{ C.}}$ 1·4740, $[\alpha]_D^{20° \text{ C.}} + 62·2°$, $[R_L]_D$ 44·60, the optical exaltation of the latter (0·75), over the theoretical value for a bicyclic monoethenoid terpene $C_{10}H_{16}$, $[R_L]_D$ 43·85 being due to conjugation of the ethylenic bond and *cyclo*propane ring. The presence of a single double bond in the molecule of our terpene was confirmed by an iodine value and by the low intensity ultraviolet absorption maximum at 210mμ (ϵ 4200), characteristic of a trisubstituted ethylenic bond[13,14]. Unfortunately $\triangle^4$-carene does not yield any well authenticated crystalline derivatives. Reaction with bromine is said to give

CH₃ (structure label)

(I)

1006
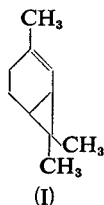
a viscid oil, which shows no sign of crystallising. Bromination of our purified monoterpene, similarly gave a colourless viscous oil, but which, after chromatography yielded a colourless crystalline dibromide, $C_{10}H_{16}Br_2$, m.pt. 89° C., $[\alpha]_D^{17°\ C.}$ + 98° identical (m.pt. and mixed m.pt.) with that obtained from fraction I. Further work on this terpene fraction is at present restricted from lack of material, but it is interesting to note, however, in connection with its tentative recognition as (—)-$\triangle^4$-carene, that, to date, only the dextrorotatory form of this terpene has been isolated from natural sources.

Constants for fraction C were in reasonable agreement with literature figures for (—)-bornyl formate ($d_{4°\ C.}^{20°\ C.}$ 1·006, $n_D^{15°\ C.}$ 1·4708, $[\alpha]_D$ —49°), but saponification with ethanolic potassium hydroxide gave the equivalent weight as 276, equivalent to only about 70 per cent. of bornyl formate. Extraction of the neutralised saponification liquors yielded neutral material, which was separated chromatographically into (—)-borneol, and a small quantity of a colourless oil, $C_{15}H_{24}$. (—)-Borneol, was confirmed by conversion to its *p*-nitrobenzoate and by oxidation to camphor. Physical constants of 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°, $[R_L]_D$ 65·46) were characteristic of a bicyclic sesquiterpene ($[R_L]_D$ 66·1) with two ethylenic bonds (*see* Simonsen and Barton[15]), and the presence of these two ethylenic bonds was confirmed by an iodine value. We have designated this sesquiterpene, *reticulene*.

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 solution was atypical, and acidification of the solution caused the deposition of oily droplets 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 distillate gave crystalline sodium formate, identified by conversion to the corresponding *p*-bromophenacyl ester, 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. The equivalent weight was found by titration to be 165. A more reliable figure of 169, obtained by gravimetric determination of barium (as sulphate) in the crystalline barium salt was in agreement with its formulation as $C_{10}H_{16}O_2$. The acid was unsaturated, containing a single ethylenic bond as indicated by the uptake of hydrogen on microhydrogenation. This conclusion was confirmed by the iodine value, equivalent to one double bond. The acid formed a crystalline benzyl*iso*thiouronium salt and with diazomethane it yielded a pleasant smelling (camphoraceous) liquid ester unfortunately, insufficient in quantity for proper characterisation.

Constants for the acid ($n_D^{20°\ C.}$ 1·5019; $d_{15°\ C.}^{15°\ C.}$ 1·050; $[R_L]_D$ 47·29) and for its reduction product ($d_{15°\ C.}^{15°\ C.}$ 1·030) suggested that the acid should be formulated with a monocyclic ($[R_L]_D$ 47·25) rather than an open chain structure

([R_L]$_D$ 49·45). This conclusion was supported by the molecular formula $C_{10}H_{16}O_2$. The low intensity ultra-violet absorption maximum at 215mμ (ϵ 2970), which was absent from the spectrum of the corresponding 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 in the purest form obtained, melted at 72° to 73° C. This substance exhibited a high intensity ultra-violet absorption maximum at 206mμ (ϵ 9980), characteristic of an α,β-unsaturated acid. The crystalline acid is similar to and is possibly identical with the acid isolated by Peacock,[11] which melted about 65° C. and gave a flesh-coloured precipitate with ferric chloride. Its formulation by Peacock as $C_5H_9O_2$ can be explained by the fact that it was based not upon a direct analysis of the acid, but merely on an analysis of the parent bornyl ester fraction, which had also been shown to contain acetic acid.

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.

EXPERIMENTAL

Melting points are uncorrected. Rotations were determined in absolute ethanol (unless otherwise stated) in a 1 dcm. tube. Ultra-violet absorption spectra were determined in absolute ethanol in 1 cm. cells using a Hilger Uvispek photoelectric spectrophotometer.

Extraction of Serpentary Root. The dried root (28 lb.) in No. 60 powder, was extracted with light petroleum (b. pt. 40° to 60° C.) by cold percolation until the percolate was just very pale yellow. Concentration of the percolate gave a dark greenish-brown oil (700 g.), still containing some solvent and having a pleasant odour similar to that of the root.

Removal of free acids. The oil from the above extraction was diluted with light petroleum and extracted with saturated aqueous sodium bicarbonate. The acids, recovered by acidification of the aqueous solution and extraction with light petroleum were obtained as a pale brown viscous oil (0·51 g.).

Aristolactone (a) The oil from a premilinary small scale extraction of *A. reticulata* (6 lb.) was steam distilled and the distillate collected in 27 fractions, each of 350 to 500 ml. The oily material present in each fraction of distillate was extracted with light petroleum. Fractions 6, 7, 9 and 10 on evaporation yielded pale yellow oils, from which aristolactone separated in varying amount, as a colourless solid, m.pt. 104° to 108° C. raised by recrystallisation from acetone-water to 110·5° to 111° C., [α]$_D^{14° C.}$ + 156·4° (C, 1). Found: C, 77·5; H, 8·8 per cent. $C_{15}H_{20}O_2$ requires C, 77·6 H, 8·7 per cent.

(b) the bulk sample of oil (700 g.) when concentrated to remove the last traces of light petroleum and seeded with a single crystal of aristolactone and cooled (refrigerator, 2 days) gave crude crystalline aristolactone (16 g.). Seeding is essential, cooling alone being insufficient.

Isolation of Carbonyl Compounds

(a) *Sodium Bisulphite Addition Compounds.* A light petroleum solution of the oil remaining after the separation of aristolactone was shaken with saturated aqueous sodium bisulphite continuously for 4 days. Small quantities of amorphous material separated at the interphase; further treatment with sodium carbonate failed to yield carbonyl compounds.

(b) *Extraction with Girard's Reagent-T.* The extract (100 g.) was refluxed for 1 hour with a solution of Girard's Reagent-T (10 g.) in a mixture of glacial acetic acid (10 ml.) and absolute ethanol (90 ml.).

After cooling and diluting with iced-water (to reduce the ethanol content of the solution to 10 per cent. w/v) neutralisation of 90 per cent. of the acetic acid (N sodium hydroxide; 158 ml.) and extraction of non-ketonic material with light petroleum, the aqueous solution of ketonic material was treated with sulphuric acid to give an approximately normal solution. This solution was allowed to stand for 1 hour and extracted with solvent ether. The ethereal solution was dried (sodium sulphate), treated with magnesium oxide (to remove acetic acid) and evaporated to give a pale brownish-yellow oil (3·14 g.), $[\alpha]_D^{17°\ C.} + 170°$ (C, 2·38).

(c) *Fractionation of the Ketonic Oil.* The oil (0·2 g.) was shaken with light petroleum (50 ml.). Insoluble material remained as a brownish-yellow viscous semi-solid (0·042 g.), $[\alpha]_D^{13°\ C.} + 62°$ (C, 0·428). Evaporation of the light petroleum gave a pale yellow oil (0·146 g.), $[\alpha]_D^{13°\ C.} + 179°$ (c, 2·07).

Steam Distillation of the Residual Oil. The oil, maintained at 120° to 130° C. was steam distilled and collected first in fractions of 1 l. (I-III) and later of 2 l. (IV and V). The oils, which readily separated were dried over sodium sulphate. Descriptions of these fractions are recorded in Table I.

Fraction I. The liquid (*ca.* 200 g.) consisting mainly of light petroleum, but containing traces of lævorotatory oil, was treated with bromine until the reagent was no longer decolourised. Evaporation of the solvent gave a trace of colourless oil (0·1 g.) which when, chromatographed on alumina from light petroleum, gave 3 fractions. The first and last fractions yielded colourless oils, which were not identified; the second fraction was obtained as a colourless, dextrorotatory crystalline solid, m.pt. 87° to 88° C. (from light petroleum).

Fractional Distillation of II and III. Fractions II and III from the steam distillation were combined and submitted to repeated fractional distillation under reduced pressure. Separation into fractions was followed by measurement of refractive index, density and optical rotation of each fraction obtained throughout the process. Constants for the 6 main fractions are given in Table II. Minor fractions, not specified in detail in Table II were also obtained at various stages of the fractionation procedure (Table III).

Fraction A. The oil (0·355 g.) was dissolved in light petroleum and treated with bromine until the reagent was no longer rapidly decolourised. Evaporation of the solvent gave a colourless oil, which when chromatographed on alumina from light petroleum gave 3 fractions, of which the second was obtained as a colourless crystalline dibromide (0·123 g.),

TABLE II

Fraction	B.pt., ° C./mm.	$n_D^{20°\,C.}$	$d_{15°\,C.}^{15°\,C.}$	$[\alpha]_D^{13°\,C.}$	Weight, g.
A	106° to 140° C. atm.	1·4770	0·856	− 30° (at 16°)	1·0
B	105° to 110° C. (bath)/75	1·4755	0·882	− 50°	1·43
C	114° C. (bath)/17	1·4765	1·000	− 44·4°	4·8
D	120° to 126° C. (bath)/18	1·4841	0·961	− 23·3°	3·8
E	130° to 132° C. (bath)/18	1·4910	0·937	− 8·3°	3·1
F	132° to 136° C. (bath)/18	1·4971	0·942	0°	2·4
Minor and intermediate fractions					8·0
Residues ..					3·5

TABLE III

B.pt. ° C./mm.	$n_D^{20°\,C.}$	$d_{15°\,C.}^{15°\,C.}$	Weight
			g.
109° to 110° C. (bath)/17	1·4755	0·992	4·0
111° to 114° C. (bath)/17	1·4764	0·998	4·0
114° to 120° C. (bath)/17	1·4802	0·979	1·0

m.pt. 89° C., $[\alpha]_D^{17°\,C.}$ + 98° (C, 0·46). Found, C, 40·65; H, 5·5; Br, 54·5 per cent. $C_{10}H_{16}Br_2$ requires C, 40·6; H, 5·5; Br, 53·9 per cent. The remaining two fractions were obtained as colourless oils, the first (0·152 g.) being odourless, and the third (0·118 g.) having a distinct camphoraceous odour.

Fraction B. (a) The oil (0·93 g.) was refluxed for 30 minutes with ethanolic potassium hydroxide (0·66N; 10 ml.) and neutralised with 0·5N hydrochloric acid. Extraction of the solution with light petroleum yielded a colourless oil, which when chromatographed on alumina from light petroleum yielded two fractions, of which the first was a colourless oil (B′;0·4 g.); $d_{15°\,C.}^{15°\,C.}$ 0·859, $n_D^{17°\,C.}$ 1·4742, $[\alpha]_D^{15°\,C.}$ − 59° (C, 6·2) λ max. 210 mμ, $E_{1\,cm.}^{1\,per\,cent.}$ 313 (ε 4260 based on $C_{10}H_{16}$). The second fraction was identified as (−)-borneol. (Note: Poor recoveries were due to deliberate loss by evaporation to ensure complete removal of light petroleum).

(b) *Iodine Value* (B′). B′(13 mg.) was treated with pyridine bromide reagent (5 ml.) for 10 minutes by the official method, and, after the addition of potassium iodide, titrated with 0·02N sodium thiosulphate. Halogen uptake was equivalent to 1·05 double bonds.

(c) *Bromination* (B′). B′(0·2 g.) was dissolved in light petroleum and treated with bromine until the reagent was no longer rapidly decolourised. Evaporation of the solvent gave a colourless oil, which when chromatographed on alumina from light petroleum gave 3 fractions, of which the second was obtained as a colourless crystalline dibromide, m.pt. 87·5° to 88° C. undepressed on admixture with that obtained from A, above.

Fraction C. (a) *Saponification.* The oil (4 g.) was refluxed for 30 minutes with ethanolic potassium hydroxide (0.66N; 50 ml), and neutralised by titration with 0·5N hydrochloric acid. Equiv. wt. 276 (equivalent to *ca.* 70 per cent. of bornyl formate). Extraction of the solution with light petroleum yielded an oily semi-crystalline solid, which when chromatographed from light petroleum on alumina yielded two fractions, of which the first, *reticulene,* was obtained as a colourless oil

(0·18 g.), $n_D^{16°\,C.}$ 1·4955; $d_{16°\,C.}^{16°\,C.}$ 0·913; $[\alpha]_D^{15°\,C.} + 1·6°$ (C, 4·47). Found C, 88·35; H, 11·6 per cent. $C_{15}H_{24}$ requires C, 88·2; H, 11·8 per cent. The second fraction, a colourless crystalline solid, was identified as $(-)$-borneol, m.pt. 206° C. (from light petroleum); *p*-nitrobenzoate, m.pt. 134° to 135° C., $[\alpha]_D^{19°\,C.} - 34·7°$ (C, 1·3 in chloroform). (Hückel and Kaluba[16] give m.pt. 136° C, $[\alpha]_D^{20°\,C.} - 34·2°$ {c, 4 in chloroform}). Found: C, 67·9, 67·9; H, 7·3, 6·8; N, 4·8 per cent. Calc. for $C_{17}H_{21}O_4N$, C, 67·3; H, 7.0; N, 4·64 per cent. Oxidation with chromic acid gave camphor, m.pt. 176° C., 2:4-dinitrophenylhydrazone, m.pt. 174° to 175° C.

(b) *The Water-insoluble Acid.* (i) *Isolation.* Acidification of the aqueous liquors from the saponification, extraction with light petroleum, drying (sodium sulphate) and evaporation of the solvent yielded a colourless, oily, optically inactive acid (0·29 g.), $n_D^{20°\,C.}$ 1·5019, $d_{15°\,C.}^{15°\,C.}$ 1·050, λ max. 215 mμ $E_{1\,cm.}^{1\,per\,cent.}$ 177. The oil on standing for several weeks slowly crystallised. Chromatography of the crystalline material from light petroleum on a column consisting of a mixture of activated charcoal (1 part) and Whatman filter paper pulp (3 parts) yielded a colourless crystalline acid, m.pt. 72° to 73° C., λ max 206 mμ $E_{1\,cm.}^{1\,per\,cent.}$ 594 (ϵ 9980 based on $C_{10}H_{16}O_2$). Equiv. wt. (titration) 165; benzyl*iso*thiouronium salt, m.pt. 124° to 125° C.

Ferric Salt, formed as a buff precipitate on addition of ferric chloride solution to an aqueous solution of the sodium salt.

(iii) *Silver Salt*, formed as a white gelatinous precipitate on the addition of silver nitrate solution to an aqueous solution of the sodium salt.

(iv) *Barium Salt*, formed as a microcrystalline solid (from aqueous ethanol) by neutralisation of an ethanolic solution of the acid with 0·05N barium hydroxide. Found (sulphated ash): Ba, 49·26 per cent. $C_{10}H_{15}O_2Ba_{\frac{1}{2}}$ requires Ba, 49·43 per cent.

(v) *Hydrogenation* of the acid (113 mg.) at a palladium charcoal catalyst gave a saturated acid, obtained as a colourless oil, $d_{15°\,C.}^{15°\,C.}$ 1·030. The absorption of hydrogen was equivalent to 1·2 mols.

(vi) *Iodine Value.* The acid (15·1 mg.) was treated with pyridine bromide reagent (3 ml.) for 10 minutes by the official method and, after the addition of potassium iodide, titrated with 0·02N sodium thiosulphate. Halogen uptake was equivalent to 0·89 double bonds.

(c) *Identification of Formic Acid in the Residual Saponification Liquors.* Distillation of the residual liquors gave an aqueous solution, which exhibited the typical properties of formic acid. The acid was isolated as its sodium salt and characterised by (*a*) reduction of neutral silver nitrate solution, (*b*) production of a red colour with ferric chloride solution (buff precipitate on heating with excess reagent), (*c*) conversion to sodium oxalate by rapid heating to 360° C., (*d*) *p*-bromophenacyl ester, m.pt. 137° to 139° C. undepressed on admixture with the *p*-bromophenacyl ester of an authentic sample of sodium formate (m.pt. 135° to 138° C.). Found (sulphated ash): Na, 32·8 per cent. Calc. for H.COONa; Na, 33·8 per cent.

Fraction D. Saponification of the oil (3·714 g.) and separation of neutral and acidic fractions, as described above for fraction C, gave a further

0·353 g. of water-insoluble acid. The neutral fraction, after chromatography from light petroleum on alumina as described above, gave borneol (0·51 g.) and 3 oily fractions (2·5 g. in all; the first of these oils (1·0 g.) after repeated chromatography from light petroleum on alumina gave reticulene (0·409 g.), $n_D^{15°\,C.}$ 1·4944, $d_{15°\,C.}^{15°\,C.}$ 0·912 [α]$_D^{15°\,C.}$ + 1·5° (C, 3·92).

Fraction E. Saponification of the oil (3·075 g.) and separation of neutral and acidic fractions, as described above for fraction C, gave a further 0·272 g. of water-insoluble acid. The neutral fraction, after chromatography from light petroleum on alumina as described above, gave reticulene (1·794 g.), $n_D^{16°\,C.}$ 1·4955, $d_{16°\,C.}^{16°\,C.}$ 0·913, [α]$_D^{15°\,C.}$ + 1·6° (C, 4·47).

Fraction F. Saponification of the oil (1·897 g.) and separation of neutral and acidic fractions, as described above for Fraction C, gave only traces of water-insoluble acid. The neutral fraction, after chromatography on alumina from light petroleum, as described above, gave reticulene (1·189 g.), $n_D^{14°\,C.}$ 1·4972, $d_{15°\,C.}^{15°\,C.}$ 0·914, [α]$_D^{15°\,C.}$ + 1·1° (C, 4·2).

Iodine Value of Reticulene. Reticulene (58·8 mg.) was treated with pyridine bromide reagent (30 ml.) and, after the addition of potassium iodide, titrated with 0·1N sodium thiosulphate. Halogen uptake was equivalent to 2·2 mols.

SUMMARY

The light petroleum-soluble fraction from a batch of *Aristolochia reticulata* has been examined and found to contain the following:

1. A ketonic oil, consisting of at least 2 unidentified components.

2. A crystalline lactone, $C_{15}H_{20}O_2$, m.pt. 110·5° to 111° C., designated aristolactone.

3. A bicyclic monoterpene, which has been tentatively identified as (−)-$\triangle^4$-carene.

4. A mixture of esters, which on saponification yields (−)-borneol formic acid and an unidentified cyclic unsaturated acid, $C_{10}H_{16}O_2$.

5. A bicyclic sesquiterpene, $C_{15}H_{24}$, designated reticulene.

REFERENCES

1. Rosenmund and Reichstein, *Pharm. Acta Helvet.*, 1943 ,**18**, 243.
2. Walz, *Jahrb. F. prakt. Pharmacie*, 1852, **24**, 65.
3. Pohl, *Arch. exp. Path. Pharmak.*, 1892, **29**, 282.
4.· Hesse, *Arch. Pharm.*, 1895, **233**, 684.
5. Idem, *Pharm. J. and Transactions*, 1892, (3), **22**, 551.
6. Castille, *J. Pharm. Belg.*, 1922, **4**, 141, 569.
7. Krishnaswamy and Munjaneth, *J. Indian. chem. Soc.*, 1935, **12**, 476.
8. Krishna Rao, Munjaneth and Menon, *ibid.*, 1935, **12**, 494.
9. Bucholz, *Berl. Jahrb.*, 1807, 127.
10. Spika, *Gazz. chim. Ital.*, 1887, **17**, 313.
11. Peacock, *Amer. J. Pharm.*, 1891, **63**, 257.
12. Simonsen, *J. chem. Soc.*, 1922, **121**, 2292.
13. Henbest and Wood, *Chem. Ind.*, 1951, 866.
14. Halsall, *ibid.*, 1951, 867.
15. Simonsen and Barton, *The Terpenes*, III, 2. Cambridge University Press, 1952.
16. Hückel and Kaluba, *Ann.*, 1942, **550**, 269.

DISCUSSION

The paper was presented by DR. J. B. STENLAKE.

DR. W. M. MITCHELL (London) said the authors took the view that the light petroleum-insoluble part of the ketonic fraction was an artefact. He thought it more likely that it was an original constituent retained in petroleum solution by the presence of other constituents of the oil, a not unusual happening when fractionating natural products.

DR. J. W. ROWSON (London) asked whether it had been confirmed that the commercial sample used was an authentic specimen of *Aristolochia reticulata?*

DR. G. E. FOSTER (Dartford) asked whether there was any evidence that the bitter principle was an alkaloid.

DR. STENLAKE, in reply, said that they were undecided whether the suggestion made by Dr. Mitchell was correct. On re-examination recently, they noted that the oily fraction had undergone considerable decomposition and deposition of solid matter, and it would be investigated further. Two batches of material were used in the work and samples from each examined and the authenticity of the material confirmed. In their petroleum extracts they had found traces of basic material. In other experiments, where they had further extracted the defatted drug, a basic material of fairly high molecular weight was present in quite small amounts, being about 0·1 or 0·05 per cent. of the total solid matter.