Tetrahedron, 1958, Vol. 2, pp. 256–270. Pergamon Press Ltd., London

THE CONSTITUENTS OF *PHEBALIUM NUDUM* HOOK—I

THE BARK

L. H. BRIGGS and R. C. CAMBIE
Department of Chemistry, Auckland University College, New Zealand

(*Received* 21 *October* 1957)

Abstract—By a systematic investigation of the extractives of the bark of *Phebalium nudum* Hook twenty-one compounds have been isolated. Seventeen have been identified as D-citronellal, geranial, neral, acetic, *iso*valeric, palmitic and cinnamic acids, *iso*eugenol, β-sitosterol, α-terpineol, dictamnine γ-fagarine, evolitrine, skimmianine, kokusaginine, 9-hydroxy-4-methoxyfurano(3,2-*g*)benzopyran-7-one, and ellagic acid. The remaining four substances were obtained in a crystalline state, but in insufficient quantity for complete investigation. Two are new and appear to be of the coumarin type; they have been named phebalin and phebalarin. A partial structure has been proposed for phebalarin. The other two are aliphatic. The presence of at least a further ten phenolic substances has been shown by paper chromatography.

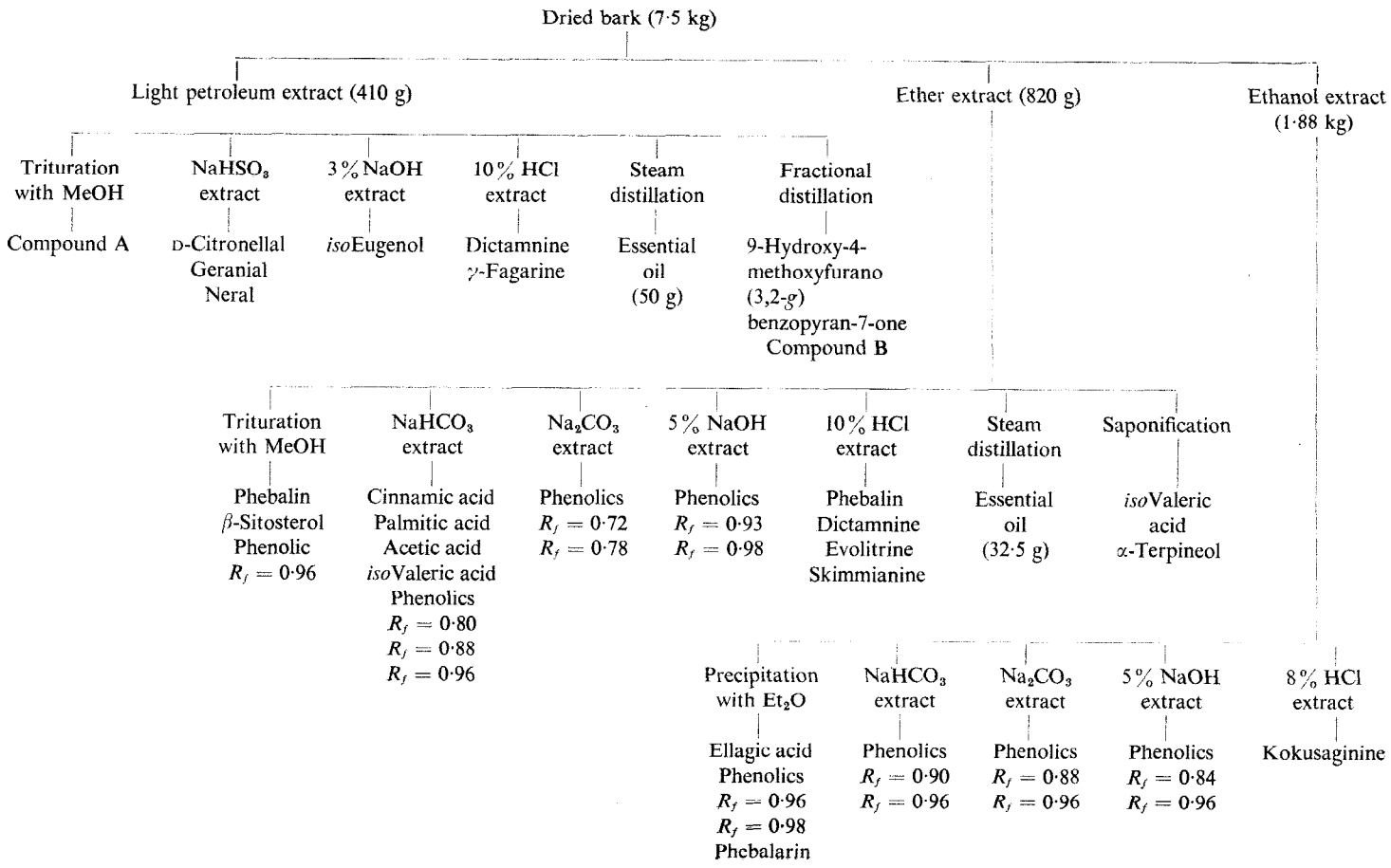
Phebalium nudum Hook (Maori name "Mairehau") is a shrub species of the Rutaceae and is the only member of the *Phebalium* genus found in New Zealand where it is endemic and confined to the northern part of the North Island. The leaves and bark possess a strong aromatic odour and the bark is coloured a pale yellow. The essential oil has been investigated by Radcliffe and Short[1] and found to contain citronellal, citral, acetic, *iso*valeric and cinnamic acids, an unidentified phenol, camphene, limonene, terpinyl acetate, and a mixture of unidentified sesquiterpenes.

The family Rutaceae is noted for its extremely diversified series of extractives, a common characteristic being the presence of methoxy and methylenedioxy groups, but seldom free phenolic groups. In view of the strong alkaloidal tests shown by the bark it was expected that the alkaloids present would be of the type related to anthranilic acid and particularly those isolated from Australian species.[2] Preliminary investigation on aliquot samples indicated that the alkaloids represented only a minor fraction of the total extracts. They could be isolated by acid extraction of the initial solvent extracts but precipitation as the picrates always gave products contaminated with large amounts of acid soluble tars. If the acidic and phenolic constituents were first removed by alkaline extraction, residues were obtained which on subsequent acid treatment, although giving fractions heavily contaminated with impurities, did lead to tractable products. Only in the case of the methanol extract were water soluble alkaloids observed by the usual test reagents, and these products represented but a minute quantity of the total alkaloidal content. A comprehensive investigation of the total extractives was therefore commenced, based on these preliminary investigations. The constituents and the extraction and fractionation scheme are summarised in Table 1.

Successive extraction with light petroleum, ether and ethanol gave a total extractive yield of 41·5 per cent of the dried bark, a considerable quantity of this consisting of phlobaphenic material. Both the light petroleum and ether extracts were mainly composed of oily products, difficult to separate and difficult to remove from solid products isolated. A complete investigation of the extractives would

[1] C. B. Radcliffe and W. F. Short *J. Soc. Chem. Industr., Lond.* **47**, 324T (1928).
[2] J. R. Price *Progress in the Chemistry of Organic Natural Products* Vol. XIII, pp. 302–345. Springer Publishing House, Austria (1956).

TABLE 1. *Phebalium nudum* BARK EXTRACTIVES



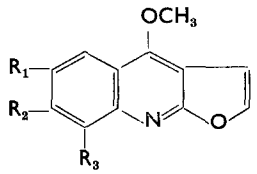
therefore entail techniques for the examination of an essential oil. Such an investigation was not attempted in the present work, attention being directed to the isolation and characterisation of the alkaloids and solid products present. By testing the products of the fractionation scheme with Mayer's and Dragendorff's reagents the location of the alkaloidal material could be determined during each step.

Trituration of the light-petroleum extract (5·47 per cent yield) with methanol gave a colourless neutral product, compound A, $C_{23}H_{44}O$, m.p. 81·5–82°, purified by crystallisation and chromatography. The infra-red spectrum showed strong bands at 1736 cm^{-1} (carbonyl) and 2924 cm^{-1} (CH_2). The substance, however, showed no unsaturation and gave no carbonyl derivatives. From these results it appears to be a monocyclic hydrocarbon possessing a sterically hindered oxo grouping. There was insufficient material for further investigation.

The aldehydes in the residual oil were extracted from their ethereal solution with aqueous sodium bisulphite and regenerated with sodium carbonate. The free aldehydes were separated by fractional distillation *in vacuo* and were identified as D-citronellal and citral. The semicarbazone of citral was further separated into geranial semicarbazone (major portion) and neral semicarbazone by fractional crystallisation from alcohol.[3] They could also be separated by the solubility of neral semicarbazone in ether, geranial semicarbazone remaining undissolved.[4]

Trial extractions of the ethereal solution, free from aldehydes, with saturated sodium hydrogen carbonate and 10% sodium carbonate solutions gave no products. These extracts were therefore omitted from the major fractionation scheme. Extraction with 3% sodium hydroxide gave no major solid products but ferric chloride tests indicated the presence of phenolic compounds. Solid derivatives were not obtained from the oily fraction by attempted purification by preparation of acetates, 3:5-dinitrobenzoates or chloracetic acid derivatives. Fractional distillation *in vacuo* however, gave as the major product, *iso*eugenol, purified by refractionation. The identity was confirmed by paper chromatography, the infra-red spectrum,[5] and by comparison of the 3:5-dinitrobenzoate with an authentic sample. A further yield was also obtained by steam-distillation of the crude oil.

The alkaloids were completely extracted from the ether solution with 10% hydrochloric acid and isolated by basification. The crude precipitate was separated by repeated fractional crystallisation from ethyl acetate–light petroleum into a crystalline alkaloid and a more soluble product. The crystalline alkaloid, further purified by chromatography, was identified as dictamnine, 4-methoxyfuro(2,3-*b*)quinoline[2] (I; $R_1 = R_2 = R_3 = H$) by circular paper chromatography in two solvent systems, the ultra-violet spectrum,[6] and by comparison of the free base with an authentic

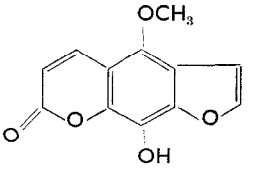


(I)

[3] E. Guenther *The Essential Oils* Vol. II, p. 326. Van Nostrand, New York (1948).
[4] E. S. Guenther and C. H. Grimm *J. Amer. Chem. Soc.* **60**, 933 (1938).
[5] L. Levi, J. L. Thompson, J. C. Evans, H. Bernstein, S. A. Forman and N. A. Miles *Proc. Sci. Sec., Toilet Goods Ass.* No. 25 (1956).
[6] J. Iriarte, F. A. Kincl, G. Rosenkranz and F. Sondheimer *J. Chem. Soc.* 4170 (1956).

sample. The more soluble alkaloid, γ-fagarine, 4:8-dimethoxyfuro(2,3-*b*)quinoline (I; $R_1 = R_2 = H$, $R_3 = OCH_3$), was isolated via the picrate and identified by comparison with an authentic sample.

A pale green essential oil (0·66 per cent yield), not further examined, was separated from the oil left after chemical fractionation, by steam-distillation. The residue was then fractionally distilled *in vacuo*, in twenty fractions which were stored at 0° for further investigation. The higher boiling fractions, after 3 months deposited a yellow phenolic substance, $C_{12}H_8O_5$, m.p. 222·5–223° identified by infra-red and ultra-violet



(II)

spectra and mixed m.p. as 9-hydroxy-4-methoxyfurano (3,2-*g*)benzopyran-7-one(II), recently found in the seeds of *Casimiroa edulis*.[7] To avoid confusion and to draw attention to the identity of the furanocoumarin with that isolated from *Casmiroa edulis* the name assigned by Rosenkranz *et al.*[7] has been adhered to for this compound. In the more usual system of naming it would be 8-hydroxy-5-methoxypsoralene.

Chromatography on alumina of the combined oil from the higher boiling fractions and the oil after separation of the furanocoumarin, gave a further neutral substance, compound B, $C_{13}H_{26}O$, m.p. 94°. It was not isolated in sufficient quantity for further investigation.

The brown viscous oil (10·93 per cent yield) obtained from the ether extract of the bark gave, on trituration with methanol and further purification, a crystalline compound, $C_{20}H_{18}O_4$, m.p. 175–176·5°. It contained one methoxyl group and from the infra-red[7] and ultra-violet[8] spectra appeared to be of the coumarin type. The compound is apparently new and has been named phebalin. Further structural studies are in progress. From an ether extract obtained during the purification of phebalin, β-sitosterol was isolated by chromatography on alumina. A phenolic impurity from this fraction was shown to be homogeneous by paper chromatography but was not obtained as a solid by chromatography on alumina.

The ether–alcohol solution remaining after trituration was fractionated between saturated sodium hydrogen carbonate, 10% sodium carbonate and 5% sodium hydroxide. The alkaloids were then removed by further extraction with 10% hydrochloric acid.

Cinnamic acid was isolated by crystallisation from a hot aqueous extract of the acidified sodium hydrogen carbonate fraction. From the aqueous phase after acidification three aliphatic acids were isolated by ether extraction and fractional distillation. The two acids boiling below 200° were identified as acetic and *iso*valeric acids and the higher boiling acid, obtained as a crystalline solid after chromatography, was identified as palmitic acid.

No solid material was obtained from the remaining phenolic fraction of the sodium hydrogen carbonate extract or the sodium carbonate and sodium hydroxide

[7] F. A. Kincl, J. Romo, G. Rosenkranz and F. Sondheimer *J. Chem. Soc.* 4163 (1956).
[8] W. L. Stanley and S. H. Vannier *J. Amer. Chem. Soc.* **79**, 3488 (1957).

fractions by attempted acetylation. Each fraction behaved as a single substance when chromatographed on magnesia–celite or alumina. However, the presence of three phenolic substances in the sodium hydrogen carbonate fraction was indicated by paper chromatography. The other phenolic extracts each contained two substances. Forestal solvent[9] was used in all the paper chromatographic investigations. Bate-Smith[10] has shown that a large number of phenolic residues can be recognised using this solvent and that it is unequalled for general purposes. By using diazo spray reagents he has shown that distinctive colours are given with particular constituents and that the most useful of such reagents was diazotised *p*-nitroaniline diluted with 2 N sodium acetate. Examination of the chromatographic strips under ultra-violet light further distinguishes coumarin compounds by their blue-fluorescing properties from the more common phenolic constituents e.g. sinapic and ferulic acids. Bate-Smith[10] has shown further, that many members of the Rutaceae give complicated patterns of blue-fluorescing constituents, few of which have been identified. All the fractions from *Phebalium nudum* Hook, examined by these methods showed phenolic constituents with high R_f values, suggesting compounds of high complexity. Blue-fluorescing coumarin derivatives were absent in all cases when strips were examined with ammonia vapour.

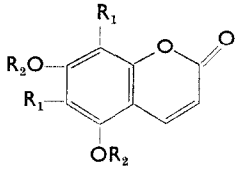
The alkaloids of the ether extract were completely contained in the 10% hydrochloric acid fraction. Dictamnine was again the principal alkaloid. By fractional crystallisation of the crude precipitate obtained on basification, a small yield of a crystalline furoquinoline alkaloid was isolated, identified by analysis of the picrate, m.p. and ultra-violet spectrum as evolitrine, 7-methoxyfuro(2,3-*b*)quinoline[2] (I; $R_1 = R_3 = H$, $R_2 = OCH_3$) previously only isolated in low yield from *Evodia littoralis*.[11] Dictamnine, evolitrine and phebalin (product of mechanical carry-over) were isolated from the mother-liquors of the fractional crystallisation by chromatography on alumina. A further furoquinoline alkaloid was isolated by ether extraction of the basified aqueous extract and precipitation as the crude picrate. The major product, after regeneration of the base by chromatography on alumina, was skimmianine, 4:7:8-trimethoxyfuro(2,3-*b*)quinoline[2] (I; $R_1 = H$, $R_2 = R_3 = OCH_3$), identified by comparison with an authentic sample.

The dark viscous oil remaining after fractionation was further separated into a steam-volatile essential oil (0·43 per cent) and a larger non-volatile fraction. Solid products were not isolated from the latter fraction by chromatography. Saponification followed by chromatography however, gave α-terpineol from the initial fractions. The major acid after saponification was *iso*valeric acid.

An aliquot of the ethanol extract (25·07 per cent yield) was dissolved in ethanol and the more insoluble constituents precipitated by the addition of ether. The material remaining in solution was then fractionated in a similar manner to that of the ether extract of the bark. The precipitated solid was dissolved in ethanol and a further phenolic fraction (two spots on chromatographic investigation) separated by the addition of water. Attempts to isolate traces of water soluble alkaloids from the aqueous ethanol solution by extended chloroform extraction were unsuccessful. However, fractionation of the residue after removal of the solvent gave a yellow

[9] E. C. Bate-Smith *Biochem. J.* **58**, 122 (1954).
[10] E. C. Bate-Smith *Sci. Proc. R. Dublin Soc.* **27**, 165 (1956).
[11] R. G. Cooke and H. F. Haynes *J. Aust. Chem.* 7, 273 (1954).

crystalline coumarin, m.p. 125–126°, which possessed a free phenolic grouping. Analysis gave the formula $C_{15}H_{18}O_6$, and the ultra-violet spectrum was very similar to that of 5-geranyloxy-7-methoxycoumarin and 5:7-dimethoxycoumarin[8] suggesting that it is a 5:7-dialkoxycoumarin. A band in the infra-red at 3546 cm^{-1} was due to free hydroxyl stretching and bands assignable to tertiary hydroxyl deformations appeared at 1157, 1399, 1385, and 1337 cm^{-1}. The compound does not correspond to any naturally occurring coumarin[12] and has been named phebalarin. The partial structure III (R_1 = H or OH; R_2 = CH_3 or $CH_2{\cdot}CH_2{\cdot}C(OH){\cdot}(CH_3)_2$) is proposed from the available information.



(III)

The second fraction from this residue gave a positive Greissmayer test[13] and was shown to be composed mainly of ellagic acid[12] by paper chromatography. Bate-Smith[14] has shown that ellagic acid can be recognised instantly by inspection of a chromatogram in Forestal solvent.

The sodium hydrogen carbonate, sodium carbonate and sodium hydroxide fractions each contained two phenolic substances, one of which was common to each fraction. Their presence was demonstrated by paper chromatographic investigation in the usual manner.

The alkaloids contained in the ethanol extract were isolated as the picrates. The major picrate analysed for a trimethoxyfuroquinoline derivative, and the free base, on regeneration, was identified as kokusaginine, 4:6:7-trimethoxyfuro(2,3-*b*)quinoline[2] (I; R_3 = H, R_1 = R_2 = OCH_3), by comparison with an authentic sample. Fractionation of the ethanol extract with more concentrated acid gave only a trace of alkaloidal material.

EXPERIMENTAL

Microanalyses were by Dr. A. D. Campbell, University of Otago, N.Z. Infra-red spectra were measured as KBr discs, unless otherwise stated, with a Beckmann IR2 instrument. Ultra-violet spectra were measured for EtOH solutions with a Beckmann DU instrument. Light petroleum was of b.p. 60–70°.

Extraction of the bark. The finely ground bark of *Phebalium nudum* Hook, (7·5 kg, dried and stored for 4 years) was successively extracted (Soxhlet) with light petroleum, ether and ethanol, each extraction taking 32 hr with 8 gal of solvent. The bark was air-dried before successive extractions. The extracts were concentrated and the solvent removed by distillation under reduced pressure. No crystalline or solid material separated on successive concentration to half volume followed by cooling overnight. The concentrates of each extract gave positive Mayer's and Dragendorff's tests for alkaloids.

[12] F. M. Dean *Progress in the Chemistry of Organic Natural Products* Vol. IX, pp. 225–291. Springer Publishing House, Austria (1952).
[13] A. G. Perkin and M. Nierenstein *J. Chem. Soc.* **87**, 1412 (1905).
[14] E. C. Bate-Smith *Chem. & Ind. (Rev.) B.I.F. Rev.* R 32 (1956).

Petroleum ether extract

Compound A. The dark green, viscous concentrate (410 g) was triturated with methanol (2·5 l.) and the green waxy solid separated and washed well with methanol. Precipitation with methanol from light petroleum gave a colourless, amorphous solid (1·25 g), m.p. 75–79°.* Successive crystallisation from light petroleum containing a few drops of benzene, ligroin, and twice from ethyl acetate, raised the m.p. to 78·5–80°.* Final purification by chromatography in light petroleum on alumina gave, as the major fraction, colourless plates (880 mg) of *compound A*, m.p. 81·5–82°, not raised on further crystallisation from ethyl acetate (Found: C, 82·2; H, 13·0. $C_{23}H_{44}O$ requires C, 82·1; H, 13·2 per cent). I.R.: 2924, 2890†, 1736, 1704, 1468, 1422, 1403, 1377, 1202†, 1185, 1182, 1121, 959, 922, 732, and 723 cm^{-1}.

The substance was soluble in light petroleum, ligroin, benzene, *cyclo*hexane, chloroform, carbon tetrachloride, dioxan and ethyl acetate. It was slightly soluble in ether and soluble in hot but not in cold alcohol, acetone, and acetic acid. It was insoluble in water and alkalies. With hot sulphuric acid it gave a reddish-brown coloration. It gave negative tests for unsaturation and the carbonyl group.

Citronellal and citral. Most of the solvent was removed from the combined filtrate and methanol washings of compound A. The green, oily concentrate (406 g) was taken up in ether (800 cm^3) and extracted with 35% aqueous sodium bisulphite containing free acetic acid (5 × 750 cm^3). The free aldehydes (8·3 g) were regenerated from the bisulphite derivatives (39 g) and from the aqueous layer, by the addition of 10% sodium carbonate (500 cm^3) and isolated by ether extraction. They possessed a strong lemon-like odour. Fractional distillation under reduced pressure gave two main fractions:

(a) B.P. 70–95°/10 mm, which gave citronellal (2·2 g), b.p. 200–210°, after refractionation. The semicarbazone crystallised as colourless plates from chloroform–ligroin, m.p. and mixed m.p. 82–83°. The 2:4-dinitrophenylhydrazone crystallised as yellow plates from methanol, m.p. and mixed m.p. 76–77°.

(b) B.P. 100–115°/10 mm, which gave citral (3·7 g), b.p. 225–230°, after refractionation. The 2:4-dinitrophenylhydrazone crystallised as orange-red needles from ethanol, m.p. 115–116°, undepressed on admixture with an authentic sample prepared from commercial citral. Fractional crystallisation of the semicarbazone, m.p. 132–135°, from methanol gave, as the major portion, needles of geranial semicarbazone, m.p. and mixed m.p. 163–164°. The minor portion, neral semicarbazone, crystallised from aqueous methanol as leaflets, m.p. and mixed m.p. 169–170°. Separation was also achieved by partial solution of citral semicarbazone in hot ether, geranial semicarbazone being insoluble.

iso*Eugenol.* The phenolic constituents were extracted from the ether solution with 3% sodium hydroxide (6 × 750 cm^3). Acidification of the extract gave a dark upper layer, separated by the addition of ether (50 cm^3). A further yield (320 mg) of a colourless material separated from the ether solution on standing. This product after crystallisation from ethyl acetate had m.p. 82–83°, undepressed by admixture with compound A, obtained above. Concentration of the ether solution, together with the ether extracts (3 × 200 cm^3) of the aqueous layer, under reduced pressure, after washing with water until the washings were neutral, gave a viscous amber oil (40·0 g).

* Melting points taken in evacuated tubes.
† Inflexion.

Steam-distillation for 10 hr and ether extraction of the distillate, gave a brown viscous oil (3·0 g). The infra-red spectrum and the chromatographic behaviour were identical with those of *iso*eugenol (see below). Fractional distillation of the residual crude oil *in vacuo* gave, as the major product, a fraction, b.p. 110–140°/10 mm, which on refractionation yielded *iso*eugenol (15 g), b.p. 266–269°. It gave a transient greenish-blue colour with alcoholic ferric chloride. Paper chromatography in Forestal solvent[9] (glacial acetic acid: concentrated hydrochloric acid: water (30 : 3 : 10 vol.) by the ascending method showed a single spot, $R_f = 0{\cdot}89$ (average of 6 determinations), in the same position as *iso*eugenol run as control. The spots were coloured orange in the presence of ammonia and brown with diazotised *p*-nitroaniline spray reagent.[10] The infra-red spectrum (contact film) was identical with that recorded by Levi and Thompson *et al.*[5] The 3:5-dinitrobenzoate, prepared in pyridine and crystallised twice from *n*-butanol, gave rosettes of long colourless needles, m.p. and mixed m.p. 156°.

Dictamnine. The ether solution was washed with concentrated brine (3 × 400 cm³) and with water (4 × 400 cm³) until the washings were neutral. Concentration under reduced pressure after drying, gave a dark green mobile oil (320 g) which gave strong positive alkaloid tests. The total alkaloid content was extracted with cold 10% hydrochloric acid after the addition of ether (50 cm³) and isolated by basification with aqueous ammonia. Repeated fractional crystallisation of the crude brown gummy precipitate (3·5 g) from ethyl acetate–light petroleum gave, as the more insoluble fraction, colourless needles of dictamnine (total yield 1·06 g), m.p. 129–130°. A sample chromatographed in benzene on alumina and eluted with benzene–ether (9 : 1), had m.p. 131–132°, unchanged on further recrystallisation from ethyl acetate–light petroleum and undepressed on admixture with an authentic sample (Found: C, 72·5; H, 4·5; N, 7·3, 6·9; OMe, 13·6, 13·2; NMe, nil. Calc. for $C_{12}H_9O_2N$: C, 72·35; H, 4·55; N 7·05; 1 OMe, 15·6 per cent). I.R. (identical with authentic dictamnine): 3175, 2985, 1621, 1580, 1543, 1506, 1466, 1449, 1416, 1385, 1366, 1340, 1297, 1266, 1235, 1209, 1179, 1160, 1121, 1088, 1052, 979, 940, 873, 791, 758, and 722 cm^{-1}. U.V.: λ_{max} 236 mμ (log ε 4·78), 309 mμ (log ε 3·95) and 329 mμ (log ε 3·88).

Circular paper chromatography of purified dictamnine showed a single band which had R_f values, 0·94 (BuOH: 2 N HCl) and 0·89 (BuOH: 5% HAc) (in reference 11 $R_f = 0{\cdot}89$). The bands were coloured orange with a modified Dragendorff's reagent spray, prepared by suspending bismuth oxychloride (2·61 g) and potassium iodide (10 g) in water (10 cm³) and adding 10% hydrochloric acid (6 cm³) with shaking.

Dictamnine picrate separated from methanol as light yellow plates, m.p. 164–165° (in reference 6 m.p. 165–166°) (Found: C, 50·75; H, 2·8. Calc. for $C_{12}H_9O_2N$, $C_6H_3O_7N_3$: C, 50·45; H, 2·85 per cent).

γ-Fagarine. The more soluble alkaloid fraction was isolated by precipitation as the crude picrate (780 mg), m.p. 165–171°. Repeated crystallisation from ethanol gave, as the major fraction, pale yellow needles of γ-fagarine picrate, m.p. 177–178°, with softening at 170–171°. (In reference 15 m.p. 177°) (Found: C, 50·9; H, 3·1; N, 12·2; OMe, 13·2; NMe, nil. Calc. for $C_{13}H_{11}O_3N$, $C_6H_3O_7N_3$: C, 49·8; H, 3·1; N, 12·2; 2 OMe, 13·5 per cent).

† Inflexion

[15] V. Deulofeu, R. Labriola and J. de Langhe *J. Amer. Chem. Soc.* **64**, 2326 (1942).

The alkaloid was regenerated from the purified picrate with dilute aqueous lithium hydroxide. Two recrystallisations from ethyl acetate–ether gave small yellow needles of γ-fagarine, m.p. and mixed m.p. 140–141° I.R. (identical with authentic γ-fagarine): 3175, 2976, 2874†, 1515, 1460, 1395, 1381, 1337†, 1304, 1261, 1238†, 1189, 1159, 1094, 1053†, 908, 868, 816, 771, 751, and 726 cm^{-1}. U.V.: λ_{max} 238 mμ (log ε 4·76), 370 mμ (log ε 3·89) and 332 mμ (log ε 3·88). The hydrochloride crystallised as colourless needles from chloroform–ether, m.p. 158–159°. It decomposed when dried at 85°/P_2O_5/vac. for analysis.

Steam-volatile neutral oil. The ether solution was washed well with water (4 × 400 cm^3) until the washings were neutral and the solvent removed after drying. The dark green mobile oil (298 g) was then steam-distilled for 32 hr after addition of sodium chloride. The steam-volatile fraction was separated by addition of ether (20 cm^3) and by ether extraction (2 × 100 cm^3) of the aqueous distillate after saturation with sodium chloride. Removal of the solvent, under reduced pressure, from the dried solution, gave a pale green essential oil (50 g). It possessed the characteristic aromatic odour of the dried bark and had d_4^{25} 0·9949, n_D^{25} 1·5040, and $[\alpha]_D^{25}$ + 3·6° (l = 1, c = 1·95 in $CHCl_3$).

Non steam-volatile fraction. The volatile constituents of the residual oil (228 g) after steam-distillation, were separated by distillation *in vacuo* (100–350°/5 mm) after drying in ether solution. The dark brown distillate (162·3 g) had d_4^{25} 0·9982, n_D^{25} 1·6562, and $[\alpha]_D^{25}$ + 0·24° (l = 1, c = 5·38 in $CHCl_3$).

The oil was fractionally distilled; fractions 1–14, 115–252°/10 mm, fraction 15, 252–262°/10 mm, fractions 16–17, 230–300°/4 mm and fractions 18–20, 300–250°/1 mm.

9-*Hydroxy*-4-*methoxyfurano*(3,2-*g*) *benzopyran*-7-*one*. A yellow crystalline solid separated from fractions 15–17 on standing at 0° for 3 months. The compound (982 mg), m.p. 196–214°, was separated from adhering oil by washing with ether. It crystallised from ethanol as thick yellow rods, m.p. 221·5–222°, undepressed on admixture with an authentic sample of 9-hydroxy-4-methoxyfurano(3,2-*g*) benzopyran-7-one (Found: C, 62·1; H, 3·3; OMe, 13·0. Calc. for $C_{12}H_8O_5$: C, 62·1; H, 3·5; 1 OMe, 13·35 per cent). I.R.: 3390, 3175, 2976, 2874, 1709, 1647, 1613, 1595, 1484, 1451†, 1441, 1389, 1355, 1316, 1263, 1220, 1205, 1148, 1095, 1072, 1048, 1000, 962, 895, 871, 833, 808, 746 and 720 cm^{-1}. U.V.: λ_{max} 224 mμ (log ε 4·24), 272 mμ (log ε 4·28) and 316 mμ (log ε 4·03).

The furanocoumarin was readily soluble in 5% sodium hydroxide, with a yellow coloration which changed to purple on standing. Red and yellow colours were produced by concentrated sulphuric acid and ferric chloride respectively. The acetate, prepared by use of pyridine and acetic anhydride (1 hr, 90°), crystallised from methanol as colourless needles, m.p. 180–181° (in reference 7 m.p. 181–182°) (Found: C, 61·5; H, 3·4. Calc. for $C_{14}H_{10}O_6$: C, 61·3; H, 3·7 per cent).

Compound B. The combined oil from fractions 15–17 after separation of the furanocoumarin, and from fractions 18–20, was chromatographed in benzene on alumina columns. Elution with benzene and benzene–ether (9 : 1), gave greenish oils which on trituration with acetone deposited a colourless amorphous solid (187 mg) melting over a range, 50–60°. Crystallisation from methanol gave colourless, feathery needles of *compound B*, m.p. 94°,* with shrinking at 84° (Found, for sample dried at room temperature: C, 78·6; H, 12·9. $C_{13}H_{26}O$ requires C, 78·7; H, 13·2 per cent).

Solid products were not obtained by continued elution of the columns with ether and ether–methanol mixtures.

Ether extract

Phebalin. The brown viscous oil (820 g) from the ether extract was triturated with methanol (2·5 l.) and the solid separated. The deposit, after washing well with methanol and with light petroleum, consisted of a yellow amorphous material admixed with large, colourless, hexagonal crystals (15·2 g). Extraction with hot ether (A; 200 cm^3) left as residue the crystalline material (1·6 g), m.p. 158–161°. Repeated crystallisation from chloroform–methanol gave thick clusters of shining prismatic rods of *phebalin*, m.p. 171–172°, raised by chromatography in benzene–chloroform (9 : 1) on alumina to m.p. 175–176·5° (Found: C, 74·6, 74·55; H, 5·8, 5·8; OMe, 9·7, 9·6. $C_{20}H_{18}O_4$ requires C, 74·5; H, 5·6; 1 OMe, 9·65 per cent). I.R.: 3106, 2959, 2874†, 1730, 1603, 1560, 1497, 1458, 1437, 1404, 1397, 1340, 1284, 1253, 1182†, 1167, 1122, 1093, 1049, 1029, 994, 937, 891, 837, 803, 772 and 703 cm^{-1}. U.V.: λ_{max} 255 mμ (log ε 4·06) 285 mμ (log ε 4·15) and 322 mμ (log ε 4·29).

Phebalin was soluble in benzene, toluene, dioxan, dimethylformamide and chloroform. It was insoluble in water, light petroleum and ligroin and only slightly soluble in ether, ethyl acetate, alcohol and acetone. The compound was insoluble in hot 5% sodium hydroxide and insoluble in concentrated hydrochloric acid. With hot nitric acid it gave a yellow coloration and a yellow colour in cold concentrated sulphuric acid turning to dark red on warming. It gave a negative ferric chloride test, a negative cryptophenolic test[16] and a negative flavonoid test with magnesium and concentrated hydrochloric acid. The Molisch test was negative as was also the Labat test for methylenedioxy groupings with both phloroglucinol and gallic acid. Phebalin showed negative unsaturation tests with 1% permanganate, bromine in chloroform or acetic acid and also with tetranitromethane.

β-Sitosterol. The hot ether extract (A) of the triturated solid obtained above, on slow cooling deposited a pale yellow oily micro-crystalline solid (6·03 g), m.p. 63·5–67°. Partial solution in hot methanol (50 cm^3) followed by decantation of the hot solution separated the major fraction from a brown gum (B; 2·0 g). The cream amorphous solid (4·03 g), m.p. 62–70°, obtained from the methanol solution was chromatographed in benzene on alumina columns. From the fractions eluted with benzene–ether (5 : 1) colourless feathery plates of β-sitosterol were obtained, m.p. 134°, which separated from methanol as plates, m.p. and mixed m.p. 136–137° (Found: C, 83·8; H, 12·1. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·1 per cent). The acetate had m.p. and mixed m.p. 127–128°.

Chromatography of the residue (B) in ethyl acetate on alumina gave no useful results, gummy products being obtained from all fractions after elution with ethyl acetate, ethyl acetate–acetone (1 : 1), and acetone. Paper chromatographic investigation in Forestal solvent in the usual manner showed a single brown spot $R_f = 0{\cdot}96$ (brown-red with the usual spray-reagent).

Ether extract fractionation. Removal of the solvent from the ether–alcohol solution gave a brown viscous oil (804·5 g) which, when dissolved in ether (600 cm^3), was fractionated with saturated aqueous sodium hydrogen carbonate (8 × 800 cm^3), 10% sodium carbonate (10 × 800 cm^3), 5% sodium hydroxide (10 × 800 cm^3) and

[16] L. Claisen *Liebigs Ann.* **418,** 69 (1919).

10% hydrochloric acid (12 × 800 cm^3). The alkaline extractions were taken as complete when the last two extracts were colourless and the acid extract taken as complete when the last two extractions gave negative Mayer's tests. Only a trace of alkaloid material was obtained on further extraction with 20% hydrochloric acid, in insufficient quantity to isolate as picrate.

Cinnamic acid; *phenolics.* The acidified sodium hydrogen carbonate extract gave a brown oily upper layer which was separated by the addition of ether (50 cm^3). Removal of the solvent after washing with water (2 × 1 l.) and drying, gave a dark brown hygroscopic tar (33·4 g). Extraction of this fraction with hot water (2 × 250 cm^3) gave, on cooling, a crystalline acid (300 mg). Two recrystallisations from ethanol gave colourless plates of cinnamic acid, m.p. and mixed m.p. 132–132·5°.

The residual brown tar (32·68 g) after drying, gave no solid products on attempted purification by acetylation or by chromatography on a magnesia–celite (1 : 1) column; an apparently homogeneous brown band was obtained which showed no fluorescence under ultra-violet light. Chromatography in benzene on alumina showed the presence of two bands which on elution with benzene and benzene–ether mixtures, gave only gummy products.

Paper chromatographic investigation in Forestal solvent showed the presence of three phenolic compounds $R_f = 0{\cdot}80$, 0·88 and 0·96, respectively (average of 3 determinations). Strips were run by the ascending method (cyanidin, control $R_f = 0{\cdot}50$) and examined for spots with (a) ammonia vapour (b) ammonia vapour under ultra-violet light and (c) diazotised *p*-nitroaniline spray reagent. The spots were coloured blue-purple, orange, and red-brown, respectively with the spray reagent.

Acetic, isovaleric and palmitic acids. The aqueous layer, after acidification, was extracted with ether (2 × 2 l.) and the extract washed twice with water, dried, and the solvent removed. The brown viscous residue (17·7 g) possessed an unpleasant odour and, on fractional distillation, gave three main fractions;

(a) B.P. 106–120°/760 mm, which on refractionation gave acetic acid (9·1 g), b.p. 115–120°. The silver salt was prepared and analysed in the usual manner (Found: Ag, 64·3; equiv., 61·0. Calc. for $C_2H_3O_2Ag$: Ag, 64·6 per cent; equiv. ≡ *M*, 60·0). The *s*-benzyl*iso*thiuronium salt separated as needles from dilute alcohol, m.p. and mixed m.p. 142–142·5°.

(b) B.P. 168–180°/760 mm, which on refractionation gave *iso*valeric acid (3·2 g), b.p. 175–179°. The silver salt was prepared and analysed in the usual manner. (Found: Ag, 51·9; equiv., 107. Calc. for $C_5H_9O_2Ag$: Ag, 51·7 per cent; equiv. ≡ *M*, 102). The *s*-benzyl*iso*thiuronium salt separated as needles from dilute alcohol, m.p. and mixed m.p. 156–157°.

(c) B.P. 210–230°/10 mm. (2·68 g), which on chromatography in benzene on alumina and elution with the same solvent gave a pale yellow oil. Slow evaporation of the solvent left a discoloured waxy solid (30 mg), b.p. > 360, which on repeated crystallisation from benzene–methanol as waxy scales, had m.p. 60–63°, undepressed on admixture with palmitic acid. The acid was insoluble in water but readily soluble in sodium hydrogen carbonate. The anilide, prepared with ice-cold aniline via the acid chloride, and crystallised twice from alcohol and finally from benzene had m.p. 89–90°, undepressed by palmitanilide prepared in the same manner.

Ether extract; *phenolics.* The 10% sodium carbonate and 5% sodium hydroxide extracts on acidification, collection of the brown tarry precipitates and ether extraction

of the aqueous layer, gave in both cases, black sticky residues (9·73 g and 24·31 g, respectively), after washing, and drying in acetone solution. The extracts contained large quantities of phlobaphenic material. No crystalline products were isolated in attempted purification by acetylation or by chromatography on magnesia–celite (1 : 1) or alumina columns, apparently homogeneous brown bands being obtained in all cases.

Paper chromatographic investigation in Forestal solvent in the usual manner gave the following results; Na_2CO_3 extract, R_f 0·72 (orange) and 0·78 (orange); NaOH extract, R_f 0·93 (brown) and 0·98 (brown-red).

Evolitrine and skimmianine. The 10% hydrochloric acid extract was basified with aqueous ammonia and the gummy precipitate (4·43 g) isolated by filtration. Repeated fractional crystallisation from ethyl acetate and again from ethyl acetate–light petroleum gave two main fractions;

(a) Thick, colourless rods (274 mg), m.p. 164–168°, raised to m.p. 175–175·5° by three recrystallisations from chloroform–methanol. The substance was identified as phebalin by mixed m.p. and the infra-red spectrum. The product gave a negative qualitative test for nitrogen and had identical properties with those recorded earlier for phebalin.

(b) Rosettes of needles of evolitrine (22 mg), m.p. 114–115°, unchanged on further recrystallisation from light petroleum (in reference 11 m.p. 114–115°). U.V.: λ_{max} 247 mμ (log ε 4·84), 308 mμ (log ε 4·01), 320 mμ (log ε 3·98) and 333 mμ (log ε 3·90). The picrate crystallised from ethanol as pale yellow needles, m.p. 191–192° (in reference 11 m.p. 191–192°). Found: C, 49·8; H, 3·1; OMe, 14·0; NMe, nil. Calc. for $C_{13}H_{11}O_3N$, $C_6H_3O_7N_3$: C, 49·8; H, 3·1; 2 OMe, 13·5 per cent).

Chromatography of the gummy residue from the mother-liquors from fraction (b) above, in benzene on alumina, gave three major fractions;

(c) Colourless needles (150 mg), m.p. 127–128°, raised by further recrystallisation from ethyl acetate, to m.p. 131–132°, undepressed by dictamnine isolated from the light petroleum extract. The picrate formed yellow plates from methanol, m.p. and mixed m.p. 164–165°.

(d) Colourless needles (51 mg), m.p. 102–110°, raised by further recrystallisation from light petroleum to m.p. 114–115°, undepressed by evolitrine obtained from (b) above. The picrate formed yellow needles from ethanol, m.p. and mixed m.p. 191–192°.

(e) Colourless rods (40 mg), m.p. 162–165°, raised on repeated recrystallisation from ethyl acetate to m.p. 174–175°, undepressed by phebalin.

The basified aqueous solution was extracted with ether (5 × 2 l.). It then gave a negative alkaloid test. The brown oil (9·40 g) obtained from the ether extract after washing and drying and removal of the solvent, was taken up in ethanol (20 cm^3) and treated with excess of hot ethanolic picric acid. The crude picrate mixture (1·84 g), m.p. 162–164° (decomp.), on extraction with hot ethanol (2 × 50 cm^3) gave, as the major fraction, pale yellow needles (800 mg), m.p. 190–191·5°. Three recrystallisations from dioxan gave fine pale yellow needles of skimmianine picrate, m.p. and mixed m.p. 195–196° (Found: C, 49·4; H, 3·25; N, 11·5. Calc. for $C_{14}H_{13}O_4N$, $C_6H_3O_7N_3$: C, 49·2; H, 3·3; N, 11·5 per cent).

Regeneration of the base from the picrate by chromatography in ethyl acetate on alumina gave skimmianine, colourless prisms from aqueous ethanol, m.p. and mixed

m.p. 177°. I.R. (identical with authentic skimmianine): 3175, 2976, 2874, 1623, 1580, 1550, 1508, 1497†, 1451, 1391, 1383, 1323, 1295, 1271, 1238, 1217, 1152, 1117†, 1094, 1058, 1043, 996, 987, 951, 888, 873, 826, 809, 776, and 741 cm^{-1}.

Steam-volatile neutral oil. The dark ether solution remaining after chemical fractionation was washed with water until the washings were neutral, dried, and the solvent removed. The dark viscous oil (122 g) was then steam-distilled for 36 hr after addition of sodium chloride. The steam-volatile fraction was separated by addition of ether (50 cm^3) and by ether extraction (2 × 2 l.) of the aqueous distillate after saturation with sodium chloride. Removal of the solvent under reduced pressure, from the dried solution, gave a dark green essential oil (32·5 g).

Non steam-volatile fraction. The residual oil (88·2 g) after steam-distillation was washed well with water, dried, and the volatile constituents (51·9 g), separated by distillation *in vacuo* (100–300°/10 mm). Considerable charring occurred above 300°. Preliminary chromatography in benzene on alumina gave no useful results. The oil was saponified in ethanol (30 cm^3), by refluxing with 2 N alcoholic KOH at 100° for 2 hr. Ethanol was removed by distillation under slightly reduced pressure in a slow stream of carbon dioxide, water (200 cm^3) added, and the mixture extracted with ether (2 × 200 cm^3). The black oil obtained on removal of the solvent from the dried extract was chromatographed in benzene on alumina. The initial fractions, eluted with the same solvent, gave α-terpineol as a brown oil (500 mg), b.p. 212–220°/760 mm. Treatment with dry hydrogen chloride in anhydrous ether solution and crystallisation from aqueous methanol gave colourless plates, m.p. 48·5–49°, undepressed by dipentene bis-hydrochloride prepared from α-terpineol in the same manner.

Acidification of the aqueous phase, after saponification and ether extraction gave, as the major fraction, *iso*valeric acid (500 mg), b.p. 174–179°. The *s*-benzyl-*iso*thiuronium salt had m.p. and mixed m.p. 156–157°.

Ethanol extract

Ethanol extract fractionation. The black sticky tar (1·88 kg) from the alcohol extract contained a large amount of phlobaphenic material and gave only weak alkaloid tests. An aliquot (910 g) was dissolved in hot ethanol (3 l.), and ether (7 l.) added to the cooled solution which was then allowed to stand at room temperature overnight. The solution was decanted from the thick syrupy deposit (A) and successively fractionated between saturated sodium hydrogen carbonate (3 × 2·75 l.), 10% sodium carbonate (3 + 2 l.), 8% hydrochloric acid (4 × 2 l.), 12% hydrochloric acid (2 × 2 l.), 20% hydrochloric acid (5 × 2 l.) and 36% hydrochloric acid (2 × 1·5 l.) in a similar manner to the ether extract.

The more ether–alcohol insoluble syrup A (213·5 g) was dissolved in ethanol (1 l.) and poured into water (6 l.). The washed and dried flocculent precipitate (35·6 g) contained a large amount of phlobaphenic material (9·76 g). Extraction of the dried deposit with hot acetone (4 × 100 cm^3) and with hot ethanol (3 × 100 cm^3), separated two further fractions. These extracts on removal of the solvent, gave brown resins (15·1 g) and (10·8 g), respectively, which could not be obtained in a crystalline state or purified by acetylation. Paper chromatographic investigation in the usual manner gave the following results; acetone soluble fraction, $R_f = 0{\cdot}96$ (red); ethanol soluble fraction $R_f = 0{\cdot}98$ (blue-brown).

Phebalarin. The brown gum (17·8 g), remaining after removal of the solvent from

the aqueous ethanol solution obtained from A, was treated with hot ethanol (B; 2 × 25 cm³).

The sparingly ethanol soluble residue (4·92 g), m.p. >360°, on further extraction with hot acetic acid (20 cm³) left phlobaphenic material (4·5 g). Addition of water (10 cm³) to the cooled solution deposited a pale yellow crystalline solid (373 mg), m.p. 116–118°. Recrystallisation from ethanol gave pale yellow needles of *phebalarin*, m.p. 125–126° (Found: C, 61·3; H, 6·1. $C_{15}H_{18}O_6$ requires C, 61·2; H, 6·2 per cent). I.R.: 3546, 2985, 1709, 1621, 1582, 1563, 1546, 1520, 1475†, 1460, 1425, 1399, 1385, 1337, 1256†, 1196†, 1157, 1117, 1034, 997†, 920, 841, and 795 cm^{-1}. U.V.: λ_{max} 225 mμ (log ε 4·24), 236 mμ (log ε 4·16), 245 mμ (log ε 4·18) and 332 mμ (log ε 4·25).

Phebalarin was insoluble in sodium hydrogen carbonate but dissolved in aqueous sodium carbonate with a strong yellow coloration. It gave a green colour with ferric chloride. The substance dissolved in concentrated sulphuric acid to give a yellow solution, changed on warming to a blue-green colour. The magnesium–hydrochloric acid test for flavonoids was negative.

Ellagic acid. The ethanol soluble fraction B, on slow evaporation deposited ellagic acid as a yellow microcrystalline powder (1·66 g), m.p. >360. The product gave a dark red colour with nitric acid containing nitrous acid, and, in methanol solution, gave a yellow colour with acetic acid and sodium nitrite,[14] changing to orange-brown on standing. Paper chromatographic investigation in Forestal solvent in the usual manner gave a single spot, R_f = 0·32 (in reference 14 R_f = 0·33). The spot was violet under ultra-violet light, yellow in the presence of ammonia/u.v. and red-brown when sprayed.

Phenolics. The sodium hydrogen carbonate, 10% sodium carbonate and 5% sodium hydroxide extracts on acidification all gave dark brown tarry precipitates, 59·6 g, 23·72 g, and 10·05 g, respectively. The extracts contained large quantities of phlobaphenic material. No crystalline products were isolated in attempted purification by chromatography on magnesia–celite (1 : 1) or alumina columns, apparently homogeneous, brown bands being obtained in all cases. Paper chromatographic investigation in Forestal solvent by the previous method gave the following results; $NaHCO_3$ extract, R_f = 0·90 (orange), Na_2CO_3 extract, R_f = 0·88 (blue); NaOH extract, R_f = 0·84 (orange–red). Each extract gave a further spot R_f = 0·96 (brown-red).

The aqueous filtrate after acidification of the sodium hydrogen carbonate fraction gave a faint positive Mayer's test for water soluble alkaloids. Although they could be separated from the basified solution by continuous extended chloroform extraction for 72 hr, the brown concentrate (15·8 g) retained a large quantity of gummy impurities. Further purification by extraction with 10% hydrochloric acid (4 × 250 cm³) from chloroform solution (50 cm³) followed by basification and chloroform extraction separated non-nitrogenous impurities. The crude alkaloid residue (2·3 g) still retained a large quantity of oily impurities. The alkaloids were not present in sufficient quantity for isolation as insoluble hydrochlorides or by precipitation as the picrates from alcoholic or chloroform solution.

Kokusaginine. The 8% hydrochloric acid extract was basified with ammonia with cooling, and the crude alkaloid gum (1·20 g) separated and washed well with water (the aqueous layer, and washings gave alkaloid tests). The gum was separated from black non-nitrogenous impurities (210 mg), by extraction with hot ether (4 × 25 cm³)

containing chloroform (5 cm^3) and the alkaloid content precipitated by treatment with excess of ethanolic picric acid. The crude picrate (833 mg), m.p. 184°, crystallised once from ethanol and twice from dioxan as large yellow needles, had m.p. 212–213°, undepressed on admixture with kokusaginine picrate (m.p. 217–218°) (Found: C, 49·3; H, 3·1; N, 11·5; OMe, 18·6. Calc. for $C_{14}H_{13}O_4N$, $C_6H_3O_7N_3$: C, 49·2; H, 3·3; N, 11·5; 3 OMe, 19·0 per cent.) Regeneration of the free base by chromatography in ethyl acetate on alumina gave kokusaginine, colourless prisms from benzene, m.p. and mixed m.p. 171°. I.R. (identical with authentic kokusaganine): 3125, 2941, 1623, 1585, 1543, 1506, 1479, 1458, 1418, 1395, 1360, 1318, 1312, 1253, 1208, 1167, 1155, 1138, 1089, 1046, 1008 988 945, 858, 840, 794, 771, and 744 cm^{-1}.

The 12, 20 and 36% hydrochloric acid extracts, on working up in the previous manner, contained only traces of alkaloidal material, in insufficient quantity to isolate picrates.

Acknowledgements—Acknowledgement is made to Professor P. A. S. Smith and Dr. B. F. Cain for preliminary work on the plant. We are indebted to Dr. J. R. Price, C.S.I.R.O., Melbourne, Australia, for samples of dictamnine, γ-fagarine, skimmianine and kokusaginine and to Dr. G. Rosenkranz, Syntax S. A., Mexico for a sample of 9-hydroxy-4-methoxy(3,2-*g*)benzopyran-7-one. Assistance is also gratefully acknowledged from the Chemical Society, The Rockefeller Foundation of New York, The Australian and New Zealand Association for the Advancement of Science, the Research Grants Committee of the University of New Zealand, and one of us (R. C. C.) for a University Research Fund Fellowship.