Tetrahedron, 1963, Vol. 19, pp. 1293 to 1305. Pergamon Press Ltd. Printed in Northern Ireland

HAWAIIAN PLANT STUDIES—IX*

THE ALKALOIDS OF *PLATYDESMA CAMPANULATA* MANN

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Abstract—Six alkaloids were isolated from the endemic Hawaiian shrub *Platydesma campanulata* Mann: the known furoquinolines evolitrine and kokusaginine and the new 6-methoxydictamnine (Ia); a new dihydrodictamnine named platydesmine (III); and two derivatives of 4-quinolone. One of these, 1,2-dimethyl-4-quinolone was known heretofore as a synthetic product. The other, pilokeanine, could not be isolated in crystalline form. On the basis of physical and chemical evidence tentative structure V is proposed.

THE rutaceous genus *Platydesma*, which comprises four species, is endemic to the Hawaiian Islands. *P. campanulata*[1], which is the subject of the present investigation, occurs on the four largest islands of the chain at an elevation of 1,500–5,000 feet. It is a shrub or small tree with large (20 × 50 cm) leaves. The leaves when crushed reveal the presence of essential oils while the bark and wood emit a semeniferous odor on breaking. In Engler and Prantl[2] *Platydesma* is placed among the Xanthoxylae of the subfamily Rutoideae between the Mexican genus *Choisya* and the New Caledonian genus *Dutaillyea*. Stone[1], however, considers the genus to be the most closely related to the Australian genus *Medicosma*. Our earlier survey[3] had revealed the presence of alkaloids in *P. campanulata* and it seemed therefore desirable to study in detail a member of this endemic genus.

Plant material for preliminary investigation was collected in the Pupukea area of the Koolau range on Oahu along the Ridge Trail and identified by Mr. I. E. Lane. Collections for the bulk of this work were made in the Kokee region on Kauai (taxonomic identification by Dr. B. C. Stone) and in the Kohala mountains on Hawaii, south of Hawi (identification by Mr. I. E. Lane). For processing, root and bark from each collection were combined but kept separate according to geographical origin; the leaves from all collections were extracted together. In this fashion the following six bases were isolated: evolitrine, kokusaginine and 6-methoxydictamnine, m.p. 134–135°, from the root and bark originating on Kauai and Hawaii; platydesmine, m.p. 137–138°, and 1,2-dimethyl-4-quinolone from the Hawaii root and bark only; and the combined leaves yielded kokusaginine, 1,2-dimethyl-4-quinolone and pilokeanine, picrate m.p. 216° (dec).

Dictamnine derivatives

The new alkaloid 6-methoxydictamnine proved to be the major basic constituent of *P. campanulata*. It was isolated after column chromatography on Florisil and

* Part VIII: P. J. Scheuer, M. Y. Chang and C. E. Swanholm, *J. Org. Chem.* **27**, 1472 (1962).

† From the Ph.D. Dissertation of Frank Werny, University of Hawaii (1962).

[1] B. C. Stone (*Pacific Sci.*, in press) kindly informed us that *P. campanulata* Mann will be known as *P. spathulatum* (A. Gray) B. C. Stone in his proposed taxonomic revision of the genus.

[2] A. Engler and K. Prantl, *Die natürlichen Pflanzenfamilien* (2nd Edition) Vol. 19a; pp. 187–359. W. Engelmann, Leipzig (1931).

[3] C. E. Swanholm, H. St. John and P. J. Scheuer, *Pacific Sci.* **14**, 68 (1960).

had m.p. 134–135° after recrystallization from benzene–petroleum ether. Its composition, $C_{13}H_{11}NO_3$, which included two methoxy groups, and its U.V. spectrum (Fig. 1) suggested a furoquinoline alkaloid.[4]

It seemed unlikely that our compound was the isomeric evolitrine, m.p. 114–115°, but it was conceivable that we had isolated the second known monomethoxydictamine, γ-fagarine, m.p. 142–143°. Direct comparison with an authentic sample[5]

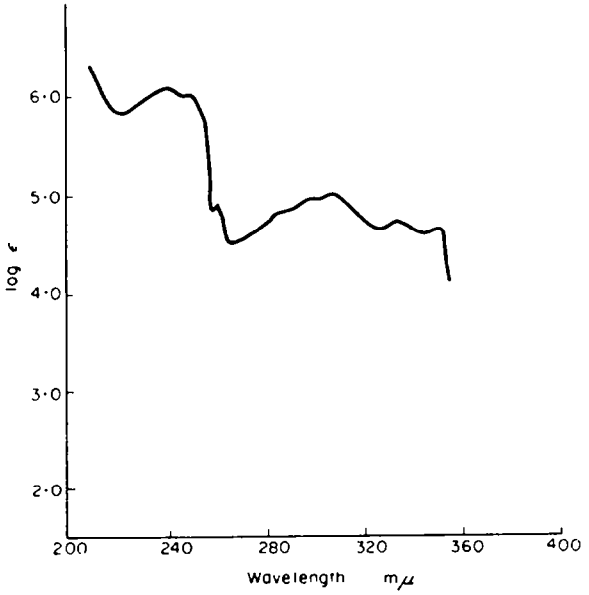
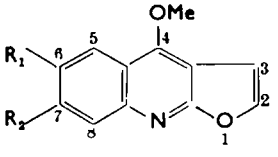
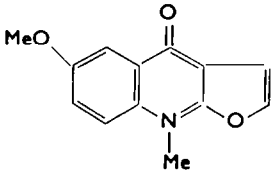


FIG. 1. U.V. spectrum of 6-methoxydictamnine (Ia) in 95% ethanol

disproved this possibility and suggested that our compound was the hitherto unknown 5- or 6-methoxydictamnine. The U.V. spectrum of our base which resembled closely that of maculosidine (6,8-dimethoxydictamine),[6] suggested that we had in hand 6-methoxydictamine (Ia). This was proven by isomerizing our compound with methyl iodide to 6-methoxyisodictamnine (II), m.p. 214–216°, which had been obtained by Lamberton and Price[7] as a degradation product of medicosmine. Identity was proved by direct comparison with an authentic sample.[8]



Ia: R_1 = MeO, R_2 = H
Ib: R_1 = R_2 = MeO
Ic: R_1 = H, R_2 = MeO



II

[4] J. R. Price in L. Zechmeister, *Fortschritte der Chemie organischer Naturstoffe* Vol. 13; p. 317 ff. Springer-Verlag, Wien (1956).
[5] Kindly supplied by Prof. V. Deulofeu, Buenos Aires.
[6] R. H. Prager, E. Ritchie and W. C. Taylor, *Austr. J. Chem.* **13**, 280 (1960).
[7] J. A. Lamberton and J. R. Price, *Austr J. Chem.* **6**, 173 (1953).
[8] Kindly furnished by Dr. J. R. Price, Melbourne.

Kokusaginine (Ib) was encountered in extracts from all parts of the plant material from Hawaii and Kauai. Crystalline material which was obtained after chromatography on silica gel G was recrystallized from absolute ethanol, m.p. 169·0–169·5°. Melting point, elemental analysis and U.V. spectrum as well as melting point of the picrate, 205–207° (dec) suggested identity with the known alkaloid kokusaginine. This was proven by direct comparison with authentic kokusaginine picrate,[8] m.p. 204–206° (dec); the two compounds had identical I.R. spectra and a mixture had m.p. 205–208° (dec).

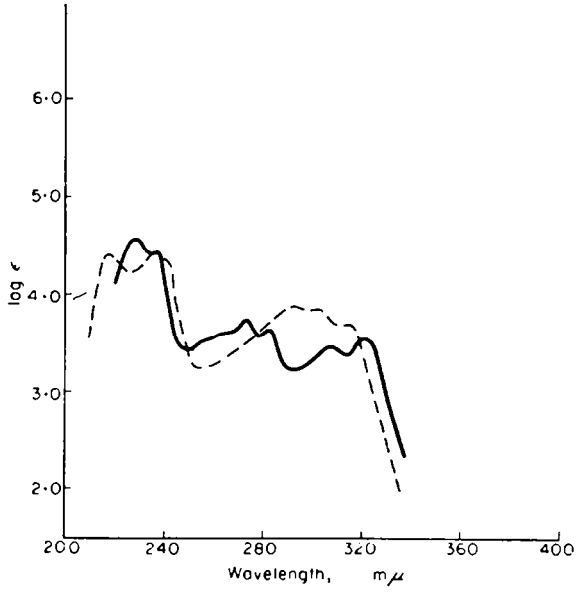


FIG. 2. U.V. spectrum of platydesmine (III): — in 95% ethanol; - - - in 0·01N ethanolic HCl

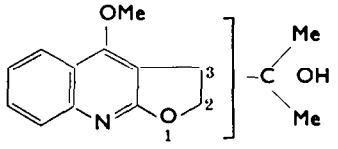
Evolitrine (Ic), which was found to occur in the root and bark of *P. campanulata*, could not be isolated as a separate crystalline entity. It was invariably eluted as a mixture with 6-methoxydictamnine. All attempts at further separation yielded pure 6-methoxydictamnine and a mixture. Thin-layer chromatography on aluminum oxide G provided a means of separation, which was used to establish the presence of evolitrine. The mixture was chromatographed concurrently with pure 6-methoxydictamnine and authentic evolitrine[9] and thereby resolved into two spots whose R_f-values and colours, after spraying with modified Dragendorff's reagent[10] were identical with those originating from 6-methoxydictamnine and evolitrine, respectively.

A dihydrodictamnine derivative

A new alkaloid, which we named platydesmine, was isolated as the picrate, m.p. 107–109°, from bark and root collected on Hawaii. The free base, m.p. 137–138°, was obtained by chromatographing the picrate on aluminum oxide G and recrystallizing the solid residue of the eluate from benzene–petroleum ether. Analysis of the picrate suggested a formula of $C_{15}H_{17}NO_3$ for the free base. The U.V. spectra in ethanol and in ethanolic hydrochloric acid (Fig. 2) were identical with the spectra

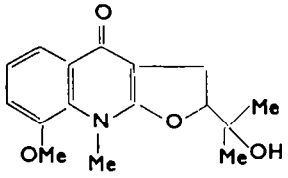
[9] Kindly supplied by Dr. R. G. Cooke, Melbourne.
[10] N. A. Robles, *Pharm. Weekblad* **94**, 178 (1959).

of dihydrodictamnine.[11] A mass spectrum of the free base[12] had only two prominent mass peaks, at 259 and at 200 m/e units. The first peak corresponds to the molecular weight of $C_{15}H_{17}NO_3$ and the second peak is that of a fragment from which a $C_2H_3O_2$ (59) or a C_3H_7O (59) moiety has been lost. Absence of infrared absorption in the 5·6 μ region excludes acetate or methyl carboxylate. Presence of a band at 2·79 μ, on the other hand, suggests an alcohol, possibly tertiary.[13] There is no evidence that the molecule contains an ether linkage in addition to those which are accounted for by a dihydrodictamnine nucleus. Since cleavage during electron bombardment occurs more readily at aliphatic and alicyclic than at aromatic linkages,[14] part structure III may be written for platydesmine. We favor attachment of the side chain at the 2-position on the basis of the existing analogy with the enantiomeric pair of alkaloids



III

balfourodine and hydroxylunacrine, which were isolated from the rutaceous plants *Balfourodendron riedelianum*[15] and *Lunasia amara* Blanco[16] and possess structure IV.



IV

4-Quinolone derivatives

A crystalline picrate, m.p. 234–236°, was isolated from the Hawaii plant material and from the combined leaves. The free base, m.p. 178–179° (change in crystal form at 174°), was obtained after chromatography of the picrate on a column of aluminum oxide G and recrystallization from chloroform–petroleum ether. Analytical data supported a formula of $C_{11}H_{11}NO$, a methoxy group being absent. The U.V. spectrum of the alkaloid (Fig. 3) exhibited twin peaks at 322 and 336 mμ, which are characteristic of 4-quinolones.[17] Furthermore, these maxima merged into a single peak at 303 mμ in ethanolic hydrochloric acid. This phenomenon has been observed in 2-alkyl-substituted quinolones.[18] Since but two carbon atoms in addition to the

[11] J. R. Price, Private Communication.
[12] Kindly determined by Prof. K. Biemann, M.I.T.
[13] A. R. H. Cole in L. Zechmeister, *Fortschritte der Chemie organischer Naturstoffe* Vol. 13, p. 30, Springer-Verlag, Wien (1956).
[14] K. Biemann, *J. Amer. Chem. Soc.* **83**, 4801 (1961).
[15] H. Rapoport and K. G. Holden, *J. Amer. Chem. Soc.* **82**, 4395 (1960).
[16] S. Goodwin, A. F. Smith, A. A. Velasquez and E. C. Horning, *J. Amer. Chem. Soc.* **81**, 6209 (1959).
[17] G. W. Ewing and E. A. Steck, *J. Amer Chem. Soc.* **68**, 2181 (1946).
[18] E. A. Steck, G. W. Ewing and F. C. Nachod, *J. Amer. Chem. Soc.* **71**, 238 (1949).

4-quinolone nucleus needed to be placed, the structure of the alkaloid had to be 2-ethyl-4-quinolone or a 2,x-dimethyl-4-quinolone. An N.M.R. spectrum[19] in deuteriochloroform showed two three-proton peaks at low field (7·66 and 6·41τ, tetramethylsilane internal standard) thus eliminating a 2-ethyl structure from consideration. The fact that no analogy exists for naturally occurring quinolones which are alkyl-substituted in the benzene ring coupled with the low field N.M.R. absorption of the two methyl groups reduces the structural problem to a choice between 1,2- or 2,3-dimethyl-4-quinolone. The ultraviolet data discussed above rule out a 1,3-dimethyl substitution pattern.

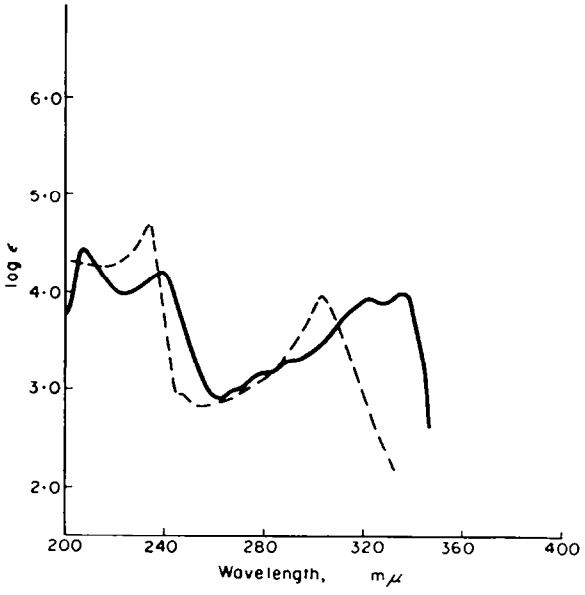


FIG. 3. U.V. spectrum of 1,2-dimethyl-4-quinolone: — in 95% ethanol; - - - in 0·01N ethanolic HCl

2-methyl-4-quinolone was synthesized from aniline and ethyl acetoacetate.[20] The product, m.p. 234–235·5°, had an U.V. spectrum which was identical with that published by Ewing and Steck.[17] N-methylation with dimethyl sulfate in base, purification by chromatography on aluminum oxide G followed by vacuum sublimation and recrystallization led to a white crystalline compound, m.p. 179·5–180·5°. This compound was identical with the natural product in all respects.

A second 4-quinolone alkaloid, which we have named pilokeanine (*pilo-kea* is the Hawaiian name of *Platydesma*), was encountered in small quantity in the leaf material only. It was isolated as a crystalline picrate, m.p. 216° (dec); the free base could be regenerated by passage of the picrate over alumina but only as an opaque oil. All attempts at crystallization failed. Analysis of the picrate suggested a composition of $C_{16}H_{19-21}NO_3$ for the free base, which also contained one methoxy group and one methyl linked to nitrogen.

[19] Kindly determined by Prof. L. Mandell, Emory University.
[20] M. Conrad and L. Limpach, *Ber. Dtsch. Chem. Ges.* **24**, 2990 (1891).

Comparison of the U.V. spectrum of pilokeanine (Fig. 4) with pertinent spectra in the literature[16–18,21] indicated as a likely structure a 1-methyl-8-methoxy-4-quinolone. As was noted by Goodwin[22] in her work on lunacrine, the long wave maximum of an 8-methoxy-4-quinolone is shifted hypsochromically in dilute acid. This shift, while less pronounced in our case, is nevertheless present. The I.R. spectrum of pilokeanine in the 6 μ region is clearly that of a 4-quinolone.

The nature and attachment of the remaining $C_5H_{9-11}O$—fragment could not be determined with certainty. Analogy with other naturally occurring 4-quinolones offers an attractive possibility that an isoprene moiety is attached to the 3-position of the 4-quinolone nucleus. Absence of I.R. bands associated with a furan ring[23]

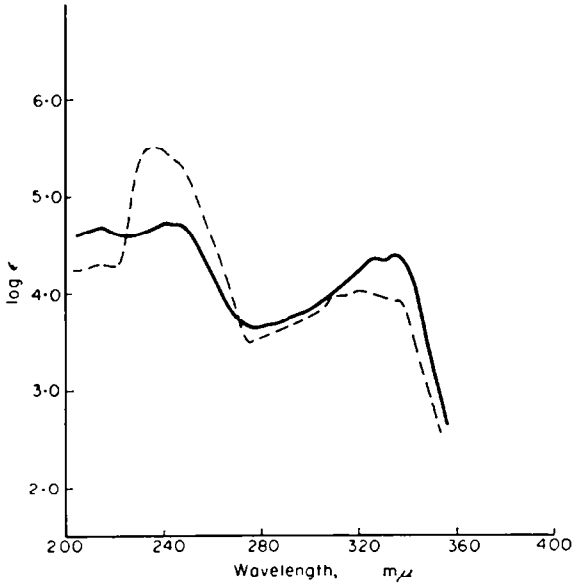
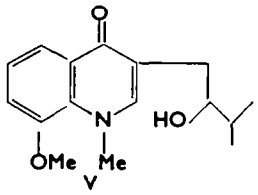


FIG. 4. U.V. spectrum of pilokeanine (V): — in 95% ethanol; - - - in 0·01N ethanolic HCl

and the presence of a band at 2·72 μ point to the remaining oxygen atom as an alcohol function, perhaps primary of secondary.[13] In order to obtain additional clues as to the nature of the side chain some of the oily base was oxidized with chromic anhydride in acetic acid. A resulting picrate, m.p. 187–190°, analyzed well for $C_{22}H_{23}N_4O_{10}$ thereby supporting the formula $C_{16}H_{21}NO_3$ for pilokeanine. The I.R. spectrum of the picrate of the oxidation product differed from that of the picrate of its precursor, but U.V. spectra of the two picrates were identical. This latter observation makes it mandatory that the hydroxy group in pilokeanine be insulated from the quinolone nucleus by at least one carbon. We are therefore proposing structure V as likely expression for pilokeanine.

[21] R. D. Brown and F. N. Lahey, *Austr. J. Sci. Research* A3, 615 (1950).
[22] S. Goodwin and E. C. Horning, *J. Amer. Chem. Soc.* **81**, 1908 (1959).
[23] L. H. Briggs and L. D. Colebrook, *J. Chem. Soc.* 2458 (1960).



V

Furoquinolines are perhaps the most characteristic class of compounds which have been isolated from Rutaceae. It is therefore not surprising that in the course of this investigation the well-known evolitrine and kokusaginine as well as the new 6-methoxydictamnine were found to occur in *Platydesma campanulata*. Isolation of pilokeanine, which is a 4-oxygenated quinoline bearing a hydroxylated isoprene side chain, and of platydesmine, which is a relatively rare dihydrodictamnine, are of greater interest. Compounds such as these may well constitute biogenetic links between quinolines bearing isoprene side chains and furoquinolines. The simple 1,2-dimethyl-4-quinolone is perhaps the most surprising alkaloid to occur in a rutaceous plant. The wide-spread echinopsine (1-methyl-4-quinolone)[24] seems to be confined to the genus *Echinops* in Compositae. Other simple quinoline derivatives, such as quinaldine or 1-methyl-2-quinolone, have been found in the rutaceous genus *Galipea*,[24] but their occurrence seems to be confined to the tribe Cuspariae.

EXPERIMENTAL*

Extraction of root and bark from Kauai. Dried and ground stem bark (6·4 kg) and whole root (4·5 kg) was extracted with hexane under reflux for 36 hr. The hexane extract gave a positive alkaloid test, but attempts to isolate alkaloids failed.

The plant material was next extracted with refluxing methanol for 48 hr. The methanolic extract was concentrated to 4 l. in a steam-jacketed vacuum evaporator. To the concentrated methanolic extract 4 l. 5% aqueous tartaric acid was added and the mixture was filtered using Kenite filter aid and sand. The resulting solid was washed with 5% tartaric acid, followed by 5% HCl. The washings were combined with the original filtrate. This combined aqueous acidic solution was then extracted with chloroform in a continuous liquid–liquid extractor for 24 hr. Removal of the chloroform in a rotary evaporator under water pump vacuum yielded 119 g of brown oil. Further extractions of the aqueous fraction at pH 7 and pH 10 yielded solutions giving positive alkaloid tests, but subsequent chromatography did not yield crystalline alkaloids. The brown oil was now taken up in 1 l. butanol and extracted with 20 × 100 ml portions 5% HCl. The acidic extract was again extracted continuously with chloroform for 48 hr. Upon concentration of the chloroform extract a brown oil, ca. 12 g, was obtained.

Isolation of alkaloids. This oil was dissolved in benzene and chromatographed in a column containing 500 g of Florisil. Thirty fractions were collected upon successive elution with the following solvents: 2 l. benzene, 1 l. 1:1 chloroform-benzene, 1 l. chloroform, 1 l. 1:1 chloroform-acetone, 1 l. acetone, 2·5 l. methanol. Only fractions 1-14 showed promising spots on ascending paper chromatograms using ethyl acetate, pyridine and water in the ration 7·5:2·3:1·65 as developing solution.[25]

These 14 fractions were therefore combined and rechromatographed in a column containing 100 g of Florisil. Elution was started with 300 ml. of benzene and continued as follows: 200 ml 3:1 benzene–chloroform, 100 ml 1:1 benzene–chloroform, 300 ml chloroform, 200 ml 1:1 chloroform–acetone,

* All m.p's determined on a Fisher–Johns block and uncorrected. Elemental and functional group analyses by Dr. A. Bernhardt, Mülheim, Germany, unless noted otherwise. I.R. spectra measured on a Beckman I.R.-5 and U.V. spectra on a Beckman DK-2 instrument. All plant material was dried in a forced draft oven at 60° for 48 hr and ground in a Wiley Mill to pass a 16 mesh screen. Alkaloid tests were considered positive when Mayer's and Dargendorff's reagents gave a precipitate.

[24] H. G. Boit, *Ergebnisse der Alkaloid-Chemie bis* 1960 p. 600 ff., Akademie-Verlag Berlin (1961).

[25] G. B. Marini-Bettolo and G. C. Casinovi, *J. Chromat* **1**, 411 (1958).

200 ml acetone, and 500 ml methanol. Twenty-two fractions were collected. The first 6 fractions gave a positive alkaloid test, and fractions 4, 5, and 6 crystallized. Fractions 1–3 were extracted with hot pet. ether (b.p. 30–60°). Upon cooling the combined extracts 30 mg of a crystalline substance, m.p. 115–116°, was isolated.

After extraction with hot pet. ether the residual fractions 1–3 were taken up in hot benzene. Upon addition of pet. ether (b.p. 30–60°) to cloudiness and subsequent cooling slightly yellow rosettes crystallized. Those from fractions 1 and 2 melted at 124–128° and those from fraction 3 melted at 134–135°.

Thin-layer chromatography on aluminium oxide G in 1:1 benzene–chloroform of the 3 crystalline fractions thus obtained showed that the crystals melting at 134–135° were a pure base, later shown to be 6-methoxydictamnine. Those melting at 115–116° and those melting at 124–128° appeared to be mixtures of two alkaloids, 6-methoxydictamnine and evolitrine.

Altogether 252 mg of chromatographically pure 6-methoxydictamnine was obtained from fraction 3 and by recrystallization of the mixed crystals from benzene–petroleum ether (b.p. 30–60°). Attempts to obtain pure evolitrine from the mixture of the two bases failed.

Trituration of fractions 4-5 with absolute ethanol yielded 18 mg of a white crystalline solid, m.p. 164–167°, subsequently shown to be kokusaginine.

Extraction of root and bark from Hawaii. A total of 12 kg of stem bark and whole root was extracted with refluxing methanol for 48 hr. The extract was concentrated to 4 l. to which 7·6 l. 5% HCl was added. A solid precipitated which was filtered off and repeatedly extracted with 5%, 10%, and 20% HCl in succession. The acidic extracts were combined with the acidic filtrate and the solid was discarded.

The aqueous acidic solution was neutralized under cooling with conc ammonium hydroxide and then extracted with chloroform for 24 hr in a liquid–liquid extractor. The chloroform solution was evaporated to near-dryness in a rotary evaporator under water pump vacuum and then triturated with ether. The original chloroform extract was thus separated into an ether-soluble fraction, weighing 190 g, and an ether-insoluble fraction, weighing 158 g.

Isolation of alkaloids from the ether-soluble fraction. The ether was removed on a rotary evaporator under water pump vacuum and the residual oil was taken up in 1 l. benzene and subsequently extracted, first with 1 l. 5% HCl and then with 1 l. 10% HCl. The acidic extracts were brought to pH 6 with solid sodium bicarbonate and extracted with chloroform for 24 hr. About 30 g of a viscous oil remained after distillation of the chloroform on a rotary evaporator under water pump vacuum. The viscous oil was dissolved in 50 ml benzene and chromatographed in a column containing 500 g Florisil. Elution was started with benzene, gradually changed to chloroform, then to ethanol and finally to methanol. Fractions of 25 ml each were collected with an automatic fraction collector. The first 50 fractions gave positive alkaloid tests. Thin-layer chromatography on aluminium oxide G showed that the 50 fractions were resolved poorly. The fractions were therefore combined, dissolved in 2:1 benzene–carbon tetrachloride and rechromatographed on basic alumina (Woelm). The column was eluted with the following solvents: 1 l. 2:1 benzene–carbon tetrachloride, 1 l. benzene, 1 l. 9:1 benzene-chloroform, 1 l. chloroform, 1 l. ethyl acetate, and 1 l. acetone. In this manner 425 fractions of 25 ml each were collected. Fractions 150–299 were combined in benzene and chromatographed on 100 g of silica gel G. Elution with chloroform–benzene mixtures (1:9, 1:4, 2:3, 1:1, 3:1) brought down several crystalline fractions. Fractions 109–124 of the silica gel G column could be shown by thin-layer chromatography (1:1 benzene–chloroform on aluminum oxide G) to contain alkaloids with R_f values of the 3 previously isolated bases. They were again combined and chromatographed in a column containing 50 g silica gel G. The alkaloids were eluted with 1:9 chloroform–benzene followed by 1:6 chloroform–benzene. Fractions 31–62 and 87–110 contained the alkaloids. Fractions 87–110 were combined and recrystallized from ethanol to yield 67 mg kokusaginine, m.p. 169–169·5°.

Hot pet. ether extracted 27·7 mg 6-methoxydictamnine from fractions 31–62. Upon recrystallization from hot pet. ether, it melted at 134–135°. An examination of the mother liquours by thin-layer chromatography showed the presence of evolitrine and 6-methoxydictamnine. Attempts to separate the two were unsuccessful.

Isolation of alkaloids from the ether-insoluble fraction. The ether-insoluble oil was mixed with 4 lbs sand, filled into a column and extracted in succession with 3 l. 5% HCl, 2 l. 10% HCl 20% HCl. The extracts were neutralized with solid bicarbonate, combined, and extracted with chloroform for 24 hr. The chloroform was distilled off and the residual oil was taken up in 80% methanol which was

extracted with carbon tetrachloride for 3 days. The methanol solution was evaporated to near-dryness and triturated with benzene. Upon evaporation of the solvent the combined carbon tetrachloride and benzene fractions gave 12 g of a viscous brown oil. The oil was separated into two fractions, one of which was eluted from 500 g aluminum oxide G with chloroform, and one which was eluted with ethanol. The ethanol eluate yielded no alkaloids. The chloroform eluate was dissolved in benzene and applied to a column containing 150 g of silica gel G. The alkaloids were eluted with 500 ml of 3:1 benzene–chloroform, 1 l. 7:3 benzene–chloroform, 500 ml 3:2 benzene–chloroform, 500 ml 1:1 benzene–chloroform, and 1 l. 5% methanol in chloroform. Three hundred and twenty-five fractions were collected. Fractions 1–38 and 274–325 contained no alkaloids. It could be shown by thin-layer chromatography (aluminum oxide G in chloroform) that fractions 39–130 contained non-alkaloidal substances and an alkaloid of R_f 0·2–0·7. Similarly, fractions 269–273 could be shown to contain an alkaloid of R_f 0·2. In order to purify the fractions picrates of the alkaloidal components were prepared by dissolving the fractions in a minimum amount of hot ethanol and then adding 1–2 ml conc picric acid solution in methanol. The results of this work-up are summarized in Table I.

TABLE 1. RESULTS OF WORK-UP

Fraction	M.p.	Wt of Picrate	M.p. after 1st recryst. from EtOH	After 2nd recryst.
1–38	—	—	—	—
39–65	160–170°	48 mg	hot cryst.	—
66–69	172–178° (dec)	39 mg	177–180°	193–195°
70–89	171–172°	91 mg	185–185·5°	—
90–130	205–207°	54 mg	—	—
211–216	92–96°	168 mg	98–99°	107–109°
269–273	234–245°	105 mg	234–235° (dec)	—
274–325	—	—	—	—

By thin-layer chromatography, comparison of U.V. and I.R. absorption spectra and m.p it was established that (1) the picrates from fractions 39–65 and 66–69 were mixtures of the picrates of evolitrine and 6-methoxydictamnine; (2) the picrate from fractions 70–89 was a mixture of picrates of evolitrine, 6-methoxydictamnine and kokusaginine; (3) the picrate from fractions 60–130 was identical with kokusaginine picrate; (4) the picrate from fractions 211–216 was the picrate of a new base, named *platydesmine;* and (5) the picrate from fractions 269–273 was the picrate of another new base, subsequently shown to be 1,2-dimethyl-4-quinolone.

Extraction of the combined leaves. A total of 4·8 kg dried and milled leaves was extracted with refluxing methanol for 48 hr. The methanolic extract was concentrated to a volume of 4 l. on a steam-jacketed vacuum evaporator. Four liters 5% HCl was added to the concentrate. The resulting oily precipitate was filtered and washed with 2 l. 5% HCl. Combined acidic extracts had a pH 2 and were extracted with chloroform for 48 hr to yield 175 g of a viscous brown oil upon evaporation of the chloroform. The pH of the aqueous solution was now raised to 13-14 with solid sodium hydroxide and once more extracted with chloroform for 48 hr. Upon evaporation of the solvent 30 g dark brown oil was obtained.

Isolation of alkaloids from the pH 2 extract. The oil (175 g) was taken up in 300 ml methanol and 1200 ml 5% HCl was added. An oily precipitate formed which was filtered off on a buchner funnel. The acidic filtrate was extracted with chloroform and chromatographed in a column containing 500 g basic alumina (Woelm). Elution was started with chloroform and gradually changed to ethanol. Only the early fractions which were eluted with pure chloroform gave positive alkaloid tests. Upon thin layer chromatography of these fractions on aluminum oxide G in 1:1 benzene-chloroform only one alkaloidal spot was noticed. The alkaloidal fractions were therefore combined, dissolved in 100 ml ethanol, and 1 g picric acid was added. The mixture was heated to boiling. Upon cooling, a non-crystalline yellow picrate was collected. Crystallization from ethanol yielded 71 mg fine yellow needles m.p. 205–207·5°. Its I.R. absorption spectrum and a m.p. showed this picrate to be identical with kokusaginine picrate.

Isolation of alkaloids from the pH 8 extract. The oil (20 g) was dissolved in 50 ml chloroform and chromatographed on 600 g basic alumina (Woelm). Fractions of 1–58 resulted from elution with pure chloroform. Fractions 59–160 were eluted with 20% ethanol in chloroform, and the remainder with ethanol. Only fractions 14–74 gave alkaloidal spots on thin-layer chromatogams. These fractions were therefore combined and dissolved in 50 ml 95% ethanol and 5 ml conc methanolic picric acid was added. The solution was brought to the b.p. and then slowly evaporated to dryness. Upon trituration with acetone, a yellow picrate remained. Recrystallization from ethanol yielded 145·5 mg yellow needles. The I.R. absorption spectrum and a mixture m.p. showed this base to be identical with the picrate of 1,2-dimethyl-4-quinolone.

Isolation of alkaloids from the pH 14 extract. The oil (30 g) was dissolved in 50 ml chloroform and chromatogaphed in a column containing 500 g basic alumina (Woelm). Chloroform eluted two bands. More polar solvents eluted no further alkaloids. The fractions eluted with chloroform were combined and dissolved in benzene. The benzene solution was chromatographed in a column containing 250 g aluminum oxide G. Elution was started with benzene, continued with 1:1 benzene–chloroform, and then with chloroform. The fractions eluted with the 1:1 benzene–chloroform showed one alkaloidal spot on a thin-layer chromatogram. They were combined in chloroform and about 4 ml methanolic picric acid was added. The solution was brought to the b.p. and then cooled resulting in precipitation of a yellow picrate. By fractional crystallization from ethanol the picrate could be separated into the picrates of the previously found 1,2-dimethyl-4-quinolone and a new base named *pilokeanine*. Pilokeanine picrate began to soften at 195° and decomposed from 203–216°. There was 71 mg of this picrate.

Evolitrine. The mixture of evolitrine and 6-methoxydictamnine which was obtained from both extractions of root and bark was chromatographed on a thin layer of aluminum oxide G in 1:1 benzene–chloroform concurrently with pure 6-methoxydictamnine and an authentic sample of evolitrine.[9] Development with modified Dragendorff's reagent[10] yielded results which are summarized in Table 2.

TABLE 2. CHROMATOGRAPHY OF THE MIXTURE OF EVOLITRINE AND 6-METHOXYDICTAMNINE

	Pure 6-methoxy-dictamnine	Authentic evolitrine	Mixture	
			Spot 1	Spot 2
R_f value	0·58	0·61	0·58	0·61
Color	Dark purple	Tan	Dark purple	Tan

6-Methoxydictamnine. The base could be recrystallized by dissolving it in a minimum amount of benzene, adding pet. ether to appearance of cloudiness, and subsequent cooling. It sublimed readily at 107°/3 × 10^{-2} mm. The analytically pure base m.p. 134–135°. It was very soluble in chloroform, alcohol, benzene, and carbon tetrachloride; sparingly soluble in acetone and pet. ether (b.p. 30–60°); and insoluble in water, and aqueous base, but it did dissolve slowly in 5% HCl. The base was chromatographically pure. The R_f values on aluminum oxide G were 0·58 in 1:1 benzene–chloroform and 0·44 in 3:1 benzene–chloroform. (Found: C, 68·37, 68·24; H, 5·06, 4·86; OCH_3, 25·77%. $C_{13}H_{11}NO_3$ requires: C, 68·11; H, 4·84; 2 OCH_3, 27·08%.)

The U.V. absorption spectrum (Fig. 1) in 4·4 × 10^{-5}M in 95% ethanol solution showed the following maxima and minima; λ_{max} 350 mμ (log ε 4·68), 333 (4·75), 307·3 (5·04), 295·5 (4·99), 284 sh (4·81), 260·7 (4·91), 248·8 (6·10); λ_{min} 342 (4·63), 324·2 (4·68), 267 (4·62), 220 (5·85).

The major peaks in the I.R. absorption spectrum occured at the following wavelengths: $\lambda_{max}^{chloroform}$ 3·40 (m), 6·18 (s), 6·33 (s), 6·49 (m), 6·64 (s), 6·83 (s), 7·07 (m), 7·33 (s). 7·67 (s), 7·92 (m), 8·11 (s), 8·23 (s), 8·67 (s), 9·01 (s). 9·15 (s), 9·68 (m), 10·20 (m), 11·78 (w), 12·06 (m), 14·30 (w).

A picrate of the alkaloid was prepared by dissolving 4·5 mg of the base in 1 ml absolute ethanol. Upon addition of 2 drops of conc methanolic picric acid, the picrate precipitated immediately as fine yellow needles. Recrystallization from ethanol gave fine needles, m.p. 195–196° (dec.)

The iso-compound. 6-Methoxydictamnine (112 mg) was sealed in a Pyrex tube with 2 ml methyl iodide and left overnight at 100°. After opening of the tube the solvent was evaporated and the residue extracted with three 5 ml portions chloroform. The extract was concentrated to 5 ml in a

stream of nitrogen and applied to a column containing 10 g basic alumina (Woelm). Three successive colored fractions could be eluted with chloroform. The third fraction upon evaporation of the chloroform gave a crystalline residue, m.p. 206–209°. The residue was recrystallized by dissolving it in a minimum amount of cold absolute ethanol, warming the solution, and then adding water until the solution appeared cloudy. Colorless crystals separated overnight in the refrigerator. This compound melted from 214–216°. The crystals slowly turned pink on standing. (Found: C, 67·82; H, 4·82%. Calc. for $C_{18}H_{11}NO_3$: C, 68·11; H, 4·84%).

Upon melting, cooling, and remelting the iso compound melted from 208–210°. The two samples when mixed by fusion followed by cooling, had on remelting a m.p. 208–210·5°.

The U.V. absorption spectrum in $4·39 \times 10^{-5}$ M 95% ethanol solution showed the following maxima and minima: λ_{max} 361 mμ (log ε 3·66), 344 (3·64), 329 (3·38), 302 (3·07), 290 (3·19), 264·5 (4·15), 255 (4·03), 243 (4·11), 216·5 (3·91); λ_{min} 350 (3·53), 310 (2·88), 260 (3·99), 250 (3·94), 225 (3·86).

The I.R. absorption spectrum gave the following peaks: $\lambda_{max}^{chloroform}$ 3·39 μ (m), 6·17 (s), 6·28 (s), 6·49 (s), 6·63 (s), 6·80 (m), 6·98 (m), 7·42 (w), 7·65 (s), 8·08 (s), 8·37 (m), 8·44 (m), 8·60 (m), 8·84 (w), 9·02 (m), 9·53 (w), 11·12 (w), 11·34 (w), 12·32 (w).

Kokusaginine. Recrystallization of the base from absolute ethanol gave needles m.p. 169–169·5°. The base was found to be soluble in chloroform, ethanol, and methanol. It was slightly soluble in benzene, ether, and 5% HCl. It was insoluble in water, aqueous base and pet. ether. (Found:[26] C, 65·12, 64·86; H, 5·39, 5·20. Calc. for $C_{14}H_{13}NO_4$: C, 64·86; H, 5·05%.)

The U.V. absorption spectrum in $3·86 \times 10^{-5}$ M 95% ethanol showed the following maxima and minima: λ_{max} 334 mμ (log ε 3·53), 323 (3·64), 352 (4·23), 246 (4·17); λ_{min} 315 (3·60), 263 (2·71).

The I.R. absorption spectrum in 0·13 M chloroform showed the following peaks: λ_{max} 3·36 μ (m), 3·52 (w), 6·13 (m), 6·43 (w), 6·62 (s), 6·71 (s), 6·81 (m), 6·90 (m), 7·53 (s), 7·91 (s), 8·38 (w), 8·50 (s), 9·60 (m), 9·46 (m), 9·60 (w), 9·82 (s), 10·04 (m), 10·52 (m), 11·63 (m), 14·22 (w), 15·13 (w).

The base (5 mg) was dissolved in 10 drops methanol and 3 drops conc methanolic picric acid were added. A precipitate formed immediately. After recrystallization from ethanol it melted 205–207° (dec). An authentic sample of kokusaginine picrate[4] had m.p. 204–206° (dec) and the m.p. of the mixed compounds was 205–208° (dec). The I.R. absorption spectra of the picrates were identical.

Platydesmine picrate. The base was isolated as the picrate. The picrate after recrystallization from methanol softened around 93° and m.p. 107–109°. (Found: C, 51·55, 51·75; H, 4·24, 4·11. $C_{18}H_{11}NO_3 \cdot C_6H_3N_3O_7$ requires: C, 51·64; H, 4·13%.)

The free base was obtained from the picrate by adsorbing 50 mg on 2 g aluminum oxide G and placing this mixture on top of a 10 g aluminum oxide G in a column. Elution with chloroform and evaporation of the solvent gave 21·3 mg of crystalline base.

Platydesmine dissolved readily in benzene, chloroform, and ethanol; it was slightly soluble in 5% HCl; and insoluble in pet. ether. It was recrystallized from benzene–petroleum ether, yielding white rosettes of needles, m.p. 137–138°.

Its U.V. absorption spectrum (Fig. 2) showed the following maxima and minima. In $3·86 \times 10^{-5}$ M 95% ethanol: λ_{max} 320·5 mμ (log ε 3·55), 307·2 (3·49), 294·5 sh (3·24), 283 (3·65), 272 (3·73), 262·3 (3·65), 253·5 (3·50), 238·1 (4·43), 229·3 (4·57); λ_{min} 314 (3·38), 292 (3·23), 278·3 (3·59), 266·5 (3·64), 248·5 (3·43), 236·5 (4·42). $3·86 \times 10^{-5}$M in 0·01N HCl in 95% ethanol: λ_{max} 315 (3·69), 302·5 (3·86), 291·5 (3·89), 240·2 (4·33), 236 (4·40), 215·8 (4·39); λ_{min} 312 (3·68), 253 (3·24), 226 (4·23).

The mass spectrum of the base showed two principal peaks at 259 and 200 e^{m}.[12]

The I.R. absorption spectrum of the base gave the following peaks: $\lambda_{max}^{chloroform}$ 2·79 (w), 3·36 (s), 6·11 (s), 6·29 (s), 6·59 (s), 6·81 (m), 700 (s), 7·16 (s), 7·31 (s), 7·49 (m), 7·64 (m), 7·72 (m), 8·08–8·31 (m), 8·47 (m), 8·58 (m), 8·72 (w), 8·91 (s), 9·08 (s), 9·82 (s), 10·04 (m), 10·50 (m).

1,2-Dimethyl-4-quinoline picrate. The picrate was soluble in acetone and 95% ethanol. Recrystallization from 95% ethanol yielded yellow needles, m.p. 234–236°, dec 237°. (Found: C, 51·19, 51·39; H, 3·79, 3·73; N, 13·93; OCH_3, 0%. Calc. for $C_{11}H_{11}NO \cdot C_6H_3NO_7$: C, 50·75; H, 3·51; N, 13·93; OCH_3, 0%.)

The picrate (60 mg) was adsorbed on 2 g aluminum oxide G by dissolving the picrate in acetone, adding the adsorbent and subsequently evaporating the solvent on a rotary evaporator under water pump vacuum. The resultant mixture was placed on top of a column containing 10 g aluminum oxide

[26] Analysis by Dr. W. Zimmermann, Australian Microanalytical Service, Melbourne.

G. The base was eluted with 10% methanol in chloroform. Evaporation of the solvent gave 23 mg of slightly yellow needles of the free base.

1,2-*Dimethyl-4-quinoline*. The free base was soluble in chloroform, hot benzene, ethanol, methanol, and 5% HCl. It was recrystallized from chloroform–pet. ether or from benzene. It crystallized as fine needles which turned to prisms at 174° and melted sharply at 178–179°. (Found: C, 75·96, 76·10; H, 6·51, 6·46. Calc. for $C_{11}H_{11}NO$: C, 76·27; H, 6·40%).

The U.V. absorption spectrum (Fig. 3) showed the following maxima and minima. In $5·78 \times 10^{-5}$ M 95% ethanol: λ_{max} 335·5 mμ (log ε 3·94), 321·5 (3·96), 310 (3·72), 290 (3·29), 279 (3·17), 266 (2·97), 239·5 (4·20),, 206·7 (4·44); λ_{min} 327·5 (3·89), 261 (2·92), 222·5 (3·97); $5·78 \times 10^{-5}$M in 0·01N HCl in 95% ethanol: λ_{max} 302·8 (3·95), 247·1 (2·95), 233·1 (4·70); λ_{min} 257·5 (2·84), 219 (4·27).

The I.R. spectrum in 0·21M chloroform showed the following peaks: λ_{max} 3·25 μ (w), 3·34 (s), 6·23 (s), 6·32 (s), 6·42 (m), 6·65 (s), 6·78 (m), 6·97 (w), 7·05 (m), 7·22 (w), 7·41 (w), 7·56 (m), 7·86 (m), 8·45 (m), 8·61 (w), 8·92 (w), 9·24 (m), 9·61 (m), 9·73 (w), 11·08 (m), 11·86 (m).

The N.M.R. spectrum showed a 3-proton peak at 7·66τ, a 3-proton peak at 6·41τ, a 1-proton peak at 3·95τ, a split 3-proton peak at 2·61τ, and a split 1-proton peak at 1·64τ. Tetramethylsilane was the standard and deuteriochloroform the solvent.

Pilokeanine Picrate. The alkaloid was isolated as the picrate. It could be recrystallized from absolute ethanol. The picrate softened at 194°, began to turn brown at 203°, and was decomposed by 216°. (Found: C, 52·51, 52·56; H, 4·35, 4·39; OCH_3, 5·69; NCH_3, 9·48. $C_{16}H_{21}NO_3 \cdot C_6H_3N_3O_7$ requires: C, 52·38; H, 4·80; 1 OCH_3, 6·16; 1 NCH_3, 5·75%).

The picrate (0·110 g) was adsorbed on 2 g aluminum oxide G and added to the top of a column containing 10 g aluminum oxide G. Elution with chloroform brought down the free base. Fifty milligrams of the free base was collected. The free base could not be obtained crystalline. Attempts were made to crystallize it from benzene, 95% ethanol, ethyl acetate, ether, benzene–pet. ether, chloroform–pet. ether, ethanol–water, and ethyl acetate–pet. ether. The base always appeared as a whitish opaque oil.

Thin-layer chromatography of the base on aluminum oxide G with chloroform as the developer gave a single spot of R_f 0·35.

The U.V. absorption spectra (Fig. 4) of this oil had the following maxima and minima. In $4·76 \times 10^{-5}$M 95% ethanol: λ_{max} 336·5 mμ (log ε 4·39), 324·8 (4·34), 291·6 sh (3·75), 281 sh (3·66), 248 (4·72), 242 (4·74), 230·8 sh (4·63), 215 (4·68); λ_{min} 276 (3·64), 223·4 (4·61), 205 (4·62); $4·76 \times 10^{-5}$M in 0·01N HCl in 95% ethanol: λ_{max} 336 (3·94), 322 (4·01), 308·7 (3·96), 248 sh (5·34), 234 (5·51), 215 (4·30); λ_{min} 275·4 (3·46), 220·5 (4·28), 210 (4·27).

The I.R. absorption spectrum showed the following peaks: $\lambda_{max}^{chloroform}$ 2·73 μ (w), 3·34 (s), 3·48 (sh), 3·53 (sh), 6·14 (s), 6·22 (s), 6·37 (m), 6·79 (m), 6·90 (w), 7·06 (w), 7·41 (w), 7·53 (w), 7·60 (w), 7·85 (w), 8·46 (w), 8·61 (w), 9·23 (w), 9·68 (s), 10·29 (w), 11·56 (w), 11·71 (w).

Oxidation of pilokeanine.[27] The base (50 mg) was dissolved in 6 ml glacial acetic acid and a solution of 100 mg chromic anhydride in 10 ml 90% acetic acid was added dropwise. No immediate change in color was noted. Upon standing, the solution turned dark. After 1 hr at room temp the solution was slowly poured into 30 ml conc ammonium hydroxide with cooling. The basic solution was extracted with 25 ml portions chloroform. The chloroform solution was washed with 100 ml water and dried by pouring it through a bed of sodium sulfate on a funnel. The solution was evaporated to dryness on a rotary evaporator under water pump vacuum. The resulting brown oil was dissolved in 10 ml 95% ethanol and treated with 15 ml 2,4-dinitrophenylhydrazine reagent. After refrigeration overnight the solution became cloudy but no crystals could be detected. The cloudy solution was slowly neutralized with 5% sodium bicarbonate solution. At pH 1·5 the salt of the unreacted 2,4-dinitrophenylhydrazine precipitated. Neutralization was continued with potassium hydroxide pellets and the solution was thus brought to pH 10 and extracted with three 50 ml portions chloroform. Upon evaporation of the chloroform the residual solid was taken up in 10 ml absolute ethanol and 2 ml conc methanolic picric acid was added. No picrate precipitated. Addition of 5 ml 10% acetic acid and cooling overnight yielded a crystalline picrate. Recrystallization from absolute ethanol gave fine yellow needles, melting without decomposition 187–190°. The U.V. spectrum of this picrate was identical with that of pilokeanine picrate, but their I.R. spectra differed. (Found: C, 52·57, 52·66; H, 4·32; 4·31; $C_{16}H_{19}NO_3 \cdot C_6H_3N_3O_7$ requires: C, 52·58; H, 4·42%.)

[27] R. E. Marker and E. E. White, *J. Amer. Chem. Soc.* **61**, 1329 (1939).

Synthesis of 1,2-*dimethyl-4-quinolone by the Conrad-Limpach method*[20]

a. 2-*Methyl-4-quinolone*. To a mixture of 51·5 g (0·55 mole) aniline and 66 g (0·51 mole) ethyl acetoacetate methylene chloride was added to a volume of 250 ml. After 4 days at room temp the solution was washed successively with 100 ml 2% HCl, 100 ml water, 100 ml 0·5N sodium hydroxide and again with 100 ml water. Subsequently, the solution was dried over magnesium sulphate and the solvent was evaporated. The residual light brown oil was added to 500 ml paraffin oil at 205° while stirring and distilling off the alcohol as it was formed. After the addition the temp was raised to and kept at 240° for 10 min. The mixture was then cooled with stirring. A solid separated on cooling. It was filtered and washed with benzene. Washing with boiling chloroform removed the final traces of color and left 6·29 g (7·8%) of a white solid m.p. 234–235·5°.[22] The U.V. absorption spectrum was identical with that published by Ewing and Steck[17] for 2-methyl-4-quinolone.

b. 1,2-*Dimethyl-4-quinolone*. To a solution of 0·71 g (0·013 mole) potassium hydroxide in 50 ml hot methanol 2 g (0·013 mole) 2-methyl-4-quinolone was added. The methanol was then distilled off on a rotary evaporator under water pump vacuum and 5 ml dimethyl sulfate was added to the residue. The reaction mixture was refluxed 0·5 hr and then taken up in an excess aqueous potassium hydroxide. The resulting purple solution was extracted with chloroform and the extract was passed through a column containing 50 g of aluminum oxide G. The eluant was evaporated to dryness and then taken up in a minimum of chloroform. Upon addition of 3–4 times as much pet. ether as there was chloroform purple crystals appeared. These were sublimed at 120°/0·3 mm. A total of 0·3 g (13%) white crystals, m.p. 179° (Lit. 174–175°[22b]), was collected. Two recrystallizations from benzene raised the m.p. to 179·5–180·5°. (Found: C, 76·22, 75·99; H, 6·42, 6·44; O, 9·78; N, 8·15; NCH_3, 7·94; OCH_3, 0%. Calc. for $C_{11}H_{11}ON$: C, 76·27; H, 6·40; O, 9·24; N, 8·09; NCH_3, 8·66; OCH_3, 0%.)

The U.V. and I.R. absorption spectra were identical with those of the natural product in all respects.

Acknowledgements—The authors wish to express their gratitude to Dr. J. R. Price for communicating unpublished data and for numerous comparison samples and to the National Science Foundation (G-9915) and the National Institutes of Health (RG-5095) for generous financial support.