585. *Alkaloids of* Daphnandra *Species. Part II. Daphnandrine, Daphnoline, and Aromoline.*

By I. R. C. Bick, E. S. Ewen, and A. R. Todd.

Daphnandrine and daphnoline, alkaloids first isolated by Pyman from the bark of *D. micrantha*, are shown to have structures (VI) or (VII), and (VIII) or (IX), respectively, in which, of the groups R and R′, one is H and the other Me. Evidence is also presented that aromoline, which accompanies daphnoline in the bark of *D. aromatica*, is *N*-methyldaphnoline (VIII; R = R′ = Me) or (IX; R = R′ = Me). All three alkaloids on complete methylation with methyl iodide furnish *O*-methyloxyacanthine dimethiodide, from which it follows that they belong to the same stereochemical series as oxyacanthine. The only other alkaloid known to belong to this series is trilobamine, and a comparison of the published data for this rare alkaloid suggests strongly that it contains only one methylimino-group and is identical with daphnoline. This view is supported by the apparently identical behaviour of daphnoline and trilobamine on paper chromatograms.

Pyman (*J.*, 1914, **105**, 1619) recorded the isolation of three alkaloids, daphnandrine, daphnoline, and micranthine, from the bark of the Australian tree *Daphnandra micrantha* but, although he characterised them by preparation of their salts with mineral acids, he made no study of their constitution. In 1938 the late Prof. G. Barger obtained Pyman's original specimens of the alkaloids but his projected investigation of their structure was prevented by his untimely death. Subsequently two of us (E. S. E. and A.R.T.) took up the investigations using the material which was left by Prof. Barger. The quantities available were insufficient to complete the structural work on daphnandrine and daphnoline, and the amount of micranthine was too small to permit any degradative studies. Further supplies could not be obtained owing to the outbreak of war, and the investigations were interrupted for several years. When, after the war, it became possible to obtain further supplies of plant material, the investigations were resumed (by I. R. C. B. and A. R. T.) and their scope was extended to include a study of the new alkaloids repandine, repanduline, and aromoline, isolated by Bick and Whalley (*Univ. Queensland Papers, Dept. Chem.*, 1946, **1**, No. 28; 1947, **1**, No. 30; 1948, **1**, No. 33) in the course of an examination of the alkaloidal content of other *Daphnandra* species occurring in Queensland. In Part I of this series (Bick and Todd, *J.*, 1948, 2170) we described an investigation of repandine in which it was shown that this alkaloid is a diastereoisomer of oxyacanthine (I; R = H) or (II; R = H); whereas in the latter both asymmetric centres (marked *) are of the same configuration, in repandine one of them is inverted.

Examination of the new supply of *D. micrantha* bark showed that it contained neither daphnandrine nor daphnoline but contained micranthine in much greater quantity than had been found by Pyman. Pyman's material was supplied to him by the Director of the Sydney Botanic Gardens and was presumably collected in northern New South Wales, and it is quite possible that its alkaloidal content may have differed from that of the same species growing in southern Queensland where our material was collected. We are at present awaiting a sample of *D. micrantha* bark from New South Wales in order to examine this matter further. Fortunately we were able, through the kindness of Dr. C. H. Kellaway and Mr. T. M. Sharp

of the Wellcome Research Foundation, to obtain a small quantity of Pyman's original specimen of daphnandrine which was in their possession, and this enabled us to complete our studies. Daphnoline was available to us in substantial quantity since it occurs in the bark of *D. aromatica*, together with small amounts of aromoline (Bick and Whalley, *loc. cit.*). The present memoir deals with the structure of these three alkaloids which are closely related to one another; the structure of micranthine will form the subject of a separate communication.

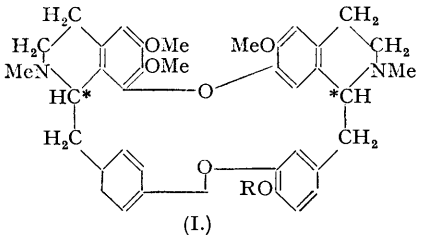
Analyses of daphnandrine were in accord with the formula $C_{36}H_{38}O_6N_2$ advanced by Pyman (*loc. cit.*) and confirmed the presence of three methoxy-groups and one methylimino-group. It was found, however, that daphnandrine had weak phenolic properties and showed the reactions of a secondary amine. The molecular formula and general properties of daphnandrine suggested a relationship to the bisbenzyl*iso*quinoline alkaloids, and this relationship became evident when daphnandrine was degraded by the procedure employed by von Bruchhausen and Gericke (*Arch. Pharm.*, 1931, **269**, 115) for oxyacanthine. *ON*-Dimethyldaphnandrine dimethiodide, formed by methylating daphnandrine with methyl iodide and methanolic sodium methoxide, gave *ON*-dimethyldaphnandrinemethine on Hofmann degradation. A portion of this methine, which was optically inactive, was converted into its crystalline dimethiodide and further degraded by the Hofmann method to O-*methylde*-N-*daphnandrine*. Another portion was ozonised, yielding 2-methoxy-5 : 4′-diformyldiphenyl ether (III; R = Me) and an amino-aldehyde, the dimethiodide of which was converted by a further Hofmann degradation into 2 : 3 : 2′-trimethoxy-6 : 5′-diformyl-5 : 4′-divinyldiphenyl ether (IV). A third portion of the methine base on oxidation gave the dicarboxylic acid (5 : 4′-dicarboxy-2-methoxydiphenyl ether) corresponding to (III).

The two above-mentioned crystalline aldehydes were identified by direct comparison with specimens prepared by an analogous series of reactions from oxyacanthine (von Bruchhausen and Gericke, *loc. cit.*) and kindly supplied by Prof. von Bruchhausen. It follows that *ON*-dimethyldaphnandrine must have structure (I; R = Me) or (II; R = Me). One of these structures also represents *O*-methyloxyacanthine, while the other represents *O*-methylberbamine (von Bruchhausen and Gericke, *loc. cit.*). It was found that *X*-ray powder photographs of *ON*-dimethyldaphnandrine dimethiodide and *O*-methyloxyacanthine dimethiodide were identical; the methine dimethiodides formed from them also gave identical powder photographs. As in the investigation of repandine (Bick and Todd, *loc. cit.*), it was found that m.p.s are frequently of little value for comparisons of methiodides. It follows that daphnandrine belongs to the oxyacanthine rather than the berbamine series, and moreover that it has the same stereochemical arrangement about the two asymmetric centres (marked *) as oxyacanthine itself (I; R = H) or (II; R = H).

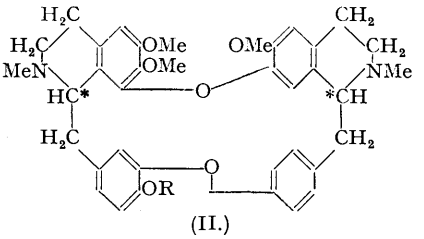
In order to locate the phenolic group in daphnandrine, a sample of the alkaloid was ethylated with diazoethane and the product converted in the usual way into N-*methyl*-O-*ethyldaphnandrine dimethiodide*. The reaction with diazoethane was very sluggish and gave a poor yield, owing presumably to the feebly acidic nature of the phenolic group; it was found that the above-mentioned dimethiodide could be prepared more satisfactorily by ethylation of *N*-methyldaphnandrine dimethiodide with ethyl iodide in ethanolic sodium ethoxide. On being submitted to Hofmann degradation, *N*-methyl-*O*-ethyldaphnandrine dimethiodide gave a methine base which on ozonolysis yielded 2-methoxy-5 : 4′-diformyldiphenyl ether (III; R = Me), identical with that obtained previously. Thus the phenolic group in daphnandrine must be situated in one of the tetrahydro*iso*quinoline nuclei and cannot occupy a position in one of the benzyl residues as it does in oxyacanthine and repandine (I; R = H) or (II; R = H).

In the structurally related series of bisbenzyl*iso*quinoline alkaloids derived from *Chondrodendron* species, such as tubocurarine chloride (V), it was found by King (*J.*, 1937, 1472; 1940, 737) that the Millon reaction could be used in certain cases to determine the position of phenolic groups. From a study of the reactions of a number of substituted phenols to Millon's reagent, he showed that alkaloids containing phenolic groups at position 7 of an *iso*quinoline residue, or position 4 of a benzyl residue, give a positive reaction, whereas those with a phenolic group at position 6 of an *iso*quinoline residue show no reaction. On this basis, King (*loc. cit.*) and Dutcher (*J. Amer. Chem. Soc.*, 1946, **68**, 419) have assigned positions to the phenolic groups in a number of alkaloids belonging to this series, and the positions have proved correct in those cases where further evidence from degradative studies has become available (King, *J.*, 1939, 1157; 1948, 265). It seems reasonable to suppose that these conclusions regarding the Millon test will apply equally to bisbenzyl*iso*quinoline alkaloids of the type found in *Daphnandra* species. Of these, repandine, daphnandrine, daphnoline, and aromoline give positive reactions, and the test is also positive with oxyacanthine. The last base

(Späth and Pikl, *Ber.*, 1929, **62**, 2251) and its diastereoisomer, repandine (Bick and Todd, *loc. cit.*), have been shown to contain a phenolic group at position 4 in one of the benzyl residues. Since daphnandrine gives the Millon test and has no phenolic group in this position, it follows that its phenolic group must be situated at position 7 of a tetrahydro*iso*quinoline residue, and daphnandrine is therefore represented by structure (VI; of R and R′, one is H and the other Me), if oxyacanthine is (I; R = H), or (VII; of R and R′, one is H and the other Me) if oxyacanthine is (II; R = H).



(I.)



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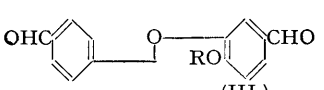

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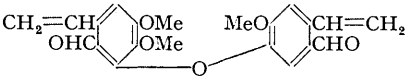

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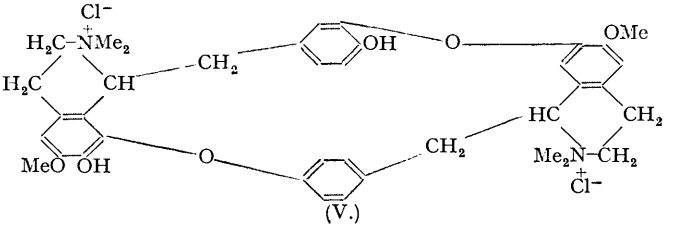

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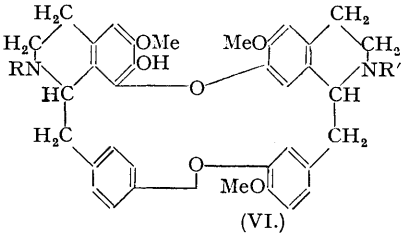
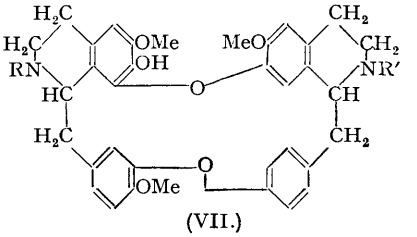


(VI.)



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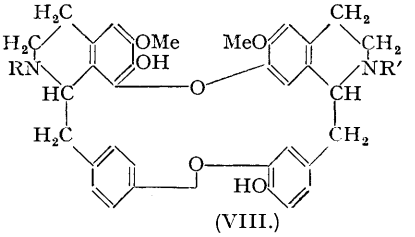
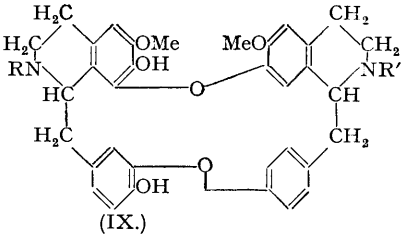


(VIII.)



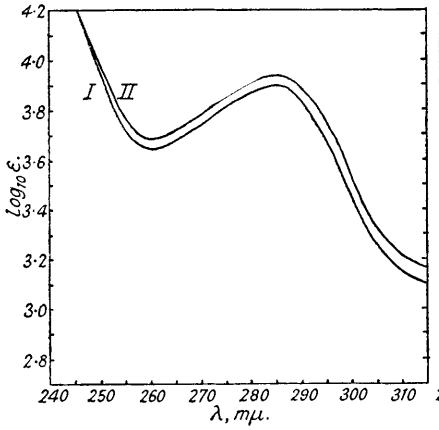
(IX.)

The alkaloid daphnoline was found by Pyman (*loc. cit.*) to be phenolic and to contain two methoxy-groups and one methylimino-group. This made it seem likely that daphnoline was a nordaphnandrine, and Pyman considered the correct formula was most probaby $C_{35}H_{36}O_6N_2$, although his analytical results agreed better with $C_{34}H_{34}O_6N_2$. Further analyses support this view. It has been found that daphnoline retains solvent of crystallisation very tenaciously and it is not possible to remove it completely without decomposition, so that satisfactory analytical results cannot be obtained on solvent-free daphnoline. A positive test was obtained for a secondary amino-group, and the greatly enhanced phenolic properties of daphnoline, compared with daphnandrine, suggested that

it contained two phenolic groups. One of these could be selectively methylated with diazomethane, and the product, converted into its hydrochloride, was identical with daphnandrine hydrochloride; the corresponding hydrobromides were also identical. Thus daphnandrine is a methyl ether of daphnoline; in conformity with this finding, *X*-ray powder photographs of *OON*-trimethyldaphnoline dimethiodide, *ON*-dimethyldaphnandrine dimethiodide, and *O*-methyloxyacanthine dimethiodide were identical. One phenolic group in daphnoline is therefore situated as in daphnandrine at position 7 of a tetrahydro*iso*quinoline residue. To determine the position of the other, daphnoline was converted into N-*methyl*-OO-*diethyldaphnoline dimethiodide* by either of the methods used in the ethylation of daphnandrine. The product was degraded by the Hofmann method to *N*-methyl-*OO*-diethyldaphnolinemethine, identified as its *dimethiodide*. This methine on ozonolysis gave 2-ethoxy-5 : 4′-diformyldiphenyl ether (III; R = Et) identical with that obtained from *O*-ethylrepandine dimethiodide by an analogous series of reactions (Bick and Todd, *loc. cit.*); oxidation of the methine gave the corresponding dicarboxylic acid.

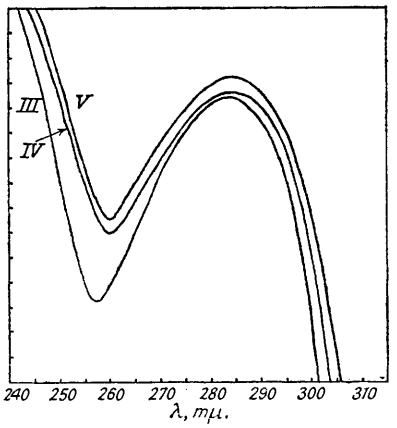
The second phenolic group in daphnoline is therefore located at position 4 of the benzyl group, and daphnoline must therefore have structure (VIII; of R and R′ one is H and the other Me) or (IX; of R and R′ one is H and the other Me). It is interesting to compare the

FIG. 1.



Absorption spectra of aromoline (I) and daphnoline (II) in methanol.

FIG. 2.



Absorption spectra of repandine (III), oxyacanthine (IV), and daphnandrine (V) in methanol.

properties of daphnoline with those of trilobamine, the only other diphenolic alkaloids of the oxyacanthine–berbamine group so far known and hitherto the only alkaloid which has been shown to have the same stereochemical configuration as oxyacanthine. Kondo and Tomita (*Arch. Pharm.*, 1931, **269**, 433; 1936, **274**, 70; *J. Pharm. Soc. Japan*, 1935, **55**, 104), who isolated this alkaloid from the Far Eastern menispermaceous plant *Cocculus trilobus*, proposed for trilobamine a formula $C_{36}H_{38}O_6N_2$ (VIII; R = R′ = Me) or (IX; R = R′ = Me), which differs from the structure now deduced for daphnoline only in having another methylimino-group in place of the secondary amino-group. Their published analytical figures for trilobamine, however, are low even for one methylimino-group, and there is better agreeement with the daphnoline formula $C_{35}H_{36}O_6N_2$ than with that proposed, particularly when allowance is made for solvent of crystallisation which, as noted in the case of daphnoline, cannot be removed completely without decomposition of the alkaloid.

The m. p. recorded for daphnoline by Pyman (*loc. cit.*) is 190—215°, and by Bick and Whalley (*loc. cit.*) 194—196°. According to Kondo and Tomita (*loc. cit.*), trilobamine melts at 195° and decomposes at 212° and has $[\alpha]_D^{15}$ +356·6° (*c*, 1·1 in dilute acetic acid). The corresponding figure for daphnoline, determined for the hydrochloride and calculated in terms of basic ion, is given by Pyman as $[\alpha]_D$ +355° (*c*, 2·1). The solubilities and colour reactions described for the two alkaloids are similar and the absorption spectrum of trilobamine as determined by Kondo and Tomita ($\lambda_{max.}$ = 285 mμ., $\lambda_{min.}$ = 263 mμ.) closely resembles that of daphnoline (Fig. 1; $\lambda_{max.}$ = 285 mμ., $\lambda_{min.}$ = 261 mμ.). From these data it seemed highly probable that daphnoline and trilobamine were identical, and in order to allow a direct

comparison to be made a small amount of trilobamine was kindly presented by Prof. Kondo. When received, however, this sample was found to have resinified almost completely and to contain very little material soluble in dilute acids or organic solvents. Since no crystalline trilobamine could be isolated from it, a comparison with daphnoline by the usual methods was not possible and recourse was had to chromatography. Paper chromatography of a chloroform extract of the specimen of trilobamine in butanol–acetic acid at three different concentrations gave R_f values which were identical in each case with those given by daphnoline and different from those of other structurally similar alkaloids of the bisbenzyl*iso*quinoline series. We therefore conclude that trilobamine and daphnoline are almost certainly identical, and that both have structure (VIII; of R and R′, one is H and the other Me) or (IX; of R and R′, one is H and the other Me).

Analyses of aromoline gave values corresponding to a formula $C_{36}H_{38}O_6N_2$ and showed the presence of two methylimino-groups and two methoxy-groups (Bick and Whalley, *loc. cit.*). Like daphnoline, aromoline retains solvent of crystallisation very tenaciously and it is not possible to remove it without partly decomposing the alkaloid. Aromoline has phenolic properties comparable to those of daphnoline and contains two phenolic groups, of which at least one must occupy the same position as one of those in daphnoline, since aromoline gives a positive Millon reaction. Methylation with methyl iodide in methanolic sodium methoxide yielded *OO*-dimethylaromoline dimethiodide, the Debye–Scherrer diagram of which was identical with those of *OON*-trimethyldaphnoline dimethiodide and *O*-methyloxyacanthine dimethiodide. Ethylation of aromoline dimethiodide gave an amorphous OO-*diethylaromoline dimethiodide*, and on Hofmann degradation this substance was converted into a methine which, with methyl iodide, yielded *OO*-diethylaromolinemethine dimethiodide. An *X*-ray powder photograph of this material was identical with that of *N*-methyl-*OO*-diethyldaphnoline methine dimethiodide prepared as described above. It follows that aromoline is *N*-methyldaphnoline and has structure (VIII; R = R′ = Me) or (IX; R = R′ = Me). The close similarity between these two alkaloids is reflected in their absorption spectra (Fig. 1). For comparison, the absorption spectra of daphnandrine, repandine, and oxyacanthine are given in Fig. 2.

Experimental.

Daphnandrine.—After recrystallising from chloroform and from methanol–ether and drying at 100°/0·1 mm., a specimen of daphnandrine had m. p. 280°. It gave no colour with ferric chloride but gave a pink colour with Millon's reagent. On addition of excess of alkali to a solution of daphnandrine hydrobromide, the precipitate first formed redissolved to give a clear solution; the latter solution, when saturated with carbon dioxide, gave a white precipitate which after recrystallising from methanol–ether and drying at 100°/0·1 mm. had m. p. 280°, undepressed by the original daphnandrine. To a solution of daphnandrine (0·2 g.) in dilute hydrochloric acid (5 c.c.; N/2) an aqueous solution of sodium nitrite (0·05 g.) was added drop by drop. An immediate red colour appeared, followed by precipitation of a brown amorphous nitroso-derivative, m. p. 80—90°. A solution of daphnandrine (0·5 g.) in glacial acetic acid (20 c.c.) was shaken in an atmosphere of hydrogen in the presence of palladised charcoal. No absorption of hydrogen took place, and after removal of the solvent daphnandrine was recovered unchanged (Found: C, 72·5; H, 6·4; N, 4·7; MeO, 15·3; MeN, 5·0. Calc. for $C_{36}H_{38}O_6N_2$: C, 72·7; H, 6·4; N, 4·7; 3MeO, 15·7; MeN, 4·3%).

ON-*Dimethyldaphnandrine Dimethiodide.*—To a solution of daphnandrine (5·0 g.) in methanol (100 c.c.), methyl iodide (8 c.c.) and a methanolic solution of sodium methoxide (0·3 g.) were added. After this mixture had been heated under reflux during 4 hours, a further addition of methyl iodide and methanolic sodium methoxide (0·2 g.) was made and heating was continued for a further 3 hours. The solvent was removed *in vacuo*, and the residue recrystallised from hot water. In order to remove periodides, an aqueous solution of the product was boiled with a little copper powder during 10 minutes, filtered, and set aside. Colourless silky needles separated, m. p. 255—265° (decomp.), identical (Debye–Scherrer diagram) with a specimen of *O*-methyloxyacanthine dimethiodide, m. p. 255—265° (decomp.) (Found: C, 49·5; H, 5·8; N, 3·3; loss at 110°/0·1 mm., 7·1. Calc. for $C_{40}H_{48}O_6N_2I_2,4H_2O$: C, 49·1; H, 5·8; N, 2·9; $4H_2O$, 7·4%).

ON-*Dimethyldaphnandrinemethine Dimethiodide.*—An aqueous solution of *ON*-dimethyldaphnandrine dimethiodide (5 g.) was heated on the water-bath with aqueous potassium hydroxide (50 c.c. of 50%) during 30 minutes. The solution was cooled and the brownish resin which separated was extracted with ether. The heating and extraction were repeated until no more solid was formed, and the combined ethereal extracts were washed with water, dried (Na_2SO_4), and evaporated, leaving *ON*-dimethyldaphnandrinemethine as a brownish resin, $[\alpha]_D^{20}$ 0·0° (*c*, 0·9 in chloroform). To a dry ethereal solution of this resin (2 g.) methyl iodide was added. A white solid separated which on recrystallisation from aqueous methanol formed silky needles, m. p. 250—260°, identical (Debye–Scherrer diagram) with *O*-methyloxyacanthinemethine dimethiodide, m. p. 250—260° (decomp.) (Found: C, 53·8; H, 5·1; N, 3·3. Calc. for $C_{42}H_{52}O_6N_2I_2$: C, 53·9; H, 5·6; N, 3·0%).

The corresponding methochloride and methohydroxide were prepared by shaking an aqueous solution of the above dimethiodide with silver chloride or oxide; they could not be obtained crystalline.

O-*Methylde*-N-*daphnandrine.*—Aqueous *ON*-dimethyldaphnandrinemethine dimethiodide (10 c.c. containing 1 g.) was heated on the water-bath with sodium hydroxide solution (10%) until no further

evolution of trimethylamine took place. The oil which separated was extracted with chloroform, and the solution washed with water, dried (Na_2SO_4), and evaporated. The residue crystallised from chloroform–ethanol in colourless prisms, m. p. 218—220° (Found: C, 77·3; H, 5·3; MeO, 13·3; active H, 0·0. $C_{36}H_{32}O_6$ requires C, 77·1; H, 5·7; 3MeO, 16·2%).

Ozonolysis of ON-*Dimethyldaphnandrinemethine.*—A clear solution of *ON*-dimethyldaphnandrinemethine in a slight excess of dilute sulphuric acid (4 g. in 100 c.c.) was cooled in ice, and a stream of ozone (4%) was bubbled through it during 30 minutes. The sticky yellow resin which separated was extracted with ether, and the ozonolysis and extraction repeated until no further solid separated. The ethereal extracts were combined, extracted with sodium carbonate solution, dried, and evaporated. The resinous residue crystallised from light petroleum–ethanol in thick prismatic needles, m. p. 74—77° (Found: C, 70·0; H, 5·1; MeO, 13·5. Calc. for $C_{15}H_{12}O_4$: C, 70·3; H, 4·7; MeO, 12·1%). A mixed m. p. with 2-methoxy-5 : 4′-diformyldiphenyl ether, m. p. 77—78°, prepared by a similar series of reactions from oxyacanthine showed no depression. The disemicarbazone, obtained in the usual manner, crystallised from ethanol as colourless needles, m. p. 235°.

The aqueous acid solution from the ozonolysis, after extraction with ether, was shaken with palladised charcoal to remove peroxides, and then shaken with hydrogen for 15 minutes, filtered, and treated with excess of sodium hydroxide. The precipitated amino-aldehyde was extracted with ether, and the extract dried and evaporated. A solution of the resinous residue in methanol (4 c.c.) was heated under reflux with methyl iodide (3 c.c.) during 1 hour. The crystalline product which separated was filtered off and recrystallised from light petroleum–ethanol, giving colourless needles, m. p. 217°, identical (Debye–Scherrer diagram) with the corresponding amino-aldehyde methiodide derived from repandine (Bick and Todd, *loc. cit.*) (Found: C, 42·7; H, 5·8; N, 3·9; loss at 110°/0·1 mm., 2·4. Calc. for $C_{27}H_{38}O_6N_2I_2,H_2O$: C, 42·6; H, 5·6; N, 3·7; H_2O, 3·0%).

5 : 4′-*Dicarboxy*-2-*methoxydiphenyl Ether.*—(a) *From* ON-*dimethyldaphnandrinemethine.* A solution of *ON*-dimethyldaphnandrinemethine (1 g.) in a slight excess of dilute sulphuric acid was diluted to 300 c.c. with water. Aqueous potassium permanganate (1%) was added until the pink colour persisted (400 c.c.). After removal of precipitated manganese dioxide by passing sulphur dioxide through the suspension, the mixture was set aside for several hours until the precipitate coagulated. The separated solid was now collected and recrystallised thrice from glacial acetic acid, giving very small prisms, m. p. 300—303° (Found: C, 62·8; H, 4·8. Calc. for $C_{15}H_{12}O_6$: C, 62·5; H, 4·2%). Späth and Pikl (*loc. cit.*) record m. p. 313—314° for this compound.

(b) *From* 2-*methoxy*-5 : 4′-*diformyldiphenyl ether.* To a solution of the dialdehyde (0·4 g.) in glacial acetic acid (4 c.c.), chromic acid (0·2 g.) dissolved in 85% acetic acid (3 c.c.) was added dropwise. The solution was warmed on the steam-bath during 30 minutes, and on cooling a white crystalline product separated, which after recrystallisation from glacial acetic acid had m. p. 300—303°, undepressed in admixture with a sample prepared by method (a) above.

2 : 3 : 2′-*Trimethoxy*-6 : 5′-*diformyl*-5 : 4′-*divinyldiphenyl Ether.*—An aqueous solution of the above amino-aldehyde was heated on the water-bath with potassium hydroxide solution (10 c.c. of 10%) till no further evolution of trimethylamine was evident. The solution was cooled, the separated oil extracted with chloroform, and the extract washed with water, dried (Na_2SO_4), and concentrated to small bulk. The product which separated was recrystallised from methanol–chloroform and formed pale yellow diamond-shaped crystals, m. p. 135—136°. It gave a postive Schiff's test and a blue colour with a mixture of sulphuric and glacial acetic acid, indicating the presence of an *O*-vinylaldehyde grouping. On catalytic hydrogenation the substance absorbed 2 mols. of hydrogen rapidly and a further 2 mols. more slowly (Found: C, 68·0; H, 5·7; active H, 0·0. Calc. for $C_{21}H_{20}O_6$: C, 68·5; H, 5·5; active H, 0·0%). A mixed m. p. with an authentic specimen prepared by an analogous series of reactions from oxyacanthine showed no depression.

The *dioxime* prepared in the usual way from the divinyl-dialdehyde had m. p. 160° (Found: C, 61·6; H, 6·1; N, 7·1; loss at 110°/0·1 mm., 3·1. $C_{21}H_{22}O_6N_2,\frac{3}{4}H_2O$ requires C, 61·3; H, 5·8; N, 6·8; H_2O, 3·3%).

N-*Methyl*-O-*ethyldaphnandrine Dimethiodide.*—(a) *By use of diazoethane as ethylating agent.* To a dry methanolic solution of daphnandrine (2 g. in 500 c.c.) was added an ethereal solution of diazoethane (1·5 g. in 50 c.c.), and the mixture set aside for 2 days. Another addition of diazoethane in ether (1·5 g. in 50 c.c.) was made, and after a further 2 days the solution was evaporated *in vacuo*, and the residue dissolved in dilute hydrochloric acid (100 c.c. of ½%). To the filtered solution aqueous sodium hydroxide (100 c.c. of 5%) was gradually added with vigorous stirring. The precipitate was separated by centrifugation, redissolved in dilute hydrochloric acid (100 c.c. of ½%), and reprecipitated as before. After reprecipitation three times in all, the clear alkaline solutions separated by centrifugation were united and neutralised by addition of small pieces of solid carbon dioxide. Unchanged starting material (1·1 g.), identical (mixed m. p.) with daphnandrine, separated and was recovered by filtration. The precipitate containing *O*-ethyldaphnandrine was dried and dissolved in absolute methanol (50 c.c.). The filtered solution was heated under reflux with methyl iodide (2 c.c.) and sodium methoxide (0·1 g.) during 6 hours. After removal of the solvents *in vacuo*, the residue was dissolved in boiling water. The yellowish resin which separated on cooling was redissolved in hot water, and the solution boiled with a little copper powder for 10 minutes, filtered, and allowed to cool. N-*Methyl*-O-*ethyldaphnandrine dimethiodide* (0·5 g.) separated as a yellowish amorphous mass; it was redissolved in boiling water and allowed to separate again, the process being repeated several times in order to purify the salt. On heating, it decomposed between 240° and 250° without melting.

(b) *With ethyl iodide as ethylating agent.* Daphnandrine (1 g.) was dissolved in hot methanol (100 c.c.) and heated under reflux with methyl iodide (2 c.c.) for 6 hours. Removal of solvent under reduced pressure gave a yellowish resin which was dissolved in hot ethanol (250 c.c.). Ethyl iodide (3 c.c.) was added, followed by ethanolic sodium ethoxide (0·08 g. of sodium in 5 c.c. of ethanol). After the mixture had been boiled under reflux for 6 hours, a further similar amount of sodium ethoxide was added to it and the heating and addition repeated until in all 5 such additions had been made. The residue left after evaporation of the solution *in vacuo* was dissolved in hot water. The crude *N*-methyl-

O-ethyldaphnandrine dimethiodide which separated on cooling was purified as described above (0·8 g.). It could not be crystallised in either case (Found : C, 50·2; H, 5·6; N, 2·6; loss at 110°/0·1 mm., 5·9. $C_{41}H_{50}O_6N_2I_2,3H_2O$ requires C, 50·3; H, 5·8; N, 2·9; H_2O, 5·5%).

Degradation of N-*Methyl*-O-*ethyldaphnandrine Dimethiodide.*—The above dimethiodide (0·5 g.), prepared by either method (*a*) or (*b*), was converted into the dimethohydroxide and submitted to a Hofmann degradation as described for *ON*-dimethyldaphnandrine dimethiodide. The crude methine was ozonised in ice-cold aqueous acid solution in the usual way, and the resin which separated was extracted with ether. The extract, after washing with aqueous sodium carbonate and then with water, was dried (Na_2SO_4) and evaporated. The residue crystallised from light petroleum as colourless needles (0·07 g.), m. p. 78—79°, undepressed in admixture with an authentic specimen of 2-methoxy-5 : 4′-diformyldiphenyl ether prepared from *O*-methylrepandine dimethiodide (Bick and Todd, *loc. cit.*) (Found : C, 70·6; H, 4·9. Calc. for $C_{15}H_{12}O_4$: C, 70·3; H, 4·7%).

Daphnoline.—A sample of daphnoline, recrystallised from chloroform, had m. p. 194—196°. It gave a positive Millon reaction and, like daphnandrine, a reddish-brown nitroso-derivative. Unlike daphnandrine, however, it gave a green ferric chloride colour and dissolved readily in cold aqueous sodium hydroxide (5%), by which it could be quantitatively extracted from chloroform solution (Found : C, 61·6; H, 5·4; N, 3·7; Cl, 15·1; MeO, 9·2; MeN, 4·0. Calc. for $C_{35}H_{36}O_6N_2,CHCl_3$: C, 61·7; H, 5·3; N, 4·0; Cl, 15·2; 2MeO, 8·9; MeN, 4·1%).

OON-*Trimethyldaphnoline Dimethiodide.*—Daphnoline (0·5 g.) was dissolved in boiling absolute methanol (150 c.c.), and methyl iodide (1·5 c.c.) was added, followed by methanolic sodium methoxide (0·06 g. of sodium in 1·5 c.c. of methanol). The mixture was heated under reflux, and similar quantities of sodium methoxide were added at intervals of 6 hours until in all five such additions had been made. Solvent was then removed under reduced pressure, and the residue dissolved in hot water. On cooling, this solution deposited a white amorphous solid which was filtered off and redissolved in hot water, and the solution boiled with a little copper powder for 10 minutes and filtered. On cooling, the dimethiodide slowly crystallised as colourless needles (0·7 g.) which decomposed at 255—260° without melting. It was identical (Debye–Scherrer diagram) with *O*-methyloxyacanthine dimethiodide and with *ON*-dimethyldaphnandrine dimethiodide (Found : C, 50·7; H, 5·7; N, 3·0; loss at 110°/0·1 mm., 5·5. Calc. for $C_{40}H_{48}O_6N_2I_2,2\frac{1}{2}H_2O$: C, 50·5; H, 5·7; N, 2·9; H_2O, 4·7%).

O-*Methyldaphnoline.*—To a warm methanolic suspension of daphnoline (0·1 g. in 25 c.c.) an ethereal solution of diazomethane (from 0·5 g. nitrosomethylurethane) was added and the mixture set aside overnight. The solution, which had become clear, was evaporated and the residue dissolved in chloroform and extracted repeatedly until no trace of alkaloid remained in the alkaline extract. The chloroform solution was washed and dried (Na_2SO_4), then divided into two portions, and each evaporated to dryness. The residues, dissolved in hot hydrochloric acid solution and hydrobromic acid solution respectively, yielded on cooling *O*-methyldaphnoline hydrochloride and hydrobromide as colourless prisms, the first melting with decomposition at 275—276° and the second at 283—284°. The melting points were not depressed on mixing these substances with specimens of daphnandrine hydrochloride and hydrobromide respectively.

N-*Methyl*-OO-*diethyldaphnoline Dimethiodide.*—This substance was prepared from daphnoline by methods corresponding to those described above for the preparation of *N*-methyl-*O*-ethyldaphnandrine dimethiodide from daphnandrine. By method (*b*), daphnoline (0·5 g.) after *N*-methylation with methyl iodide was *O*-ethylated with ethyl iodide and ethanolic sodium ethoxide, to give the required N-*methyl*-OO-*diethyl-dimethiodide* (0·4 g.), m. p. 250—260° (decomp.) (Found : C, 50·7; H, 5·7; I, 25·4; loss at 110°/0·1 mm., 5·6. $C_{42}H_{52}O_6N_2I_2,3\frac{1}{2}H_2O$ requires C, 50·6; H, 6·0; I, 25·4; H_2O, 5·6%). The degradation of this substance was carried out by the methods already described for the corresponding daphnandrine compound. *N*-Methyl-*OO*-diethyldaphnoline dimethiodide (0·3 g.) on Hofmann degradation gave a methine base, a portion of which was converted into its crystalline *dimethiodide*, m. p. 230—240° (decomp.), for identification purposes (Found : C, 52·5; H, 6·2; loss at 110°/0·1 mm., 6·4. $C_{45}H_{56}O_6N_2I_2,3\frac{1}{2}H_2O$ requires C, 52·1; H, 6·1; H_2O, 6·1%). On ozonolysis this methine gave 2-ethoxy-5 : 4′-diformyldiphenyl ether, m. p. 60°, identical (mixed m. p.) with that obtained from *O*-ethylrepandine (Bick and Todd, *loc. cit.*) (Found : C, 70·8; H, 5·3. Calc. for $C_{16}H_{14}O_4$: C, 71·1; H, 5·2%). On chromic acid oxidation of the crude product of ozonolysis, 5 : 4′-dicarboxy-2-ethoxydiphenyl ether, m. p. 285—287°, depressed on admixture with the corresponding methoxy-compound, was obtained after recrystallisation from glacial acetic acid (Found : C, 63·2; H, 4·8. Calc. for $C_{16}H_{14}O_6$: C, 63·6; H, 4·7%).

Aromoline.—Recrystallised from chloroform, aromoline formed small plates, m. p. 174—175°, containing chloroform of crystallisation. It gave a positive Millon reaction and a green ferric chloride colour. It was readily soluble in aqueous sodium hydroxide (5%) and could be quantitatively extracted from solution in chloroform by the same reagent (Found : C, 61·8; H, 5·4; N, 4·0; Cl, 15·4. Calc. for $C_{36}H_{38}O_6N_2,CHCl_3$: C, 62·2; H, 5·4; N, 3·9; Cl, 14·9%). Aromoline had $[\alpha]_D^{17}$ 327° (*c*, 0·5 in chloroform, calculated for solvent-free base); the hydrochloride had $[\alpha]_D^{22}$ 295° (*c*, 0·3 in water).

OO-*Dimethylaromoline Dimethiodide.*—The method used to form *OON*-trimethyldaphnoline dimethiodide was employed to convert aromoline (0·7 g.) into its crystalline *OO*-dimethyl dimethiodide (0·75 g.), identical (Debye–Scherrer diagram) with *O*-methyloxyacanthine dimethiodide and with *OON*-trimethyldaphnoline dimethiodide (Found : C, 49·0; H, 5·7; N, 2·8; H_2O, 8·2. Calc. for $C_{40}H_{48}O_6N_2I_2,4\frac{1}{2}H_2O$: C, 48·7; H, 5·8; N, 2·8; H_2O, 8·8%).

OO-*Diethylaromoline Dimethiodide.*—This substance was prepared from aromoline (0·5 g.) by method (*b*) described for *N*-methyl-*O*-ethyldaphnandrine dimethiodide. The product (0·4 g.), m. p. 250—260°, could not be obtained crystalline (Found : C, 49·1; H, 6·6; N, 3·1; loss at 110°/0·1 mm., 8·8. $C_{42}H_{52}O_6N_2I_2,5H_2O$ requires C, 49·2; H, 6·1; N, 2·7; H_2O, 8·4%).

OO-*Dimethylaromolinemethine Dimethiodide.*—This substance was prepared by Hofmann degradation of *OO*-diethylaromoline dimethiodide (0·3 g.) to give the methine, which was converted into its crystalline dimethiodide (0·1 g.), m. p. 230—240° (decomp.), identical (Debye–Scherrer diagram) with *N*-methyl-*OO*-diethyldaphnolinemethine dimethiodide.

Paper Chromatography of Trilobamine and Related Alkaloids.—The resinified sample (40 mg.) containing trilobamine was exhaustively extracted with boiling chloroform, and the solution evaporated to dryness *in vacuo*. The residue (<1 mg.) was dissolved in chloroform (5 drops), and a portion of the solution (1 drop) chromatographed on Whatman No. 1 paper in butanol–acetic acid–water. The paper was dried and sprayed with an ethanolic solution of the potassium salt of tetrabromophenolphthalein ethyl ester (0·1%) (Feigl, "Spot Tests," 1939, 2nd Edn., p. 345) and dried again. After a spraying with aqueous oxalic acid (0·05%) the position of the trilobamine was shown by a deep-blue spot on a yellow background. The R_f values in three different concentrations of butanol–acetic acid–water for trilobamine and other structurally related alkaloids (*ca.* 20 μg. used in each case) determined under the same conditions are given below:

	R_f in BuOH–HOAc–H_2O.				R_f in BuOH–HOAc–H_2O.		
	25–0·1–25.	25–0·5–25.	20–5–25.		25–0·1–25.	25–0·5–25.	20–5–25.
Repandine	0·39	0·45	0·81	Aromoline.....	0·23	0·25	0·67
Oxyacanthine ...	0·33	0·37	0·77	Daphnoline	0·27	0·31	0·71
Daphnandrine ...	0·31	0·36	0·79	Trilobamine	0·27	0·31	0·71

Absorption Spectra of Daphnandrine, Daphnoline, Aromoline, Repandine, and Oxyacanthine.—The absorption spectra of the above alkaloids were determined in methanol solution (*ca.* M/10,000) using a Beckman quartz spectrophotometer. The curves are shown in Figs. 1 and 2. The values obtained for wave-length and, in parentheses, extinction coefficient at the minima and maxima, respectively, were: repandine 2570 (1050), 2840 (6760); oxyacanthine 2600 (2040), 2850 (7090); daphnandrine 2590 (2160), 2850 (8130); aromoline 2620 (4370), 2850 (7580); and daphnoline 2610 (4790), 2850 A. (8420).

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