956. *Alkaloids of* Daphnandra *Species. Part VI.*[1] *The Structures of Daphnandrine, Daphnoline, and Aromoline.*

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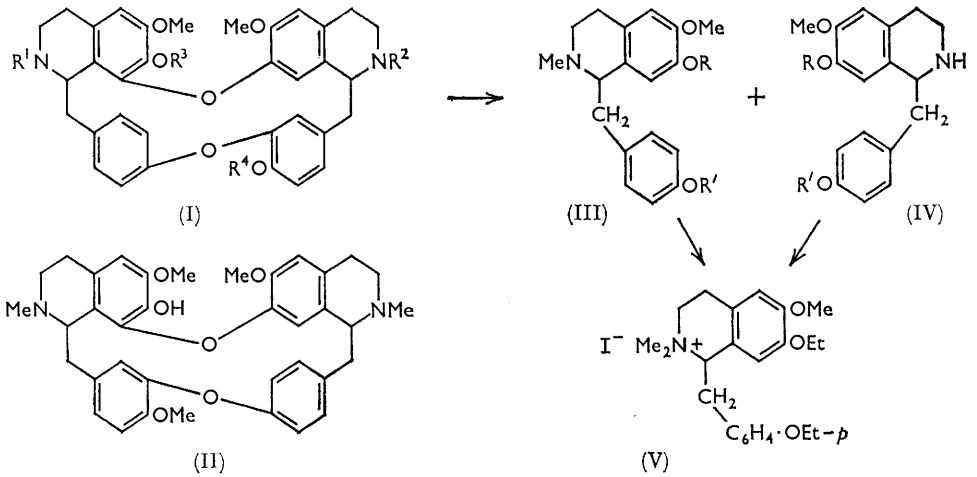
Daphnandrine, daphnoline, and aromoline are shown to have structures (I; $R^1 = R^4 = Me$, $R^2 = R^3 = H$), (I; $R^1 = Me$, $R^2 = R^3 = R^4 = H$), and (I; $R^1 = R^2 = Me$, $R^3 = R^4 = H$), respectively. Observations are made concerning the variability of alkaloid content of *Daphnandra micrantha* and *D. aromatica*.

In a previous paper,[2] daphnandrine, daphnoline, and aromoline were shown to have the same skeleton as oxyacanthine[3] (I; $R^1 = R^2 = R^3 = Me$, $R^4 = H$) and to differ from it only in the number and relative positions of the methoxy-, hydroxy-, and methylimino-groups. Aromoline proved to be a de-*O*-methyloxyacanthine, and structure (I; $R^1 = R^2 = Me$, $R^3 = R^4 = H$) was suggested for it although the position of the extra hydroxy-group remained uncertain.

Daphnoline (I; $R^1 = H$, $R^2 = Me$ or *vice versa*, $R^3 = R^4 = H$) was shown to be a de-*N*-methylaromoline, but it was not possible to determine which methylimino-group it lacked. Daphnandrine (I; $R^1 = H$, $R^2 = Me$ or *vice versa*, $R^3 = H$, $R^4 = Me$) is an *O*-methyldaphnoline and has a methoxy-group in the benzyl portion of the molecule.



However, the position of the hydroxyl in the isoquinoline residue, as with aromoline and daphnoline, was uncertain, and its tentative location at position 7 depended on the fact that daphnandrine gave a positive reaction with Millon's reagent. It had been found by King[4] in the analogous series of bisbenzylisoquinoline alkaloids derived from curare that a positive test is given by a hydroxy-group in position 4 of a benzyl group or in the position 7 of an isoquinoline, but not in position 6 of the latter. Although no exception to these findings is known in the curare series, their extension to the oxyacanthine–berbamine series is of doubtful validity since fangchinoline (II),[5] a Chinese alkaloid structurally similar to berbamine, has recently been shown to have a hydroxy-group at position 7 of an isoquinoline residue although it does not give Millon's test.

[1] Part V, Bick, Taylor, and Todd, *J.*, 1953, 695.
[2] Bick, Ewen, and Todd, *J.*, 1949, 2767.
[3] Reviewed by Kulka, "The Alkaloids," ed. Manske and Holmes, Academic Press Inc., New York, 1954, Vol. IV, p. 213.
[4] King, *J.*, 1937, 1472; 1940, 737.
[5] Chuang Chang-Kong, Hsing Chi-Yi, Kao Yee-Sheng, and Chang Juo-Jen, *Ber.*, 1939, **72**, 519; Hsing Chi-Yi and Chang Ching-Hsiang, *Acta Chim. Sinica*, 1957, **23**, 405.

To clarify the structures of these three *Daphnandra* bases we decided to apply the method developed by Tomita and his co-workers [6] for degrading bisbenzylisoquinoline bases, by which they are split with sodium in liquid ammonia into two simple benzylisoquinolines of the armepavine (III; R = Me, R′ = H) type.

Daphnoline was therefore methylated with diazomethane, then cleaved to produce a mixture of phenolic bases. One of these, separated as a crystalline oxalate, proved identical with (+)-armepavine (III; R = Me, R′ = H). The other, after methylation with diazomethane gave (−)-*OO*-dimethylcoclaurine (IV; R = R′ = Me). From these reactions, *OO*-dimethyldaphnoline must have structure (I; $R^1 = R^3 = R^4 = Me$, $R^2 = H$), and the position of the methylimino-group in daphnoline is fixed.

Daphnoline was next ethylated with diazoethane, and the product was treated with sodium in liquid ammonia. The mixture of phenols thus obtained could not be conveniently separated, so it was first ethylated with diazoethane, then the secondary base was separated from the tertiary one by chromatography on alumina. Unfortunately, neither the resulting bases nor their methiodides could be obtained crystalline so that a full comparison could not be made; however, while the bases themselves had significantly different R_F values when chromatographed on paper, the values for their methiodides were identical. This result is in accord with the structure (I; $R^1 = Me$, $R^2 = R^3 = R^4 = H$) for daphnoline, since the methiodides can be identical, or enantiomorphic, only if *OO*-diethyldaphnoline has structure (I; $R^1 = Me$, $R^2 = H$, $R^3 = R^4 = Et$), which on cleavage with sodium in liquid ammonia would give *O*-ethylcoclaurine (IV; R = H, R′ = Et) and *O*-ethyl-*N*-methylcoclaurine (III; R = Et, R′ = H); each of these compounds on ethylation of the hydroxy-groups and quaternisation would yield structure (V).

In order to provide more rigid evidence for the above structure for daphnoline and the corresponding structure (I; $R^1 = R^4 = Me$, $R^2 = R^3 = H$) for daphnandrine, the latter was ethylated with diazoethane and cleaved with potassium in liquid ammonia.[7] The phenolic tertiary base was separated from the product as a crystalline oxalate, which by mixed melting point determination and a comparison of the infrared spectra proved identical with an authentic specimen of the oxalate of (+)-*O*-ethyl-*N*-methylcoclaurine (III; R = Et, R′ = H). Thus the structures of daphnandrine, daphnoline, and aromoline are respectively (I; $R^1 = R^4 = Me$, $R^2 = R^3 = H$), (I; $R^1 = Me$, $R^2 = R^3 = R^4 = H$), and (I; $R^1 = R^2 = Me$, $R^3 = R^4 = H$), and the position previously assigned to the hydroxy-groups in the isoquinoline rings is confirmed.

In seeking additional supplies of daphnandrine for this work, a quantity of *D. micrantha* bark, collected in the East Dorrigo district of New South Wales, was extracted by methods similar to those previously described.[8,9] However, the only base found, and that in substantial amount, was micranthine, an alkaloid which had been recorded by Pyman [8] as a minor constituent of this plant. Variations in alkaloid content have already been noted [1,2,9] for samples of *D. micrantha* at different stages of growth and from different localities. The daphnoline used in this work was obtained from the bark of *D. aromatica* (collected in the Atherton district of North Queensland), a species from which daphnoline and aromoline had previously been isolated.[10] The alkaloid content is evidently variable in this plant also, for daphnoline alone was isolated from it, and aromoline could not be detected.

Experimental

Extraction of D. aromatica *Bark.*—Sun-dried bark (6·5 kg.) was extracted with methanol (Soxhlet apparatus) during 5 days. After acidification with aqueous tartaric acid the extract

[6] Reviewed by Tomita, *Fortschr. Chem. org. Naturstoffe*, 1952, **9**, 184.
[7] Djerassi, Figdor, Bobbitt, and Markley, *J. Amer. Chem. Soc.*, 1957, **79**, 2203.
[8] Pyman, *J.*, 1914, **105**, 1679.
[9] Bick and Todd, *J.*, 1950, 1606.
[10] Bick and Whalley, *Univ. of Queensland Papers, Dept. of Chem.*, 1948, **1**, no. 32.

was evaporated under reduced pressure until free from methanol, and then the syrup was diluted with water (to 10 l.). A quantity of non-basic precipitate was filtered off, washed thoroughly with dilute aqueous tartaric acid, and discarded. The acid extracts were combined and basified (to pH 11) with aqueous ammonia. After being filtered off and dried (vacuum oven), the precipitate (220 g.) was extracted exhaustively with chloroform (Soxhlet apparatus). The chloroform solution was extracted with aqueous sodium hydroxide (5%) until all the phenolic bases had been removed, then washed thoroughly with water and dried (Na_2SO_4). Removal of the solvent *in vacuo* left a residue (50 g.) which gave a very strong test with Mayer's reagent. Attempts to purify this material by chromatography on alumina and by partition between chloroform and aqueous acid failed to yield any pure crystalline substance.

The aqueous alkali extract from above was acidified with hydrochloric acid, and the non-basic precipitate was removed, washed thoroughly with dilute acid, and rejected. The bases were reprecipitated from the combined acid solutions with aqueous ammonia, and extracted with chloroform. After being dried (Na_2SO_4) and concentrated somewhat, the extract slowly deposited crystalline daphnoline (6·5 g.). The mother-liquor gave a strong test with Mayer's reagent but attempts to detect and separate aromoline by methods similar to those described above were not successful.

OO-*Dimethyldaphnoline.*—Daphnoline (0·8 g.) was dissolved in methanol (150 c.c.) and methylated with diazomethane in ether (from 3 g. of methylnitrosourea in all) in four additions during a fortnight. After removal of the solvents *in vacuo* the oil which remained was dissolved in benzene and purified by chromatography on alumina. Elution with benzene–chloroform (1 : 1) yielded *OO*-dimethyldaphnoline (0·4 g.) which was insoluble in Claisen's reagent, but could not be obtained crystalline.

Fission of OO-*Dimethyldaphnoline.*—*OO*-Dimethyldaphnoline (0·3 g.) was dissolved in toluene (30 c.c.) and cleaved with sodium (1 g. in all) in liquid ammonia (400 c.c.). After evaporation of the ammonia the phenolic products (0·25 g.) were separated from the non-phenolic material (0·03 g.) by extraction with alkali and neutralisation of the solution; they were dissolved in ethanol (2 c.c.), a saturated solution of oxalic acid in ethanol (2 c.c.) was added, and the mixture was kept overnight in a refrigerator. A crystalline oxalate (0·1 g.) separated; after repeated recrystallisation from ethanol it had m. p. 209°, with or without admixture of an authentic sample of (+)-armepavine oxalate (Found: C, 62·2; H, 6·8; O, 27·4; MeN, 5·9. Calc. for $C_{19}H_{23}NO_3,\frac{1}{2}C_2H_2O_4,1\frac{1}{2}H_2O$: C, 62·3; H, 7·1; O, 27·0; MeN, 7·5%). Dissolved in warm water, the oxalate on addition of ammonia yielded the free base, which was extracted with ether, then crystallised and recrystallised from ether–acetone, forming needles, m. p. 143°. Tomita, Fujita, and Saijoh [11] give m. p. 145° for armepavine.

The mother-liquors after removal of the armepavine oxalate were evaporated to dryness *in vacuo*, and the residue was dissolved in warm water. The base, precipitated from this solution with ammonia, was removed by ether extraction, then the extract was dried (Na_2SO_4) and evaporated. A solution of the residue (0·11 g.) in methanol was methylated with ethereal diazomethane (from 1 g. of methylnitrosourea). Two further such additions of diazomethane were made at 3-day intervals, then the solvents and excess of diazomethane were removed *in vacuo*. The residue was dissolved in benzene and chromatographed on alumina; elution with benzene–chloroform (1 : 1) and evaporation of the eluate yielded a residue (50 mg.) which crystallised when moistened with ethanol; recrystallisation gave needles, m. p. 200°. Tomita, Fujita, and Murai [12] report m. p. 202—203° for *OO*-dimethylcoclaurine.

Formation and Fission of OO-*Diethyldaphnoline.*—Daphnoline (1 g.), in methanol (200 c.c.), was ethylated with ethereal diazoethane by a method similar to that described above for the dimethyl compound. The oil (0·7 g.) was chromatographed as before, but it could not be obtained crystalline. It was insoluble in Claisen's reagent. *OO*-Diethyldaphnoline (0·6 g.) was cleaved with sodium (1 g.) in liquid ammonia (400 c.c.) to yield a mixture of phenols (0·58 g.), which was dissolved in methanol (30 c.c.) and ethylated with ethereal diazoethane during 10 days. Evaporation of the solvents left a residue which was dissolved in benzene and chromatographed on alumina. Elution with benzene afforded a yellow oil (0·24 g., Fraction A), and with chloroform–benzene (1 : 1) another oil (0·22 g., Fraction B). Neither could be obtained crystalline. They were converted into methiodides which also failed to crystallise.

[11] Tomita, Fujita, and Saijoh, *J. Pharm. Soc. Japan*, 1952, **72**, 1232.
[12] Tomita, Fujita, and Murai, *J. Pharm. Soc. Japan*, 1951, **71**, 1035.

The bases and their methiodides were chromatographed on Whatman No. 1 paper, butanol–acetic acid–water (4 : 1 : 5) being used as solvent, with the following results: R_F of Fraction A base, 0·79; methiodide, 0·84. R_F of Fraction B base, 0·88; methiodide, 0·84.

O-*Ethyldaphnandrine*.—Daphnandrine (1·68 g.) was dissolved in methanol (100 c.c.) and ethylated with ethereal diazoethane as for daphnoline. *O*-Ethyldaphnandrine (0·82 g.) was obtained as an amorphous powder which although purified chromatographically would not crystallise. It gave a single spot when chromatographed on Whatman No. 1 paper [R_F 0·56 in butanol–acetic acid–water (63 : 10 : 27)] different from that for daphnandrine (R_F 0·48, same solvent), and it formed an amorphous *picrate* which decomposed without melting (Found, in material dried at 100°/0·1 mm. for 4 hr.: C, 54·25; H, 4·8; N, 10·5. $C_{38}H_{42}N_2O_6,2C_6H_3N_3O_7,H_2O$ requires C, 54·6; H, 4·6; N, 10·2%).

Fission of O-*Ethyldaphnandrine*.—*O*-Ethyldaphnandrine (0·75 g.) was dissolved in toluene–benzene (3 : 2; 50 c.c.) and cleaved with potassium (2·1 g.) in liquid ammonia (200 c.c.). The non-phenolic (0·19 g.) and phenolic (0·49 g.) products were separated as before and the latter, dissolved in ethanol (5 c.c.), treated with a solution of oxalic acid (0·43 g.) in ethanol (2 c.c.). After being warmed on the water-bath, then set aside in a refrigerator, the mixture deposited a crystalline oxalate (0·19 g.) which was repeatedly recrystallised from ethanol, forming needles, m. p. 214—218° (decomp.), $[\alpha]_D^{20}$ +95·7° (*c* 0·397, in water), which were identical (mixed m. p. and infrared spectrum of Nujol mull) with an authentic specimen of (+)-7-ethoxy-1,2,3,4-tetrahydro-1-4′-hydroxybenzyl-6-methoxy-2-methylisoquinoline oxalate. The free base (82 mg.) was recovered from the oxalate as above and recrystallised from petroleum (b. p. 80—100°), giving needles, m. p. 130—132°, $[\alpha]_D^{20}$ +87·1° (*c* 0·426, in chloroform). Its infrared spectrum was identical with that of an authentic sample of (+)-*O*-ethyl-*N*-methylcoclaurine (III; R = Et, R′ = H).

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