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SHORT COMMUNICATION

ALKALOIDS OF *DAPHNIPHYLLUM CALYCINUM* AND *D. GLAUCESCENS* OF HONG KONG

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Abstract—From the bark or leaves of the Hong Kong species of *Daphniphyllum calycinum* or *D. glaucescens* the new alkaloids calycine, $C_{23}H_{31}O_3N$, glaucescine, $C_{19}H_{27}O_3N$, and glaucescinine, $C_{19}H_{27}O_3N$, have been obtained. These alkaloids are different from those recently obtained from the seeds of the Mainland Chinese species of *D. calycinum*. The Hong Kong *D. calycinum* leaves also yield β-sitosterol.

IN VIEW of the recent publication[1] concerning the isolation of alkaloids from *Daphniphyllum calycinum* in Mainland China, the results of our current Hong Kong investigation of *D. calycinum* and *D. glaucescens* are presented for comparison.

Fang *et al.*[1] isolated three new alkaloids from the seeds of *D. calycinum*; daphnicaline, $C_{21}H_{29}O_2N$, (as a syrup), daphnicamine, $C_{21}H_{31}O_2N \cdot 2H_2O$, m.p. 221–222°, and daphnicadine, $C_{22}H_{37}O_2N$, m.p. 285–287°. From the leaves and bark of *D. calycinum* we have isolated the alkaloid, calycine, $C_{23}H_{31}O_3N$, m.p. 205° and from the bark of *D. glaucescens* calycine, together with glaucescine, $C_{19}H_{27}O_3N$, m.p. 234–235°, and glaucescinine, $C_{19}H_{27}O_3N$, m.p. 248–250°. The leaves of the latter plant yielded calycine alone. From the leaves of *D. calycinum* we have also isolated β-sitosterol in 0·003 per cent yield.

It seems very likely that all six new alkaloids are related. Although we have not yet been able to obtain samples from China the possibility that any of the alkaloids isolated from the seeds of *D. calycinum* are identical with those described here is remote.

We wish to report also that a large scale extraction of the leaves of *D. glaucescens*, which we calculated should have given approximately 20·0 g of bases for degradative work, yielded but a few crystals. Apparently seasonal or some other factors influenced the concentration of alkaloids in the leaves and this is under investigation.

Alkaloids have been reported previously from *Daphniphyllum bancanum*[2] and *D. macropodum*.[3,4] Asperuloside[5] and rutin[6] were also found in the latter species. The seed oils of *D. humila*[7] and *D. glaucescens*[8] have also been studied.

EXPERIMENTAL

Microanalyses were by the Microanalytical Laboratories of the Universities of Melbourne and Singapore. Specific rotations were measured in chloroform; i.r. spectra on a P-E model

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137 Infracord spectrophotometer; NMR spectra on an HR60 spectrometer system. Melting points of the alkaloids were taken in sealed tubes on a copper block; those of steroids were taken on a Kofler block. Alumina used was B.D.H. preparative grade.

Extraction of D. glaucescens

(*a*) *Bark*. Dried bark (8 kg) was extracted with cold ethanol. The extract was evaporated to dryness under reduced pressure; the residue was treated with 5% ammonia solution then extracted with chloroform. Combined chloroform extracts were washed with water, then distilled. The black residue was repeatedly triturated with warm 2N hydrochloric acid and the combined acid extracts after neutralizing with ammonia were extracted with chloroform. Evaporation of the chloroform gave a syrup (18 g).

Separation of Alkaloids

The syrup (18 g) was dissolved in benzene (200 ml) and applied to a column of alumina (200 g). Elution with benzene first gave a yellowish oil, followed by crystals (1·4 g) which on recrystallization from acetone yielded *calycine*, m.p. 205° (vac.), $[\alpha]_D^{20}+58{\cdot}7°$ (Found: C, 74·0; H, 8·5; N, 4·0; OMe, 8·2; CMe, 4·0. Calc. for $C_{23}H_{31}O_3N$: C, 74·8; H, 8·5; N, 3·8; OMe, 8·4; CMe, 4·1%). ν_{max} 1690 cm^{-1} (> CO); M (by mass spectrum) was 369; NMR spectrum confirms 31 protons; 1 × OMe ($\tau = 6{\cdot}24$); 1 × quaternary C Me ($\tau = 9{\cdot}08$) and 1 × C–Me (doublet, $\tau = 8{\cdot}56, 8{\cdot}97$). Calycine was converted into *calycine picrate*, which after recrystallization from methanol had m.p. 215–218° (*vac. dec.*) (Found: C, 58·3; H, 6·0; N, 9·2. Calc. for $C_{23}H_{31}O_3N \cdot C_6H_2O_7N_3$: C, 58·2; H, 5·7; N, 9·4%); into *calycine 3,5-dinitrobenzoate* which after recrystallization from ethanol or acetone had m.p. 218–221° (*vac. dec.*) (Found: C, 62·0; H, 6·0; N, 7·5. Calc. for $C_{23}H_{31}O_3N \cdot C_7H_4O_4N_2$: C, 62·0; H, 6·1; N, 7·2%); into *calycine methiodide* which on recrystallization from ethanolic acetone had m.p. 252–254° (*vac. dec.*) (Found: C, 57·0; H, 7·0; N, 2·8, I, 23·8. Calc. for $C_{23}H_{31}O_3N \cdot CH_3I$: C, 56·4; H, 6·7; N, 2·7; I, 24·8%); and into *calycine ethiodide* which on recrystallization from ethanolic acetone had m.p. 245–246° (Found: C, 57·5; H, 7·00; N, 2·8, I, 23·6. Calc. for $C_{23}H_{31}O_3N \cdot C_2H_5I$: C, 57·2; H, 6·9; N, 2·8; I, 24·2%).

Further elution of the alumina column with benzene yielded crystals (0·030 g) which on recrystallization from acetone yielded *glaucescine*, m.p. 234–235° (*vac. dec.*) $[\alpha]_D^{20}+68{\cdot}1$ (Found; C, 71·9; H, 8·1; N, 4·2; OMe, 9·3. Calc. for $C_{19}H_{29}O_3N$: C, 71·9; H, 8·5; N, 4·4; OMe, 9·7%); ν_{max} 1690 cm^{-1} (> C=O).

Further elution with 10% ether in benzene yielded a yellowish glassy substance; and finally elution with 50% ether in benzene gave crystals (0·050 g) which on recrystallization from acetone yielded *glaucescenine*, m.p. 248–250° (*vac. dec.*) (Found: C, 71·3; H, 7·9; N, 4·1; OMe, 9·1. Calc. for $C_{19}H_{29}O_3N$: C, 71·9; H, 8·5; N, 4·4; OMe, 9·7%); ν_{max} 1660, 1680 cm^{-1} (> C=O).

(*b*) *Leaves*. Dried leaves (4 kg) were extracted and worked up as above. Calycine (0·7 g) was obtained.

Extraction of D. calycinum

(*a*) *Bark*. Dried bark (9 kg) was extracted as stated for *D. glaucescens*. The syrupy alkaloid mixture (24 g) yielded calycine (2·0 g).

(*b*) *Leaves*. (i) Dried leaves (4 kg) were extracted as stated for *D. glaucescens*. Calycine (0·6 g) was obtained. (ii) Dried leaves were extracted with ethanol. The extract was concentrated under reduced pressure; the black residue obtained was then partitioned between

chloroform and 5% aqueous ammonia solution. The combined chloroform extracts were concentrated and the black-green oil was taken up in benzene and chromatographed on a column of alumina.

Elution with benzene gave first a waxy substance followed by β-sitosterol in 0·003% yield. The substance had m.p. 138–139°, $[\alpha]_D$ −34·0° (Found: C, 83·3, H, 12·4. Calc. for $C_{29}H_{50}O$: C, 83·5, H, 12·1%) and formed an acetate, m.p. 132–133° (Found: C, 81·1, H 11·5. Calc. for $C_{31}H_{52}O_2$: C, 81·5; H, 11·5%) whose i.r. spectrum was identical with that of an authentic sample of β-sitosterol acetate.

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