Alkaloids of *Cyphomandra betacea* Sendt.

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A new base, solacaproine [*NN*-bis-(4-dimethylaminobutyl)hexanamide], has been isolated from the roots of *Cyphomandra betacea*; it is the *N*-hexanoyl derivative of solamine. Other bases identified include solamine (principal component), tropinone, and cuscohygrine. The presence of hyoscyamine (atropine), tropine, ψ-tropine, tigloidine (1α*H*,5α*H*-tropan-3β-yl 2-methylcrotonate), and a homologue of solacaproine is tentatively reported.

THE plant *Cyphomandra betacea* Sendt. is cultivated for its edible fruits [1] and has been used extensively in graft combinations concerned with the investigation of the biogenesis of tropane alkaloids in the Solanaceae, where it has been regarded as a plant free of tropane alkaloids. Schröter and Neumann [2] have however suggested the presence of both steroidal and tropane alkaloids in this species. We report here the results of a study on the alkaloid mixture obtained from *C. betacea* roots.

A root extract prepared from plant material raised in Nottingham was fractionated by column chromatography (see Experimental section). Solamine (4,4'-bisdimethylaminodibutylamine) (I; R = H) constituted the principal base of the extract, and was characterised as its picrate, aurichloride, and chloroplatinate and also by spectroscopy. This appears to be the first report of the occurrence of solamine in the free state in plant material; it occurs as the basic group of the tumour-inhibiting amides,[3] solapalmitine (I; R = CO·$[CH_2]_{14}$·Me) and solapalmitenine (I; R = CO·CH:CH·$[CH_2]_{12}$·Me). Its presence in the fresh root indicates that it is not formed as an artefact during the drying process.

$$Me_2N\cdot[CH_2]_4\cdot NR\cdot[CH_2]_4\cdot NMe_2$$
(I)

The major component of the chloroform eluate was a new amide named solacaproine, which was isolated as a gum (0·08% of the dry weight of plant material) and afforded a dipicrate. Mass spectroscopy gave molecular weight **313** and molecular formula $C_{18}H_{39}N_3O$ for the base, and the spectrum resembled those of solapalmitine and solapalmitenine.[3] Signals at *m/e* 58, 71, 84, and 100 accorded with a solamine moiety, supported by mass measurement at *m/e* 100 giving *m/e* 100·112505 (= $C_6H_{14}N$). Kupchan *et al.*[3] have discussed the nature of ions *m/e* 58, 84, and 100 but have not given a mechanistic description of *m/e* 71 (presumably $C_4H_9N^+$). Loss of CH_3 was indicated by *m/e* 298 (*M* − 15). Prominent ions also occurred at *m/e* 269 (*M* − 44), 255 (*M* − 58), 242 (*M* − 71), and 227 (*M* − 86). Corresponding ions are found in the mass spectra of solamine and solapalmitine where they were attributed [3] to elimination of a dimethylamino-group together with zero, one, two, or three carbon atoms, respectively. The peak at *M* − 86 seems anomalous since fragmentation of solamine leads to the ion *m/e* 84 and not 86. Evidence for an αβ-saturated bond in the acid portion was afforded by a peak at *m/e* 270 which arises from the fragment $(Me_2N\cdot[CH_2]_4)_2\cdot N\cdot CO\cdot CH_2\cdot CH_2\cdot$ as in solapalmitine; αβ-unsaturation, as in solapalmitenine, would have been manifest by a peak at *m/e* 268. Since accurate mass measurement of *m/e* 298 indicated the fragment $C_{17}H_{36}N_3O$, presumably $(Me_2N\cdot[CH_2]_4)_2\cdot N\cdot CO\cdot[CH_2]_4\cdot$, the structure of the new base is consistent with that of a saturated C_6 acyl

[1] E. P. Hume and H. F. Winters, *Econ. Bot.*, 1949, **3**, 140.
[2] H. B. Schröter and D. Neumann, *Mitt. Chem. Ges. D.D.R.*, 1964, **11**, 199; D. Neumann, Dissertation, Halle, 1964.
[3] S. M. Kupchan, Alan P. Davies, S. J. Barboutis, H. K. Schnoes, and A. L. Burlingame, *J. Org. Chem.*, 1969, **34**, 3888.

derivative of solamine. Hydrolysis of the base with 8N-hydrochloric acid yielded solamine and caproic acid (identified by g.l.c.). The amide prepared from solamine and n-hexanoyl chloride proved identical in all respects with the natural solacaproine.

A small signal was present on the mass spectrum of the natural solacaproine at *m/e* 327 ($M + CH_2$) and a small peak having an identical retention time with that of n-heptanoic acid was evident on the gas chromatogram of the caproic acid obtained by hydrolysis. These observations suggest the possible presence, in low proportion, of the heptanoyl derivative of solamine in the plant material. However, the base has yet to be isolated.

Tropinone (0·035%) and cuscohygrine (0·004%) were also obtained from the chloroform eluate together with a base giving a positive Vitali–Morin reaction and having an R_F value similar to that of hyoscyamine (atropine) (thin-layer and paper chromatography). Tested on isolated guinea-pig ileum, the base behaved similarly to atropine. Tropine and ψ-tropine were detected as components of the ammoniacal chloroform eluate, from which the solamine was obtained.

Cyphomandra betacea appears to be the first reported example of a plant which produces both atropine-like alkaloids and edible fruits. In the majority of the relevant Solanaceae investigated, the primary synthesis of tropane alkaloids occurs in the roots with subsequent translocation to, and accumulation in, the aerial parts. The apparent absence of tropane alkaloids from the leaves of *C. betacea* could be accounted for by metabolic participation of the bases, a hypothesis supported by the observation of Neumann and Tschoepe [4] that infiltration of labelled nicotine and atropine into isolated leaves and shoots of *C. betacea* resulted in degradation of the alkaloids.

The presence of amides in *C. betacea* (solacaproine), and in *Solanum tripartitum* (solapalmitine and solapalmitenine) [3] is in keeping with the close taxonomic relationship accorded these two genera. Solacaproine is currently under test for anti-tumour activity.

EXPERIMENTAL

Plant Material.—Plants of *Cyphomandra betacea* Sendt. were grown from cuttings in a temperate greenhouse in Nottingham; the roots of non-flowering mature plants were collected in June and dried at 50° in a forced draught.

Analytical Procedures.—Chromatographic systems employed were: (A): alumina plates, ether; (B): alumina plates, ether–ethanol (1 : 1) (both visualised with a saturated solution of iodine in carbon tetrachloride); (C): silica plates, chloroform–diethylamine (9 : 1) (visualised with iodoplatinate reagent; (D): Whatman No. 1 paper, light petroleum (b.p. 60—80°)–glacial acetic acid–pentanol–distilled water (1 : 3 : 3 : 3) (visualised with a modified Dragendorff's reagent). G.l.c. was performed with a Pye 104 series chromatogram with SE30 on solid support. N.m.r. spectra were determined in $CDCl_3$ on a Perkin-Elmer R10 spectrometer at 100 MHz with tetramethylsilane as internal standard. Mass spectra were determined by the P.C.M.U., Harwell, on an AE9 MS902 spectrometer. I.r. spectra were recorded on a Unicam SP 200 spectrometer.

Extraction and Separation of Alkaloids.—The powdered root (135 g), intimately mixed with calcium hydroxide (27 g) and moistened with water (65 ml), was set aside for 2 h. It was exhaustively extracted with ether (5 l); the extract remaining after removal of the solvent was dissolved in light petroleum (b.p. 40—60°) (5 ml) and transferred to kieselguhr (30 g) loaded with 0·5M-phosphate buffer solution (20 ml), pH 6·8. The column was successively eluted with light petroleum (b.p. 40—60°) (125 ml), ether (185 ml), chloroform (400 ml), and finally ammoniacal chloroform (300 ml).

Isolation of Solamine (*4,4'-Bisdimethylaminodibutylamine*). —The ammoniacal chloroform eluate of the column contained predominantly a base R_F 0·0 (A; deep brown after spraying), R_F 0·1 (C; deep violet), and R_F 0·2 (D; orange). Trace amounts of bases with R_F values the same as those of tropine and ψ-tropine were also present and were confirmed as such by fractionation (column chromatography, pH 7·0) and subsequent characterisation (systems A and B) of their tigloyl (2-methylcrotonoyl) esters.[5] The principal component (solamine) gave a *picrate*, shining plates, m.p. 113—114° (from aqueous alcohol) [Found: C, 39·1; H, 4·3; N, 18·5. $C_{12}H_{29}N_3 \cdot (C_6H_3N_3O_7)_3$ requires C, 39·1; H, 4·1; N, 18·3%], $\nu_{max.}$ (KBr disc) 3450 and 3500 cm^{-1} (NH); *aurichloride*, small prisms, m.p. 177—178° (from dilute hydrochloric acid) (Found: C, 12·3; H, 2·7; N, 3·3. $C_{12}H_{29}N_3 \cdot 3HAuCl_4$ requires C, 11·7; H, 2·6; N, 3·4%); *chloroplatinate*, stout prisms, m.p. 212—214° (from dilute hydrochloric acid) [Found: C, 17·0; H, 4·1; N, 4·65. $(C_{12}H_{29}N_3)_2 \cdot 3H_2PtCl_6$ requires C, 17·35; H, 3·9; N, 5·1%]; *hydrochloride*, small prisms, m.p. 262° (decomp.) (from ethanol–ether), *m/e* 215·2363 (M^+ for base. Calc. for $C_{12}H_{29}N_3$: *M*, 215·2363) with fragmentation pattern identical with that reported [3] for 4,4'-bisdimethylaminodibutylamine. The base, regenerated from the picrate, had $\nu_{max.}$ (film) 1715 and 3420 cm^{-1} (NH); τ ($CDCl_3$) 7·35 (4H, m, $CH_2 \cdot NH \cdot CH_2$), 7·75 (16H, sharp s, 2 × $Me_2N \cdot CH_2$), 7·95br (1H, m, NH), 8·5 (8H, m, 2 × $[CH_2]_2$).

Isolation of Tropinone, Cuscohygrine, and an Atropine-like Alkaloid.—The chloroform eluate of the original fractionation was collected in four fractions. Fraction 1 (0·01 g base) contained principally tropinone and a trace of base having an R_F value corresponding to that of atropine or hyoscyamine (systems B, C, and D). The neutralised fraction, treated with sodium picrate solution, gave tropinone picrate, m.p. and mixed m.p. 212° (decomp.) (Found: C, 45·3; H, 4·4; N, 15·0. Calc. for $C_8H_{13}NO \cdot C_6H_3N_3O_7$: C, 45·65; H, 4·3; N, 15·2%), i.r. identical with that of authentic picrate. The minor component was purified by repeated t.l.c. (system C); it gave a positive Vitali–Morin reaction and its atropine-like activity was confirmed by antagonism of acetylcholine with guinea-pig terminal ileum in Tyrode solution at 37°. Fraction 4 contained a mixture of bases (0·008 g) which was resolved by chromatography at pH 6·8 to give a small quantity of cuscohygrine, m.p. and mixed m.p. of dipicrate 204—206° (decomp.), i.r. spectrum identical with that of authentic cuscohygrine dipicrate.

Isolation of Solacaproine [NN-*Bis*-(*4-dimethylaminobutyl*)-

[4] D. Neumann and K. H. Tschoepe, *Flora* (*Jena*), 1966, **156**, 521.

[5] W. C. Evans and M. Wellendorf, *J. Chem. Soc.*, 1959, 1406.

hexanamide].—A new base present in fractions 2, 3, and 4 above formed *solacaproine dipicrate* with difficulty from the original neutralised aqueous solutions; recrystallisation was readily effected from ethanol, fine needles, m.p. 149—150° [Found: C, 46·55; H, 5·9; N, 16·7. $C_{18}H_{39}N_3O,(C_6H_3N_3O_7)_2$ requires C, 46·7; H, 5·8; N, 16·3%], $\nu_{max.}$ (film) (determined on base regenerated from picrate) 1645 cm^{-1} (amide CO), *m/e* 313·3093 (M^+ for base. Calc. for $C_{18}H_{39}N_3O$: *M*, 313·3093), 298, 270, 269, 255, 242, 229 (picric acid), 227, 100, 84, 71, 58 (100%), a signal at *m/e* 327 represented ($M + CH_2$).

Hydrolysis of Solacaproine.—The base (3·0 mg), recovered from the picrate, was heated in a sealed tube with 8*N*-hydrochloric acid (2 ml) at 110° for 48 h and the cooled hydrolysate shaken with ether (2 × 1 ml). The dried ($MgSO_4$) extract, treated with freshly prepared ethereal diazomethane, was chromatographed (g.l.c.) with the methyl 2-methylvalerate, 3-methylvalerate, 4-methylvalerate, caproate, 2-ethylbutyrate, 3,3-dimethylbutyrate, and n-heptanoate as references. The retention time (R_t) (3·6 min) of the natural derivative was identical with that of methyl caproate whereas the other esters had R_t 1·7—2·9 min. A second minor component of the methylated natural acids had R_t 7·1 min, identical with that of n-heptanoic acid.

The aqueous, acidic layer remaining from the hydrolysate was made basic with 5N-sodium hydroxide and shaken with chloroform (4 × 5 ml). T.l.c. of the extract (systems B and C) indicated a mixture of solamine and a small quantity of unchanged amide; purification by column chromatography at pH 6·8 gave solamine [picrate identical (m.p., mixed m.p., and i.r.) with authentic material].

Partial Synthesis of Solacaproine.—To an ether solution (0°) of solamine (20·0 mg) and triethylamine (94 mg) was added, with constant stirring over 0·5 h, an ethereal solution of n-hexanoyl chloride (12·5 mg). The mixture was stirred for 1 h at 25° and the resulting precipitate was removed by filtration; evaporation gave a product which possessed chromatographic properties (systems B, C, and D) identical with those of natural solacaproine. It afforded a picrate, fine needles from ethanol, m.p. 150° and mixed m.p. with the picrate of the natural product 149—150° (Found: 45·3; H, 5·5; N, 16·55%), i.r. and mass spectra identical with those of the natural derivative, M^+ for the base 313.

Tigloidine (1αH,5αH-*Tropan*-3β-*yl* 2-*Methylcrotonate*).—The light petroleum and ether eluates of the original chromatographic fractionation of the plant extract both contained a base having an R_F value (systems A, B, and D) the same as that for tigloidine. Insufficient material prevented complete identification.

Alkaloids of the Fresh Root.—An ethanolic extract (4 × 5 ml) of the fresh roots (1·0 g) showed the same alkaloid spectrum (systems B, C, and D) as that of the dried roots.

Alkaloids of the Aerial Parts.—An ether extract (1 l) of the dried, powdered aerial parts (10 g) contained a small proportion of solamine (systems B, C, and D) as the only detectable alkaloid.

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