The Alkaloids of the Genus *Datura*, Section Brugmansia. Part IV.[1] New Alkaloids of *D. sanguinea* R. and P.

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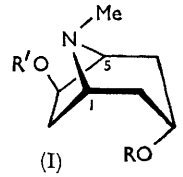
(−)-3α-Tigloyloxy-6β-acetoxytropane has been identified as a component of the alkaloidal mixture of *Datura sanguinea* leaves and young stems. Its structure was indicated by spectroscopy and by hydrolysis and confirmed by partial synthesis. 3α-Acetoxytropane, not previously recorded as a natural product, has also been isolated from the same plant material.

In Part III[1] the isolation of hyoscine, hyoscyamine, norhyoscine, and apohyoscine from the leaves of *Datura sanguinea* R. and P. was described and the occurrence of other bases noted. We record here the characterisation of two of these new bases.

The basic components of an ether extract prepared from the leaves and young shoots were progressively liberated from an aqueous solution of their sulphates in 29 fractions. Fractions 1—4 contained apohyoscine, hyoscine, and an unknown alkaloid which was isolated as a lævorotatory colourless gum by chromatography. The base afforded a crystalline lævorotatory hydrobromide $C_{15}H_{23}NO_4$,HBr and a picrate $C_{15}H_{23}NO_4$,$C_6H_3N_3O_7$. Its yield from the dried aerial parts, as determined by separate small-scale fractionations, was 0·05—0·1% for a number of samples of plant material and it comprised about 5—10% of the total alkaloids.

The ultraviolet spectrum of the hydrobromide, $\lambda_{max.}$ 217 mμ (ε 9700), suggested the new alkaloid to be a monotigloyl ester. (−)-3α,6β-Ditigloyloxytropane[2] (I; R = R′ = Me·CH:CMe·CO) and (−)-6β-tigloyloxytropan-3α-ol[3] (I; R = H, R′ = Me·CH:CMe·CO) exhibit the same maximum (ε 23,900 and 12,600, respectively).



(I)

(I) = (I; R = H–C(β)=C(α)–CO, Me, Me; R′ = Ac)

The n.m.r. spectrum of the base showed the features of a hydroxytropane nucleus[4] possessing a tigloyl moiety;[5] an acetoxy group was implicated by a singlet at τ 7·96 and a di-ester by a quartet at τ 4·5 due to the C-6 proton and a triplet at τ 4·9 due to the C-3 proton. Mass spectroscopy gave a molecular weight of 281 for the base with principal fragments consistent with a tropane nucleus.[4,6] Other fragmentations accorded with the loss of acetyl and tigloyl moieties.

On complete hydrolysis with aqueous-ethanolic barium hydroxide, the new base afforded (+)-tropane-3α,6β-diol (I; R = R′ = H) and tiglic acid; by gas chromatography, acetic acid was also identified as a component of the acid fraction. One component of the mixture which resulted from the partial hydrolysis of the base appeared to be (−)-3α-tigloyloxytropan-6β-ol (I; R = Me·CH:CMe·CO, R′ = H). This compound, prepared by partial hydrolysis of the ditigloyl ester, treated with acetic anhydride furnished (−)-3α-tigloyloxy-6β-acetoxytropane (Ia) which corresponded with the natural alkaloid. The (±)-alkaloid was similarly prepared from the (±)-ditigloyl ester. (±)-3α-Acetoxy-6β-tigloyloxytropane was prepared from the (±)-3α-acetyl mono ester and compared with the above compounds.

Fraction 28 was submitted to partition chromatography at pH 6·5 and, with chloroform as developing solvent, afforded a number of bases, one of which was characterised as 3α-acetoxytropane. The natural base was shown to be identical with 3α-acetoxytropane prepared by partial synthesis and previously described by Barger, Martin, and Mitchell.[7] It is present to the extent of about 0·02% in the dried plant material.

This appears to be the first report of the occurrence of acetic acid esters in the tropane group of alkaloids. It has yet to be ascertained whether in *Datura* they are confined to this single species. Also the biosynthetic

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[3] W. C. Evans and W. J. Griffin, *J. Chem. Soc.*, 1963, 4348.
[4] J. Parello, P. Longevialle, W. Vetter, and J. A. McCloskey, *Bull. Soc. chim. France*, 1963, 2787.
[5] H. Yamaguchi and K. Nishimoto, *Chem. and Pharm. Bull. (Japan)*, 1965, **13**, 217.
[6] E. C. Blossey, H. Budzikiewicz, M. Ohashi, G. Fodor, and C. Djerassi, *Tetrahedron*, 1964, **20**, 585.
[7] G. Barger, W. F. Martin, and W. Mitchell, *J. Chem. Soc.*, 1937, 1821.

origin of the acetic acid moiety is not without interest because all the other acids involved in the tropane ester alkaloids are plausibly derived from amino-acids.

EXPERIMENTAL

Plant Material.—Plants of *Datura sanguinea* R. and P., after wintering in a temperature greenhouse, were transferred to open land in May and collections of young leaves and stems made from August to October during the flowering period. Drying was carried out at 60° in a ventilated oven.

Extraction and Preliminary Fractionation of the Alkaloids.—The powdered plant material (4·75 kg.) was intimately mixed with calcium hydroxide (750 g.), moistened with water (2·75 l.) and set aside for 2 hr. It was exhaustively extracted with ether (about 56 l.) and the extract remaining after removal of the solvent was dissolved in ether (600 ml.) and transferred to a column of kieselguhr (600 g.) loaded with 2N-sulphuric acid (400 ml.). Pigments and other extraneous constituents were removed by elution with ether, the column was extruded and the solvent allowed to evaporate from it, and then a concentrated solution of ammonia (55 ml.) mixed with the solid. This alkaline mixture was repacked into a tube and exhaustively extracted with chloroform. Removal of the chloroform afforded a crude basic residue (82 g.).

The crude bases in ethanol (95 ml.) were neutralised with N-sulphuric acid (143 ml.), insoluble material was removed by filtration, and the alkaloids were fractionally liberated by the addition of 29 portions (5 ml. each) of N-sodium hydroxide. After each addition of alkali the bases liberated were collected in chloroform (100 ml.). Finally an excess of alkali was added and the solution again shaken with chloroform. The overall distribution of the principal alkaloids in these fractions was ascertained by thin-layer and paper chromatography.

Isolation of (−)-3α-Tigloyloxy-6β-acetoxytropane.—Fractions 1—4 each contained a similar mixture of alkaloids together with some extraneous material, and they were therefore resolved by analogous means. Thus fraction 1 (25 g. containing 7 g. alkaloids) in a mixture of ether and light petroleum (b. p. 40—60°) was transferred to kieselguhr (30 g.) supporting 0·5M-phosphate buffer (20 ml.; pH 6·0). Two bases were obtained by elution with light petroleum and a third (hyoscine [4·6 g.]) with ether. The bases obtained in light petroleum were incompletely separated and repeated chromatography on alumina (50 g.) with light petroleum–ether (55 : 45) was necessary to separate the alkaloids corresponding to the overlapping portions of the elution titration curve. Apohyoscine was the alkaloid first eluted with light petroleum.[1] The second alkaloid was obtained as a gum (1·2 g.) after collection in chloroform from the alkalinised titration liquors. It appeared to be an unknown alkaloid with an R_F value of 0·5 on alumina with ether as solvent; R_F values, under the same conditions, for other *Datura* alkaloids which might have been expected in this fraction are apohyoscine *ca.* 0·7, tigloidine 0·38, hyoscine 0·0, 3α,6β-ditigloyloxytropane 0·5, and 3α,6β-ditigloyloxytropan-7β-ol 0·35. The base failed to crystallise from light petroleum and gave a negative Vitali–Morin reaction. Dissolved in ethanol and treated with ethanolic hydrobromic acid and ether it afforded a *hydrobromide*, stout needles from ether–ethanol, m. p. 230—231° (Found: C, 49·2; H, 6·2. $C_{15}H_{23}NO_4$,HBr requires C, 49·7; H, 6·6%). The base in ethanol, neutralised with dilute sulphuric acid, gave on treatment with sodium picrate solution a *picrate*, bright yellow matted needles from aqueous ethanol, m. p. 184—185·5° (Found: C, 49·0; H, 5·1; N, 11·3. $C_{15}H_{23}NO_4$,$C_6H_3N_3O_7$ requires C, 49·4; H, 5·1; N, 11·0%).

The base (Ia), regenerated from the salt gave: $[\alpha]_D^{20}$ −11·5° (*c* 12·4 in ethanol, *l* 5 cm.); $\lambda_{max.}$ (in ethanol) 217 mμ (log ε 3·99); $\nu_{max.}$ (film) 1705, 1730 (C = O), 1650 cm.$^{-1}$ (C = C); n.m.r. in $CDCl_3$ determined on a Perkin-Elmer R10 spectrometer operating at 60 Mc./sec. with tetramethylsilane as internal reference showed signals at τ 3·1 (multiplet, olefinic proton), the multiplet consists of a 1,3,3,1-quartet, J = *ca.* 6 c./sec., due to coupling with β-methyl and each component showing further splitting due to coupling with α-methyl, τ 4·5 (1,1,1,1-quartet, sharply resolved with separations of 3·5 and 7·0 c./sec., C-6 proton), τ 4·9 (triplet with separations of about 6 c./sec. with each component broad, C-3 proton), τ 7·5 (singlet, *N*-methyl group), τ 7·96 (singlet, proton of acetoxy group), τ 8·15 (singlet, α-methyl protons), τ 8·18 (doublet, J = *ca.* 6 c./sec., β-methyl protons), τ 6·6—7·0 (multiplet, C-1 and C-5 protons), τ 7·1—8·5 (broad multiplet, C-2, 4, and 7 protons). Mass spectrum (determined on the hydrobromide) showed *m/e* (*I* per cent. in parentheses): 40(6), 41(3), 42(7) ($Me{\cdot}\overset{+}{N}{:}CH$), 43(12) ($Me{\cdot}CO^+$), 44(48), 79(3), 80(9), 81(7), 82(9), 83(7), 94(63) (*N*-methyl pyridinium ion), 95(57), 96(11), 110(6), 112(7), 113(5), 120(5), 121(16), 122(100), 123(10), 138(17), 181(13), 182(16) (loss of $Me{\cdot}CH{:}CMe{\cdot}COO$), 198(5) (loss of $Me{\cdot}CH{:}CMe{\cdot}CO$), 222(1) (loss of $Me{\cdot}COO$), 238(1) (loss of $Me{\cdot}CO$), 281(3) (M^+). It was not possible to obtain consistent mass spectra for this alkaloid; thus the relative abundance of certain ions showed considerable variation, and *m/e* 94 sometimes constituted the base peak.

Hydrolysis.—The alkaloid (0·03 g.) in ethanol (2·5 ml.) was heated in a sealed tube with a solution of barium hydroxide (0·3 g.) in water (5 ml.) at 100° for 5 hr. The alkaline solution was made acid with 10N-sulphuric acid and then shaken with ether (3 × 3 ml.). Evaporation of the ethereal solution dried over anhydrous sodium sulphate afforded tiglic acid (0·006 g.), m. p. 60—61°, undepressed on admixture with, and having the same i.r. spectrum as, authentic tiglic acid. In a subsequent reinvestigation of the hydrolysis products of the alkaloid, a similar ethereal extract was submitted to gas chromatography at 125° on Celite (60/80 mesh) loaded with phosphoric acid (2%) and silicone fluid (10%), column length 4 ft. and argon (25 ml./min.) as mobile phase. Fractions with retention times identical with those of tiglic acid (75 sec.) and acetic acid (165 sec.) were obtained. The relative proportions of the two acids in the alkaloid could not be assessed by this means because of the incomplete extraction of the acetic acid with ether. The aqueous acid solution remaining from the hydrolysis was neutralised by the addition of barium carbonate, centrifuged, and the supernatant liquid decanted. After the precipitate had been washed with water (2 × 4 ml.) and the washings added to the original solution, the water was removed. The residue, in water, treated with sodium picrate solution, afforded (+)-tropane-3α,6β-diol picrate (0·02 g.), m. p. 249—251°, undepressed on admixture with, and having the same i.r. spectrum as, the authentic material. The base recovered from the

picrate and treated with tigloyl chloride (0·013 g.) furnished (−)-3α,6β-ditigloyloxytropane (0·012 g.), picrate m. p. and mixed m. p. 151—152° and raised to 160—165° when admixed with the (+)-isomer [2] (Found: C, 52·6; H, 5·4. Calc. for $C_{18}H_{27}NO_4,C_6H_3N_3O_7$: C, 52·4; H, 5·5%). In one instance, when the hydrolysis of the alkaloid hydrobromide (0·1 g.) was discontinued before completion, a small quantity (*ca.* 0·005 g.) of base, picrate m. p. 148—153°, was obtained from the reaction mixture. The i.r. spectrum of this base was similar to that of (−)-3α-tigloyloxytropan-6β-ol; compared with the spectrum of the unhydrolysed alkaloid it exhibited only one absorption maximum (1705 cm.$^{-1}$) in the 1700—1750 cm.$^{-1}$ range and an additional absorption at 3360 cm.$^{-1}$ (hydroxyl).

Partial Synthesis of (−)- *and* (±)-3α-*Tigloyloxy*-6β-*acetoxytropane*.—(−)-3α-Tigloyloxytropan-6β-ol (0·027 g.), prepared as previously described,[3] was treated with acetic anhydride (0·012 g.) and heated under reflux at 70—80° for 2 hr. The product was mixed with dilute ammonia solution (10 ml.) and the liberated bases collected in chloroform (75 ml.). The residue remaining after the removal of the solvent was neutralised with 0·05N-sulphuric acid and treated with sodium picrate solution to give (−)-3α-*tigloyloxy*-6β-*acetoxytropane picrate* (0·030 g.), needles from ethanol, m. p. 182—186° after sintering at 170° (Found: C, 49·2; H, 4·7%). It had a similar i.r. adsorption spectrum to, and did not depress the melting point of, the picrate of the natural alkaloid.

(±)-3α-Tigloyloxytropan-6β-ol was prepared as above from (±)-tropane-3α,6β-diol and it afforded a *picrate*, needles from aqueous alcohol, m. p. 169—171° (Found: C, 48·7; H, 5·15. $C_{13}H_{21}NO_3,C_6H_3N_3O_7$ requires C, 48·7; H, 5·1%). The (±)-6β-acetoxy derivative, prepared in a similar manner to the corresponding (−)-isomer, furnished (±)-3α-*tigloyloxy*-6β-*acetoxytropane picrate*, needles from aqueous ethanol, m. p. 213° with preliminary softening at 198° (Found: C, 49·2; H, 4·8%). The i.r. spectrum of the picrate was similar to that of the (−)-picrate.

(±)-3α-*Acetoxy*-6β-*tigloyloxytropane*.—(±)-3α-Acetoxytropan-6β-ol was prepared [8] from (±)-3α,6β-diacetoxytropane; it gave a *picrate*, stout needles from aqueous ethanol, m. p. 182—184° (Found: C, 44·5; H, 4·45 $C_{10}H_{17}NO_3$, $C_6H_3N_3O_7$ requires C, 44·9; H, 4·7%). The base (0·043 g.) was treated with tigloyl chloride (0·025 g.) at 70—80° for 1·5 hr., dilute solution of ammonia (10 ml.) added, and the crude product collected in chloroform (60 ml.) and purified by chromatography on alumina using ether as solvent. The base, neutralised with dilute sulphuric acid, gave with sodium picrate solution, (±)-3α-*acetoxy*-6β-*tigloyloxytropane picrate*, tufts of needles from aqueous ethanol, m. p. 136—141° (Found: C, 49·6; H, 4·75%).

Isolation and Characterisation of 3α-*Acetoxytropane*.—Thin-layer chromatography (alumina; ether–ethanol 1 : 1) of a portion of fraction 28 (*loc. cit.*) indicated the presence of one principal base of R_F *ca.* 0·5. The entire fraction (0·94 g.) was transferred to kieselguhr (65 g.) loaded with 0·5M-phosphate buffer solution, pH 6·5 (46 ml.) and the chromatogram developed with ether and then chloroform. No basic material was eluted with ether but the titration curve of the chloroform eluate (2·1 l.) indicated the possible presence of four components. The base (0·2 g.) from the largest fraction was collected from the titration liquids in chloroform and after removal of the solvent and neutralisation afforded a *picrate*, needles from ethanol, m. p. 217° (Found: C, 46·7; H, 4·85. $C_{10}H_{17}NO_2,C_6H_3N_3O_7$ requires C, 46·6; H, 4·85%). The m. p. and i.r. spectrum of the picrate corresponded with those of the picrate of 3α-acetoxytropane prepared from tropine and acetyl chloride. The n.m.r. spectrum of the base (in $CDCl_3$) showed the following: τ 5·0 (1,2,1-triplet with separations of about 6 c./sec. and in which each component is broad, proton at C-3), τ 7·7 (singlet, *N*-methyl group), τ 7·95 (sharp singlet, proton of acetoxy group), τ 6·85 (broad singlet, protons at C-1 and C-5), τ 7·5—8·6 (broad multiplet, protons at C-2, 4, 6, and 7). Hydrolysis of the alkaloid (0·015 g.) was effected by the method indicated above and the resulting alkaline solution was shaken with chloroform (5 × 4 ml.). Removal of the chloroform gave a residue which possessed, by thin layer chromatography [(alumina; ether–ethanol (1 : 1)], an R_F value identical with that of tropine. The residue esterified with tigloyl chloride afforded 3α-tigloyloxytropane, picrate (0·012 g.) m. p. and mixed m. p. 178° (Found: C, 50·8; H, 5·4. Calc. for $C_{13}H_{21}NO_2,C_6H_3N_3O_7$: C, 50·4; H, 5·3%). The aqueous solution from the hydrolysis was evaporated to dryness, treated with orthophosphoric acid (*ca.* 0·5 ml.) and agitated with ether (0·5 ml.). The ether solution was submitted to gas chromatography as described above and a peak corresponding to that of acetic acid detected.

Quantitative Determination of New Alkaloids in Plant Material.—Samples of the aerial parts of races of *D. sanguinea* [1] were assayed by the method of Evans and Partridge [9] except that the carbon tetrachloride was replaced by light petroleum which was collected in aliquot parts (5 ml.). Each aliquot was titrated with 0·005N-sulphuric acid and the content of apohyoscine (obtained in the first fractions) and 3α-tigloyloxy-6β-acetoxytropane (eluted after apohyoscine) obtained. Hyoscine and norhyoscine were evaluated in the ether eluate and an approximate estimate of the 3α-acetoxytropane content was obtained from the complex titration curve of the chloroform eluate.

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