Phytochemistry. 1974, Vol. 13. pp. 2511 to 2513. Pergamon Press. Printed in England.

6β-[2-METHYLBUTANOYLOXY]TROPAN-3α-OL, A NEW ALKALOID FROM *DATURA CERATOCAULA* STRUCTURE AND BIOSYNTHESIS

PAMELA J. BERESFORD and JACK G. WOOLLEY

School of Pharmacy, City of Leicester Polytechnic, Leicester LE1 9BH

(*Received* 22 *February* 1974)

Key Word Index—*Datura ceratocaula*; Solanaceae; tropane alkaloids; 6β-[2-methylbutanoyloxy]tropan-3α-ol; L-isoleucine-[U-^{14}C] as precursor for 2-methylbutanoyl moiety.

Abstract—6β-[2-methylbutanoyloxy]tropan-3α-ol, a new alkaloid, has been isolated from the aerial parts of *Datura ceratocaula* (Solanaceae). Its chemotaxonomic significance within the genus is discussed. Biosynthetically, the 2-methylbutanoyl moiety is derived from L-isoleucine.

THE GENUS *Datura* is divided into four sections, (1) Stramonium, (2) Dutra, (3), Ceratocaulis and (4) Brugmansia. Sections (1) and (2) comprise the more frequently encountered annual herbaceous daturas which have erect flowers and either erect or nodding spiney fruit capsules respectively. The perennial tree-daturas of section (4), unlike the others, readily hybridise but will not self-pollinate and this has caused considerable confusion as to what constitutes a single species. Morphologically, they are distinguished in having long pendulous flowers and large spineless fruits. *Datura ceratocaula*, the only member of section (3), has erect white flowers suffused with lavender and smooth fruits similar to, but smaller than, those of section (4). Because of these similarities and the fact that *D. ceratocaula* will not readily cross with daturas of sections (1) and (2) it has been suggested that it forms a link between section Brugmansia, and sections Stramonium and Dutra.[1–3] Geographically, too, its distribution throughout Mexico and Central America, connects through a narrow isthmus the probably more ancient South American Brugmansias with the annual daturas of North America, Europe and Asia. *Datura ceratocaula*, first described by Ortega in 1798, is an aquatic, hollow-stemmed, prostrate, creeping plant long known by the Mexicans as the narcotic "Torna-loca" (maddening plant). It has received little chemical attention, paper chromatography suggesting the presence of hyoscine, hyoscyamine, cuscohygrine and four unidentified bases.[4]

Analysis of the aerial parts of mature *D. ceratocaula* plants by partition column chromatography at pH 6·8[5] has now revealed the presence of three bases in the ether eluate.

---

[1] SAFFORD, W. E. (1920) *Ann. Report Smithsonian Institute Washington*, 537.

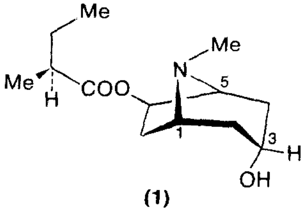
[2] SAFFORD, W. E. (1921) *J. Washington Acad. Sci.* 173.

[3] AVERY, A. G., SATINA, S. and RIETSEMA, J. (1959) in *Blakeslee: The Genus Datura* p. 35 and 220, Ronald, New York.

[4] ZIELINSKA-SOWICKA, R. and SZEPCZYNSKA, K. (1968) *Diss. Pharm. Pharmacol.* **20**, 539.

[5] EVANS, W. C. and PARTRIDGE, M. W. (1952) *J. Pharm. Pharmacol.* **4**, 769.

The third and major base present (0·007%) gave a picrate m.p. 118° and had IR absorption bands at 3420 (OH) and 1740 cm^{-1} (sat. ester carbonyl). As anticipated, the MS was characteristic of a disubstituted tropane[6] and gave the molecular formula $C_{13}H_{23}NO_2$. The base ion at *m/e* 113 (100) indicated that the free hydroxyl was at the C(3) position. PMR studies gave the usual pattern of a C(3),(6) disubstituted tropane, the triplet at higher field than usual, $\tau = 5{\cdot}95$ confirmed that the free hydroxyl was at C(3) and α-orientated.[7,8] Finally, the characteristic doublet and triplet centred at $\tau = 8{\cdot}85$ and 9·1 respectively suggested that the C(6) acyl group was 2-methylbutanoic acid and the alkaloid was therefore 6β-[2-methylbutanoyloxy]tropan-3α-ol. Hydrolysis of the base gave (+)-2-methylbutanoic acid[9] and tropan-3α,6β-diol which when esterified with tigloyl chloride afforded (−)-3α,6β-ditigloxytropane, thus confirming the 3*R* 6*R* structure assigned.[10] (**1**) The base was synthesized by esterifying (±)-2-methylbutanoyl chloride with (±)-6β-hydroxytropan-3-one and reducing the product to the 3α-ol with Raney nickel.



(**1**)

To date, 2-methylbutanoyl esters have been detected only in sect. Brugmansia[7] and the dehydro derivative of the new base 6β-tigloyloxytropan-3α-ol has been isolated from *D. cornigera*,[11] and outside the genus in *Anthocersis*,[12] which, like *Duboisia*, is placed in the sub-tribe Salpiglossideae and appears to have an affinity with the tree-daturas. Similarly, 3α-ol derivatives of di- and tri-hydroxytropane esters have hitherto only been detected in this section. The aerial parts of *D. ceratocaula*, unlike those of the tree-daturas contain little hyoscine, but the presence of this new ester as a major alkaloid would support Safford's view[1,2] that there is an affinity between sects. Brugmansia and Ceratocaulis.

The tiglic acid moiety of the ditigloyl esters of *Datura* is known to be derived from L(+)-isoleucine via 2-methylbutanoic acid (see refs 13 and 14 for scheme) but the biosynthesis of 2-methylbutanoyl esters has not previously been investigated. The isolation of dextrorotatory 2-methylbutanoic acid is in keeping with its derivation from this amino acid, and when L(+)-isoleucine-[U-^{14}C] was infiltrated into the roots of 6-month-old plants it was incorporated within 2 days. All the radioactivity was located in the 2-methylbutanoyl part.

EXPERIMENTAL

D. ceratocaula plants. Seed obtained from Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben D.D.R. were grown on open land in Leicester and had all the characters previously described.[1–3] For analy-

---

[6] Blossey, E. C., Budzikiewicz, H., Ohashi, M., Fodor, G. and Djerassi, C. (1964) *Tetrahedron* **20,** 585.
[7] Evans, W. C. and Lampard, J. F. (1972) *Phytochemistry* **11,** 3294.
[8] Parello, J., Longevialle, P., Vetter, W. and McCloskey J. A. (1963) *Bull. Soc. Chim. Fr.* 2787.
[9] Odham, G. (1963) *Ark. Kemi.* **20,** 507.
[10] Fodor, G. and Soti, F. (1965) *J. Chem. Soc.* 6830.
[11] Evans, W. C. and Griffin, W. J. (1963) *J. Chem. Soc.* 4348.
[12] Evans, W. C. and Treagust, P. G. (1973) *Phytochemistry* **12,** 2505.
[13] Basey, K. and Woolley, J. G. (1973) *Phytochemistry* **12,** 2197.
[14] Basey, K. and Woolley, J. G. (1973) *Phytochemistry* **12,** 2883.

sis, the roots and aerial parts were separately dried at 60° for 18 hr. Optical rotations were measured using a Bendix NPL automatic polarimeter type 143. L-isoleucine-[U-^{14}C] was purchased from the Radiochemical Centre, Amersham.

Counting procedures. Duplicate samples were counted in commercially available toluene or dioxane based POP/POPOP scintillators in a Packard Tri-Carb model 2002 liquid scintillation spectrometer.

Isolation of 6*β*-[*2-methylbutanoyloxy*]*tropane*-3*α*-*ol.* In a typical isolation 50 g powdered *D. ceratocaula* "top" was mixed with $Ca(OH)_2$ (15 g), H_2O (20 ml) and after 30 min exhaustively extracted with cold Et_2O. After distillation of the solvent the green residue was transferred in $CHCl_3$ (*ca* 1 ml) to a 10 g kieselguhr column containing 5 ml 0·5 M phosphate buffer pH 6·8. Development with light petrol. (100 ml) removed pigments and Et_2O eluted 3 bases. The second base (0·002%) by titre[5] was identified as hyoscine by TLC, Aluminium oxide G (Merck) Et_2O–EtOH 4:1 (R_f 0·85) and the third base $[\alpha]_D^{28}$ +5·5° (0·007%) (R_f 0·8) which was unknown easily gave a picrate (needles) m.p. 118° ν_{max}^{KBr} 3420, 1740, 1635, 1615, 1570, 1550, 1510, 1430, 1365, 1330, 1270, 1140, 1085, 1055, 907, 790, 740 and 705 cm^{-1}; MS (*m/e* with (**1**) % in parenthesis) 41 (22), 42 (26), 44 (18), 55 (8), 57 (27), 81 (9), 82 (16), 94 (30), 95 (8), 96 (47), 112 (16), 113 (100), 114 (10), 122 (11), 140 (10), 156 (7), M^+ 241 (11). Accurate mass measurement M^+ = 241·1677, $C_{13}H_{23}NO_3$ requires 241·1678. PMR (Base in $CDCl_3$ with TMS as internal standard) signals at τ = 4·37 (1H, *dd*, J = *ca* 3.3 cps), 5·9 (1H, *t*, J = *ca* 9 cps), 6·75 (*bm*), 7·5 (3H, *s*), 7·9 (OH, shift with D_2O), 8·85 (3H, *d* J = *ca* 7 cps), 9·1 (3H, *t* J = *ca* 15 cps).

Hydrolysis. The base (from 20 mg picrate) was heated at 100° in a sealed tube with 5% $Ba(OH)_2$ (10 ml) for 2 hr, when the hydrolysate was acidified (50% H_2SO_4) and extracted with Et_2O (5 × 3 ml). The Et_2O contained (+)-2-methylbutyric acid $[\alpha]_D^{24}$ +16·76° (lit.[9] $[\alpha]_D^{21\cdot2}$ +19·3°) which was identified by GLC.[15] Excess $BaCO_3$ was added to the remaining aqueous phase which was then centrifuged and the supernatant liquid was evaporated to dryness under reduced pressure. A mixture of tigloyl chloride (0·4 ml) and the residue (the alkamine sulphate) was heated under reflux for 2 hr,[16] then cooled, acidified and the excess tiglic acid was removed with Et_2O. Extraction of the basified solution with $CHCl_3$ gave a single base $[\alpha]_D^{21}$ −18·5° (lit.[17] $[\alpha]_D^{20}$ −21·5°), TLC Aluminium oxide G (Merck) Et_2O R_f identical with (−)3α,6β-ditigloyloxytropane, which gave a picrate (4·8 mg, long needles), IR (KBr), m.p., m.m.p. (151°).

Partial synthesis of 6*β*[*2-methylbutanoyloxy*]*tropan*-3*α*-*ol.* Dry 6β-hydroxytropan-3-one (28 mg) and 2-methylbutanoyl chloride (0·5 ml) heated under reflux for 2 hr. The recovered base was dissolved in EtOH and hydrogenated (at. press.) in the presence of Raney Ni. The resultant base, picrate mp 124°, had an R_f value on TLC and PMR spectrum indistinguishable from the natural base.

Feeding experiments. Thirteen 6-month-old field grown *D. ceratocaula* plants were carefully uprooted, washed free from soil and allowed to stand in blackened beakers containing an aq. soln of L-isoleucine-[U-^{14}C] (45 μCi) sp. act. 8·7 mCi/mM. After 2 days the roots and aerial parts were separately dried. The aerial parts (60 g) were extracted and the 6β-[2-methylbutanoyloxy]tropan-3α-ol (5·6 mg) from the partition column was diluted by adding authentic base picrate (11 mg) to the shake-out liquors. The re-isolated diluted picrate (9·8 mg) m.p. 118°, sp. act. 5·69 × 10^4 dpm/mM (calc. sp. incorporation 0·0006%; total incorporation 0·003%) was converted to the base and when hydrolysed gave 2-methylbutanoic acid sp. act. 5·60 × 10^4 dpm/mM (98·4% recovery of radioactivity) and tropan-3α,6β-diol, isolated as the picrate (3 mg) m.p. 247°.

Acknowledgements—One of us (P.J.B.) wishes to thank the Leicester Education Authority for a Research Assistantship. We also wish to thank Dr. W. C. Evans, Department of Pharmacy, University of Nottingham, for the generous gift of 6β-hydroxytropan-3-one, Miss V. J. Charles, Mrs. M. Warwick, Mrs A. B. Abbott and Mr. D. M. Penny for technical assistance.

---

[15] Beresford P. J. and Woolley, J. G. (1974) *Phytochemistry* **13**, 1249.

[16] Barger, G., Mitchell, W. and Martin, W. F. (1937) *J. Chem. Soc.* 1820.

[17] Evans, W. C. and Wellendorf, M. (1958) *J. Chem. Soc.* 1991.