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SHORT COMMUNICATION

BIOSYNTHESIS OF METELOIDINE

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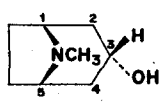
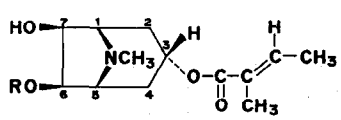
Abstract—Tropine-3β-^{3}H, *N*-methyl-^{14}C was fed to *Datura meteloides* plants. After 7 days radioactive meteloidine, scopolamine, hyoscyamine, and 7β-hydroxy-3α,6β-ditigloyloxytropane were isolated from the plants and found to have essentially the same ^{3}H/^{14}C ratio as in the administered tropine. Degradation of the meteloidine established that all its tritium was located at C-3 and all the ^{14}C was on the *N*-methyl group, indicating that tropine is a direct precursor of teloidine.

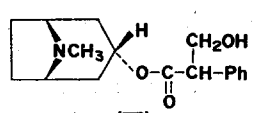
WE HAVE previously established that teloidine, the amino-alcohol moiety of the ester alkaloid meteloidine (II), is derived in part from ornithine.[1] The administration of ornithine-2-^{14}C to *Datura meteloides* afforded meteloidine labelled exclusively at the bridge-head carbons (C-1 or C-5, or both), and we suggested that tropine (I) was an intermediate since it has been established that the tropine moiety of hyoscyamine (III) is derived from ornithine[2,3] in *D. stramonium* and *metel.* We have now investigated this hypothesis by feeding labelled tropine to *D. meteloides.* The tropine was specifically labelled with ^{3}H and ^{14}C as follows. Reduction of tropinone with sodium borohydride-^{3}H in methanol afforded a mixture of tropine-3β-^{3}H and ψ-tropine-3α-^{3}H[4] which were readily separated by TLC. Tropine-*N*-methyl-^{14}C was obtained by reaction of nortropine with methyl-^{14}C iodide by modification of a previously described method.[5] Nortropine was conveniently obtained from tropine by reaction with phenyl chloroformate[6] which yielded *N*-carbophenoxy nortropine. Hydrolysis of this compound with KOH afforded nortropine.



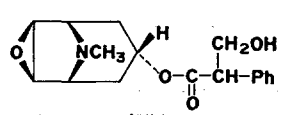
Tropine (I)



Meteloidine (II, R=H)



Hyoscyamine (III)



Scopolamine (IV)

* Contribution No. 115 from this laboratory.

[1] E. LEETE and S. J. NELSON, *Phytochem.* **8**, 413 (1969).
[2] E. LEETE, *J. Am. Chem. Soc.* **84**, 55 (1962).
[3] H. W. LIEBISCH and H. R. SCHÜTTE, *Z. Pflanzenphysiol.* **57**, 434 (1967).
[4] A. H. BECKETT, N. J. HARPER, A. D. J. BALON and T. H. E. WATTS, *Chem. & Ind.* 663 (1957).
[5] G. FODOR, G. JANZO, L. OTVOS and D. BANFI, *Chem. Ber.* **93**, 2681 (1960).
[6] A. ABDEL-MONEM and P. S. PORTOGHESE, *J. Med. Chem.* **15**, 208(1972), describe the use of this reagent for demethylating tertiary amines. Ethyl chloroformate has been similarly used for demethylating tropine and its derivatives; G. KRAISS and K. NÁDOR, *Tetrahedron Letters* 57 (1971).

The tropine-3β-^{3}H, *N*-methyl-^{14}C, having a high ratio of ^{3}H to ^{14}C to facilitate liquid scintillation counting of the doubly labelled samples, was fed to *D. meteloides* plants as its hydrochloride, dissolved in water, by means of cotton wicks inserted into the stems. After seven days the plants were harvested and the alkaloids isolated and separated by TLC. At least nine alkaloids were detected, however only four were positively identified: meteloidine, 7β-hydroxy-3α,6β-ditigloyloxytropane (V), hyoscyamine, and scopolamine (IV). All these alkaloids had essentially the same ^{3}H/^{14}C ratio as the administered tropine (see Table 1), strongly suggesting that tropine had been incorporated intact into the amino-alcohol moieties of these alkaloids. The specific activity of the hyoscyamine was significantly higher than that of the scopolamine, a result which is consistent with previous work in which it has been established that hyoscyamine is a precursor of scopolamine.[7–9] The low specific activity of 7β-hydroxy-3α,6β-ditigloyloxytropane compared with meteloidine probably indicates that this alkaloid is formed from meteloidine, a conclusion also arrived at by Evans and Woolley who were studying the origin of the tigloyl moieties of these alkaloids.[10,11]

TABLE 1

Alkaloid	Yield (mg of free base)	Specific activity (dpm/mmol)	^{3}H/^{14}C	Specific inc. (%)	Absolute inc. (%)
Meteloidine*	93	$1{\cdot}0 \times 10^8$	13·9	3·08	2·87
Scopolamine†	328	$1{\cdot}0 \times 10^7$	12·8	0·31	0·85
Hyoscyamine‡	30	$4{\cdot}4 \times 10^7$	14·1	1·36	0·36
7β-Hydroxy-3α,6β-ditigloyl-oxytropane§	113	$5{\cdot}7 \times 10^6$	13·7	0·18	0·15

* Obtained as colorless plates, m.p. 143–144°, by crystallization from benzene-pet. ether.
† Isolated as the hydrochloride, m.p. 200°, as colorless needles from EtOH-Et_2O.
‡ Isolated as the hydrochloride, m.p. 165°, as colorless prisms from EtOH–Et_2O.
§ Isolated as the hydrobromide, m.p. 217–218°, from ethanol–ethyl acetate.
All reported activities and incorporations are based on the observed ^{3}H counts. Tropine fed: $3{\cdot}24 \times 10^9$ dpm/mmol (^{3}H/^{14}C = 13·6).

The meteloidine was degraded to determine the location of the ^{3}H and ^{14}C. The 6,7-diol of meteloidine was protected as an isopropylidene derivative which was then hydrolysed with base yielding tiglic acid (completely non-radioactive) and isopropylidene teloidine which was oxidised to isopropylidene teloidinone. This ketone was devoid of ^{3}H, indicating that all the tritium had been located at C-3 of the teloidine. Demethylation of meteloidine with HI yielded MeI which was collected as triethylmethylammonium iodide by absorption in triethylamine. This derivative had the same ^{14}C specific activity as the meteloidine indicating that all the ^{14}C activity was located on the *N*-methyl group. The mechanism whereby tropine is hydroxylated at the 6 and 7-positions is unknown and is being investigated. The work of Woolley[11] suggests that the hydroxylation may occur after the formation of the tigoyl ester of tropine.

[7] A. ROMEIKE and G. FODOR, *Tetrahedron Letters* **22**, 1 (1960).
[8] A. ROMEIKE, *Planta Med.* **8**, 491 (1960); *Naturwissenschaften* **49**, 281 (1962).
[9] F. A. TURNER and J. E. GEARIEN, *J. Pharm. Sci.* **53**, 1309 (1964).
[10] W. C. EVANS and J. G. WOOLLEY, *J. Pharm. Pharmacol.* **17**, 37S (1965).
[11] J. G. WOOLLEY, *Abhandl. Deut. Akad. Wiss. Berlin Kl. Chem. Geol. Biol.* (3), 531 (1966).

Tropine which is very water soluble was not detected in the crude alkaloid extract. However, by long ether extraction of the basic aqueous extract of the plant it was isolated and found to have one tenth of the specific activity of the administered tropine. Since this reisolated tropine and all the other identified alkaloids maintained the same $^3H/^{14}C$ ratio as the administered tropine, it seems clear that there is little, if any, oxidation of the 3-hydroxyl group to a ketone, which would have resulted in loss of ^3H relative to the ^{14}C.

EXPERIMENTAL

General methods. Radioactivity measurements were carried out in a Nuclear Chicago Mark II liquid scintillation counter, using as solvents either toluene or dioxane, with the usual scintillators.[12] The addition of a drop of 2 N HCl to solutions of picrates rendered them almost colorless, with resultant much improved counting efficiency, especially of ^3H. Elementary analyses were determined by Fay Thompson at the University of Minnesota.

Nortropine. Tropine (1 g) and phenyl chloroformate (2·5 g) were dissolved in CH_2Cl_2 (25 ml) and the mixture stirred at room temp. for 24 hr. The clear solution was then evaporated to dryness and the residue dissolved in 2 N HCl (10 ml) and extracted with ether (3 × 100 ml). The ether extract was washed with K_2CO_3 solution and dried over $MgSO_4$. Evaporation yielded a white residue which was crystallized from a mixture of EtOAc and light petroleum (b.p. 60–70°) affording colorless needles of *N*-carbophenoxynortropine (1·45 g) m.p. 147–148°. (Calc. for $C_{14}H_{17}NO_3$: C, 67·99; H, 6·93; N, 5·66. Found: C, 67·91; H, 6·94; N, 5·48%.) *N*-Carbophenoxynortropine (0·75 g) was refluxed in N_2 with a mixture of EtOH (40 ml) and 50% aq. KOH (10 ml) for 24 hr. The solution was then diluted with H_2O (30 ml) and the oil which separated extracted with $CHCl_3$ (4 × 100 ml). The $CHCl_3$ extract was extracted with 2 N HCl (3 × 80 ml) which on evaporation yielded nortropine hydrochloride (350 mg), obtained as needles from EtOAc–Et_2O, m.p. 290–291°. Nortropine hydrochloride (220 mg) was dissolved in dilute KOH and extracted with $CHCl_3$. Evaporation of the dried (Na_2SO_4) extract afforded nortropine (137 mg), m.p. 161–162°.

Tropine-N-methyl-^{14}*C.* Methyl-^{14}C iodide (72 mg, 0·50 m-mol), having a nominal activity of 0·5 mCi, was added to a solution of nortropine (100 mg, 0·79 mmol) in benzene (3 ml), and the mixture stirred for 20 hr at room temp. Inactive tropine (50 mg) was added to the reaction mixture which was then evaporated to dryness. The residue was made basic with 10% NaOH and extracted with $CHCl_3$. The extract was evaporated and the residue dissolved in MeOH (3 ml). The addition of a solution of picric acid (120 mg) in EtOH (3 ml) resulted in the separation of tropine-*N*-methyl-^{14}C picrate which was obtained as fine yellow needles on recrystallization from EtOH (178 mg) m.p. 270°, having a total activity of $3·9 \times 10^8$ dpm (36% radiochemical yield).

Tropine-3β*-*^{3}*H.* Sodium borohydride-^{3}H (34 mg, 0·92 mmol) having a nominal activity of 25 mCi (purchased from The Radiochemical Centre, Amersham) was added to a solution of tropinone[13] (128 mg, 0·92 mmol) in dry MeOH (5 ml), and the mixture refluxed for 12 hr. The solution was then evaporated to dryness and the residue dissolved in 1% NaOH and extracted with $CHCl_3$ in a continuous extractor for 18 hr. The $CHCl_3$ extract, having an activity of 10 mCi, was evaporated and applied to a 20 × 20 × 0·2 cm plate of Silica Gel PF-254 (Merck). The plate was developed three times with $CHCl_3$–EtOH–conc. NH_3 (7:5:1). The zones corresponding to tropine and ψ-tropine were detected by spraying a thin strip at the side of the plate with a solution of I_2 in hexane. The tropine zone (having a lower R_f than ψ-tropine) was extracted in a Soxhlet with $CHCl_3$–MeOH (1:1). The evaporated extract ($5·5 \times 10^9$ dpm) was dissolved in EtOH (10 ml). Inactive tropine (100 mg) was added, followed by picric acid (229 mg) when tropine-3β-^{3}H picrate separated (287 mg) as fine yellow needles, having an activity of $4·6 \times 10^9$ dpm/mmol.

Administration of tropine-3β*-*^{3}*H, N-methyl-*^{14}*C to D. meteloides plants and isolation of the alkaloids.* Tropine-*N*-methyl-^{14}C picrate (42·4 mg) was dissolved in 2 N HCl (30 ml) and the solution extracted with Et_2O to remove picric acid. After radioactive assay this solution was mixed with a similarly prepared solution of tropine-3β-^{3}H obtained from tropine-3β-^{3}H picrate (102·6 mg). The total ^{3}H activity of the combined solution was $1·27 \times 10^9$ dpm, and the ^{14}C activity was $9·36 \times 10^7$ dpm ($^3H/^{14}C = 13·6$). The solution was evaporated to dryness and the residue exposed to a high vacuum at room temp. to remove traces of HCl. The residual tropine hydrochloride was dissolved in H_2O (20 ml) and administered to twenty 4-month-old *D. meteloides* plants growing in soil in a greenhouse, by means of cotton wicks inserted into the stems near to ground level. 7 days later the whole plants (fresh wt 1710 g) were harvested and macerated with a (1:1) mixture of $CHCl_3$ and Et_2O (4 l.) and conc. NH_3 (100 ml). Washings from the beakers which had contained the radioactive tropine were assayed and found to contain less than 0·002% (^{3}H activity) of the administered activity. After standing for 1 day the plant mixture was filtered through cloth and the $CHCl_3$-Et_2O layer separated. The aq. alkaline layer had a ^{14}C activity of $2·7 \times 10^7$ dpm (29% of the amount fed to the plants)

[12] A. R. FRIEDMAN and E. LEETE, *J. Am. Chem. Soc.* **85**, 2141 (1963).
[13] C. SCHÖPF and G. LEHMANN, *Ann.* **518**, 1 (1935).

and it was from this fraction that the tropine was isolated (see below). The organic layer was evaporated to dryness, the residue dissolved in Et_2O (200 ml) and extracted with 0·5 N HCl (4 × 50 ml). This solution was made basic with K_2CO_3 and extracted with $CHCl_3$ (5 × 100 ml), dried (Na_2SO_4) and evaporated to afford the crude alkaloids (924 mg) having an activity of $7·8 \times 10^7$ (^3H), $5·6 \times 10^6$ (^{14}C) ($^3H/^{14}C = 13·9$), representing an incorporation of 6%.

Separation and isolation of the individual alkaloids. The crude alkaloids were dissolved in $CHCl_3$ and separated on several 20 × 20 × 0·2 cm plates of Silica Gel PF-254 (Merck), developing with $CHCl_3$–EtOH–conc. NH_3 (100:20:1). Nine distinct zones were observed having R_fs 0·04 (A), 0·15 (B), 0·26 (C), 0·35 (D), 0·46 (E), 0·57 (F), 0·73 (G), 0·88 (H), 0·93 (I). By comparison with authentic specimens zones C, E, G, and H were identified as hyoscyamine, meteloidine, scopolamine, and 7β-hydroxy-3α, 6β-ditigloyloxytropane (picrate m.p. 185–186°, lit.[14] 184–185°, hydrobromide m.p. 217–218°, lit.[14] 215–216°, MS had a molecular ion peak at $m/e = 337$) respectively. The yields and activities of the isolated alkaloids are recorded in Table 1.

The absolute incorporation of activity into the unidentified alkaloids was approximately as follows: A (0·06%), B (0·07%), D (0·27%), F (0·1%), and I (0·03%). All these alkaloids had a $^3H/^{14}C$ ratio between 11·5 and 13·6, except B which had a ratio greater than 20 indicating that this alkaloid may be derived from nortropine, e.g. norhyoscyamine.

Degradation of the radioactive meteloidine. The radioactive meteloidine (^3H activity: $1·0 \times 10^8$ dpm/mmol, $^3H/^{14}C = 13·9$) was converted to isopropylideneteloidine (^3H activity: $1·05 \times 10^8$ dpm/mmol, $^3H/^{14}C = 14·1$) and tiglic acid (negligible activity) by previously described methods.[1] The isopropylideneteloidine was oxidized with CrO_3 in pyridine[1] yielding isopropylideneteloidinone (no ^3H activity, ^{14}C activity: $7·1 \times 10^6$ dpm/mmol). The meteloidine was demethylated by heating with HI at 360° using the procedure of Brown and Byerrum.[15] The evolved MeI was washed with cadmium sulfate and sodium thiosulfate, and then absorbed in a cooled ethanolic solution of triethylamine to yield triethylmethylammonium iodide ($7·0 \times 10^6$ dpm/mmol).

Isolation of tropine. The aq. alkaline layer obtained from the initial extraction of the plants was subjected to a continuous extraction with Et_2O for 4 days. The resultant extract had a ^3H activity of $8·1 \times 10^7$ dpm (6·4% of the activity fed to the plants) and a $^3H/^{14}C$ ratio of 13·6. This ether solution was extracted with 0·5 N HCl (4 × 50 ml) which was then made basic with K_2CO_3 and extracted again with Et_2O in a continuous extractor. TLC of the extract on Silica Gel PF-254, developing with $CHCl_3$–EtOH–conc. NH_3 (7:7:1) indicated the presence of tropine, hyoscyamine, meteloidine, and scopolamine, having R_fs 0·16, 0·5, 0·72, and 0·90, respectively. The zone corresponding to tropine was extracted with $CHCl_3$–MeOH, which was evaporated and the residue sublimed, affording tropine (3·2 mg), which was converted to its picrate. Its activity (^3H) was $3·20 \times 10^8$ dpm/mmol ($^3H/^{14}C = 13·0$).

Acknowledgement—This investigation was supported by a research grant GM-13246 from the National Institutes of Health, U.S. Public Health Service.

[14] W. C. EVANS and M. W. PARTRIDGE, *J. Chem. Soc.* 1102 (1957).
[15] S. A. BROWN and R. U. BYERRUM, *J. Am. Chem. Soc.* **74**, 1523 (1952).

Key Word Index—Datura meteloides; Solanaceae; biosynthesis; meteloidine; tropine as precursor.