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BIOSYNTHESIS OF THE TELOIDINE MOIETY OF METELOIDINE IN *DATURA METELOIDES*

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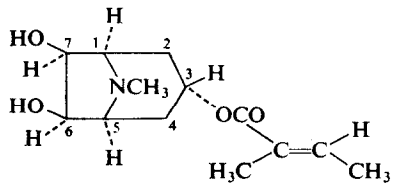
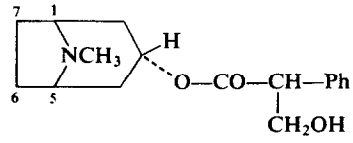
Abstract—DL-Ornithine-2-^{14}C was administered to 3-month-old *Datura meteloides* plants. The meteloidine isolated 12 days later was radioactive (0·3 per cent incorporation). A systematic degradation carried out on the alkaloid indicated that essentially all the activity was located at one or both of the bridgehead carbons (C-1 and C-5) of the teloidine moiety of the alkaloid. This result is consistent with the hypothesis that teloidine is formed by the hydroxylation of tropine, perhaps via 6,7-dehydrotropine.

METELOIDINE (I) is one of the main alkaloids in *Datura meteloides*, and occurs in this plant along with hyoscyamine (II), hyoscine (IV),[1] and norhyoscyamine.[2] Its stereochemistry is as illustrated in formula I.[3] It has previously been established that carbons 1, 5, 6, and 7 of the tropine moiety of hyoscyamine are derived from the amino acid ornithine.[4–6] The



(I)



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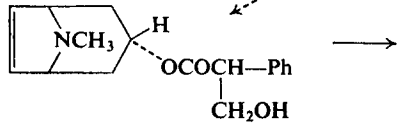

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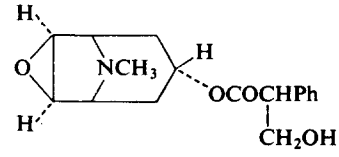

(IV)

administration of ornithine-2-^{14}C to *D. stramonium* plants afforded tropine which was labelled stereospecifically at C-1. Recently it has been shown that only the δ-amino nitrogen of ornithine is incorporated into tropine.[7] Carbons 2, 3, and 4 of tropine are derived from

[1] F. L. PYMAN and W. C. REYNOLDS, *J. Chem. Soc.* **93**, 2077 (1908).
[2] F. H. CARR and W. C. REYNOLDS, *J. Chem. Soc.* **101**, 946 (1912).
[3] G. FODOR, J. TOTH and I. VINCZE, *Helv. Chim. Acta* **37**, 907 (1954).
[4] E. LEETE, L. MARION and I. D. SPENSER, *Can. J. Chem.* **32**, 1116 (1954).
[5] E. LEETE, *J. Am. Chem. Soc.* **84**, 55 (1962).
[6] E. LEETE, *Tetrahedron Letters* 1619 (1964).
[7] H. W. LIEBISCH and H. R. SCHÜTTE, *Z. Pflanzenphysiol.* **57**, 434 (1967).

acetic acid.[8] It has also been shown that hygrine serves as a precursor of tropine.[9,10] A biosynthetic scheme for tropine, which is consistent with these tracer studies, is illustrated in Fig. 1. Transamination of ornithine (V) at the α-position affords the α-ketoacid (VI) which on decarboxylation yields 4-aminobutanal (VII). This aminoaldehyde could also be formed by the oxidation of putrescine (IX). This compound is incorporated into tropine when fed to *Datura* plants;[11–13] however, it seems unlikely that the biosynthetic route from ornithine proceeds via putrescine since the symmetry of putrescine would cause the label from ornithine-2-^{14}C to be distributed equally between C-1 and C-5 of the ultimate tropine.

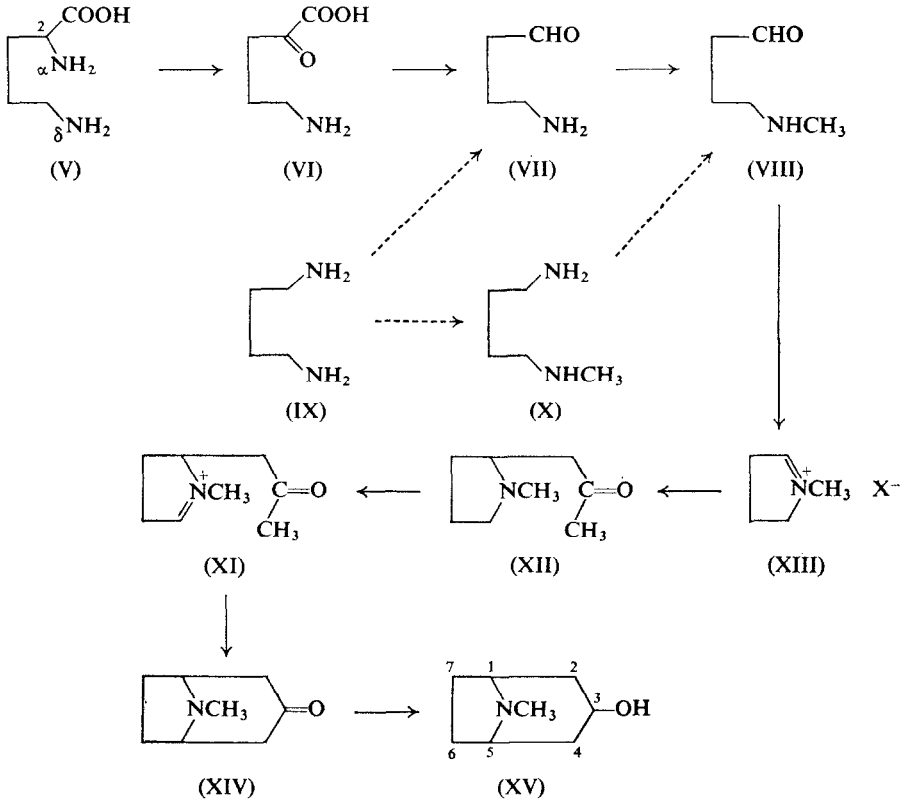

FIG. 1. BIOSYNTHESIS OF TROPINE.

Methylation of VII yields 4-methylaminobutanal (VIII). This compound, labelled with ^{14}C, has been detected in the roots of *D. stramonium* plants which had been fed ornithine-2-^{14}C.[14] An alternate source of this compound would be *N*-methylputrescine (X), which is in fact

[8] K. MOTHES, J. KACZKOWSKI and H. R. SCHÜTTE, *Biochim. Biophys. Acta* **46**, 588 (1963).
[9] H. R. SCHÜTTE, Abstracts of the 5th International Symposium on the Chemistry of Natural Products, London, July 8–13, p. 86 (1968).
[10] D. G. O'DONOVAN and M. F. KEOGH, *ibid.* p. 90.
[11] E. LEETE and M. C. LOUDEN, *Chem. & Ind.* 1725 (1963).
[12] H. W. LIEBISCH, H. R. SCHÜTTE and K. MOTHES, *Annalen* **668**, 139 (1963).
[13] J. KACZKOWSKI and L. MARION, *Can. J. Chem.* **41**, 2651 (1963).
[14] S. MIZUSAKI, T. KISAKI and E. TAMAKI, *Plant Physiol.* **43**, 93 (1968).

an efficient precursor of tropine.[15] It is conceivable that the plant could utilize administered *N*-methylputrescine even though it may not be a normal metabolite of ornithine. The administration of α-*N*-methylornithine (labelled with ^{14}C on the *N*-methyl group) to *Datura* species yielded hyoscyamine and hyoscine which were labelled with ^{14}C on their *N*-methyl groups.[16] On the other hand, δ-*N*-methylornithine was a much poorer precursor of the alkaloids and activity was not localized on the *N*-methyl groups of the alkaloids. These results are apparently in conflict with the reported incorporation of the δ-amino group of ornithine into tropine.[7] It may be that the administered *N*-methylornithines were not authentic.[17] Cyclization of 4-methylaminobutanal affords the *N*-methyl-Δ^1-pyrrolinium salt (XIII). Reaction of this compound with acetoacetic acid (derived from two molecules of acetic acid) yields hygrine (XII), a condensation which has been accomplished *in vitro*.[18] Compound XI is formed by the dehydrogenation of hygrine, and an aldol condensation yields tropinone (XIV). Finally, tropine is formed by reduction of the ketone.

Ornithine is also a precursor of hyoscine, and degradation of the radioactive alkaloid derived from ornithine-2-^{14}C indicated that all the activity was located at the bridgehead carbons of the tropane base.[19] This result is consistent with the hypothesis that hyoscyamine is a precursor of hyoscine, intermediates in this transformation apparently being 6,7-dehydrohyoscyamine (III), and 6-hydroxyhyoscyamine.[20,21] With this information it seemed reasonable to expect that the 6,7-dihydroxytropine (teloidine) moiety of meteloidine would also be formed from tropine, possibly via a 6,7-dehydro-derivative. Wenkert[22] has suggested that the pyrrolidine ring of this hydroxylated tropine is derived from erythrose.

We have administered DL-ornithine-2-^{14}C to 3-month-old *D. meteloides* plants by means of a cotton wick inserted into the stems. After 12 days the plants were harvested, and meteloidine separated from the crude alkaloids by thin-layer chromatography. The meteloidine was radioactive, with an activity representing an incorporation of 0·3 per cent. Hydrolysis of the alkaloid afforded radioactive teloidine and inactive tiglic acid. We initially attempted to convert teloidine to tropine, since we had already developed a degradative scheme for tropine to determine activity at the bridgehead carbons.[5] Various methods[23,24] for the conversion of 1,2-glycols to the corresponding alkenes or alkanes, were unsuccessful when applied to teloidine or teloidinone (6,7-dihydroxy-3-tropinone). A new method of degradation was devised and this is illustrated in Fig. 2. Meteloidine was converted to its acetonide by treatment with acetone and hydrochloride acid. Hydrolysis with methanolic potassium hydroxide yielded tiglic acid and isopropylideneteloidine (XVI), which was oxidized to the ketone XVII with chromium trioxide in pyridine. Reduction of this ketone by the Wolf-Kischner method yielded the isopropylidene derivative of 6,7-dihydroxytropane (XVIII). The 6,7-dihydroxytropane (XIX) formed on acid hydrolysis, was cleaved with sodium metaperiodate affording *N*-methylpiperidine-2,6-dialdehyde (XX). This dialdehyde

[15] H. W. LIEBISCH, W. MAIER and H. R. SCHÜTTE, *Tetrahedron Letters* 4079 (1966).
[16] D. NEUMAN and H.-B. SCHRÖTER, *Tetrahedron Letters* 1273 (1966).
[17] The *N*-methylornithines were prepared by an ambiguous method: D. NEUMAN and H.-B. SCHRÖTER, *Z. Chem.* **5**, 385 (1965), and their syntheses have been critically evaluated: T. J. GILBERTSON and E. LEETE, *J. Am. Chem. Soc.* **89**, 7085 (1967).
[18] F. GALINOVSKY, A. WAGNER and R. WEISER, *Monatsch.* **82**, 551 (1951).
[19] F. A. TURNER and J. E. GEARIEN, *J. Pharm. Sci.* **53**, 1309 (1964).
[20] A. ROMEIKE, *Planta Med.* **8**, 491 (1960); *Naturwiss.* **49**, 281 (1962).
[21] A. ROMEIKE and G. FODOR, *Tetrahedron Letters* **22**, 1 (1960).
[22] E. WENKERT, *Experientia* **15**, 165 (1959).
[23] E. J. COREY and R. A. E. WINTER, *J. Am. Chem. Soc.* **85**, 2677 (1963).
[24] H. L. SLATES and N. L. WENDLER, *J. Am. Chem. Soc.* **78**, 3749 (1956).

was not isolated, but subjected to a Wolf-Kischner reduction yielding *cis*-1,2,6-trimethylpiperidine (XXI). This piperidine derivative was surprisingly resistant to a Kuhn-Roth oxidation. It was recovered unchanged after refluxing for 1 hr in 2 N sulphuric acid containing 25% chromium trioxide. However, a 65 per cent yield of acetic acid was obtained by

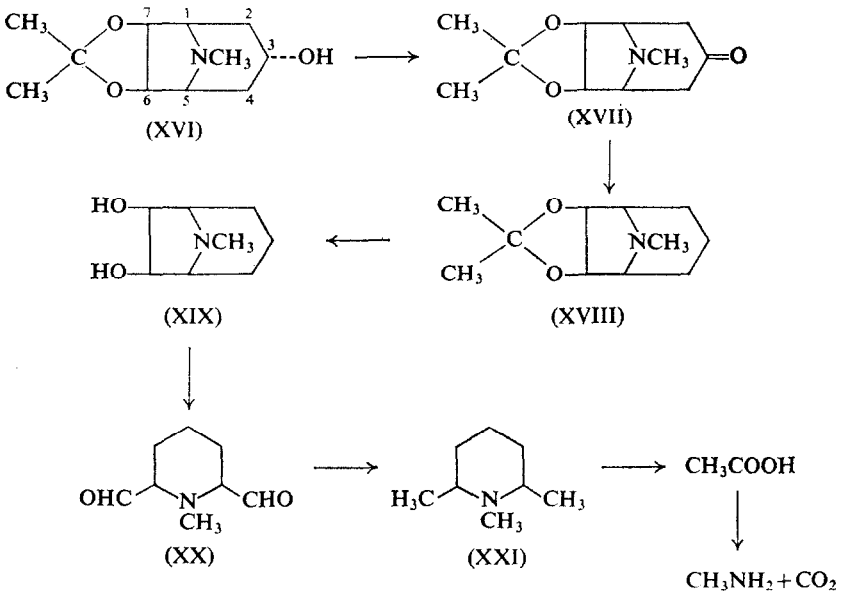

FIG. 2. DEGRADATION OF THE METELOIDINE.

TABLE 1. ACTIVITY OF METELOIDINE AND ITS DEGRADATION PRODUCTS

	Specific activity (dpm/mM × 10^{-5})
Meteloidine	1·15
Tiglic acid	<0·02
Isopropylideneteloidine	1·11
Isopropylideneteloidinone	1·06
6,7-Dihydroxytropane	0·97
cis-1,2,6-Trimethylpiperidine perchlorate	0·97
1-Acetamidonaphthalene	0·51
Barium carbonate [C-1 + C-5/2]	0·48
N-Methylbenzamide [C-6 + C-7/2]	<0·01

carrying out the reaction in refluxing 50% phosphoric acid containing 50% chromium trioxide. The acetic acid was subjected to a Schmidt reaction affording methylamine, isolated as its benzoyl derivative, and carbon dioxide collected as barium carbonate. Table 1 records the activities of meteloidine and its degradation products. This method of degradation does not differentiate between the bridgehead carbons C-1 and C-5, since the acetic acid formed in the final Kuhn-Roth oxidation is derived from carbons 1–7 and 5–6 in the original teloidine.

However, all the activity of the acetic acid was located on its carboxyl group, indicating that only the bridgehead carbons contained activity.

Since the pattern of labelling in teloidine is the same as that found in tropine when ornithine-2-^{14}C was fed to *D. stramonium* plants, we feel that the present results favour a biosynthetic route to teloidine via tropine, and we plan to examine tropine as a precursor of teloidine in *D. meteloides*.

EXPERIMENTAL

General Methods

Elementary analyses were determined by Clark Microanalytical Laboratory, Urbana, Illinois. Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation system, Model 724, using as solvents either toluene or dioxane–water, with the usual scintillators.[25]

Administration of DL-*Ornithine-2-*^{14}*C to* Datura meteloides *and Isolation of the Meteloidine*

DL-Ornithine-2-^{14}C monohydrochloride (18·8 mg, $4{\cdot}7 \times 10^8$ dpm)[26] was dissolved in water (5 ml) and divided between five *D. meteloides* plants (3-month-old) growing in soil in a greenhouse (March, 1966). The feeding was carried out by means of cotton wicks inserted into the stems of the plant by means of a darning needle. After 12 days the whole plants were harvested (fresh wt. 880 g) and macerated in a Waring blendor with a 1:1 mixture of $CHCl_3$ and ether (2 l.) and concentrated ammonia (100 ml). After standing for 2 days the mixture was filtered through cloth. The organic layer was evaporated to small volume (300 ml) and extracted with 0·5 NHCl (3×100 ml). The acidic extract was made basic with sodium carbonate and extracted with $CHCl_3$ (6×50 ml). Evaporation of the dried (sodium sulphate) extract yielded the crude alkaloids, having a total activity of $3{\cdot}2 \times 10^6$ dpm. The extract was subjected to TLC on Silica gel-G plates (Merck). Elution with a 7:1 mixture of $CHCl_3$ and ethanol yielded the following alkaloids, having the R_f values indicated in parentheses: hyoscyamine (0·16), meteloidine (0·25), and hyoscine (0·50). The zone containing meteloidine was extracted with 95% ethanol, and u.v. spectroscopy indicated that there was 10·6 mg of meteloidine in the extract. The solution was diluted with inactive meteloidine (137 mg), evaporated, and the residue crystallized from a mixture of benzene and petroleum ether (b.p. 60–70°) affording colourless needles, m.p. 141·5–143°, having a specific activity of $1{\cdot}15 \times 10^5$ dpm/mM.

Degradation of the Meteloidine

Dilutions were carried out as required. The activities reported in Table 1 are for undiluted material.

Isopropylideneteloidine

Meteloidine (66·8 mg) was stirred in acetone (5 ml) containing conc. HCl (0·2 ml) for 6 hr at room temperature. KOH (0·6 g) in water (1 ml) was added and the acetone removed *in vacuo*. The residue was dissolved in methanol (5 ml) and the solution refluxed for 18 hr. The solution was then evaporated and a saturated solution of K_2CO_3 (5 ml) added to the residue. The mixture was extracted with ether in a continuous extractor for 4 days. Evaporation of the dried ether extract yielded isopropylideneteloidine, which was sublimed (120°, 0·01 mm), and crystallized from a mixture of ethyl acetate and isooctane affording colourless needles (53·7 mg), m.p. 129–131°, lit.[27] 131–133°. The K_2CO_3 solution from which the teloidine derivative had been extracted was acidified with HCl and extracted with ether. Evaporation of the dried ether extract yielded tiglic acid (12 mg), purified by sublimation (50°, 0·05 mm), having a m.p. 60–61°, lit.[28] 65°.

Isopropylideneteloidinone

CrO_3 (53 mg) was dissolved in pyridine (1·5 ml) and the mixture stirred for 15 min. Isopropylideneteloidinine (51·5 mg) in pyridine (0·5 ml) was then added and the mixture stirred for 24 hr at room temperature. The reaction mixture was then diluted with ether (10 ml) and the precipitated salts removed. The solution was then evaporated to dryness and the residue sublimed (50°, 0·05 mm) affording the ketone (44·2 mg) m.p. 75–80°. Resublimation yielded material, m.p. 87–89°, lit.[27] m.p. 89–90°.

6,7-*Dihydroxytropane*

Isopropylidine teloidinone (174 mg) was refluxed with 95% hydrazine (5 ml) for 3 hr. The hydrazine was then removed *in vacuo* and the residual oil heated in a sealed tube with ethanol (7 ml), in which sodium (0·4 g)

[25] A. R. FRIEDMAN and E. LEETE, *J. Am. Chem. Soc.* **85**, 2141 (1963).
[26] Purchased from Tracerlab, Waltham, Mass.
[27] J. C. SHEEHAN and E. R. BISSEL, *J. Org. Chem.* **19**, 270 (1954).
[28] A. MICHAEL and J. ROSS, *J. Am. Chem. Soc.* **55**, 3684 (1933).

had been dissolved, for 18 hr at 180–190°. The contents of the tube were diluted with water (10 ml) and acidified with HCl. The solution was heated at 100° for 30 min, and then made alkaline with K_2CO_3. Continuous ether extraction (2 days) of the solution afforded 6,7-dihydroxytropane (87·2 mg), purified by sublimation (70°, 0·01 mm), and crystallization from a mixture of ethyl acetate and isooctane, from which it was obtained as colourless needles, m.p. 92–94°.

Anal. Calc. for $C_8H_{15}NO_2$: C, 61·12; H, 9·62; N, 8·91. Found: C, 61·38; H, 9·44; N, 8·56 per cent.

cis-1,2,6-*Trimethylpiperidine*

6,7-Dihydroxytropane (77·8 mg) was dissolved in water (2 ml) and neutralized with 0·1 N HCl. Sodium metaperiodate (127 mg) was added and the solution allowed to stand for 15 min at room temperature. Barium chloride dihydrate (145 mg) was added and the precipitated iodate and periodate salts removed. The solution was evaporated below 40° to a small volume. Hydrazine (5 ml) was added and the mixture refluxed for 2·5 hr. The hydrazine was removed *in vacuo* and the residue heated in a sealed tube with ethanol (5 ml) containing dissolved sodium (0·3 g) at 180–190° for 22 hr. The contents of the tube were diluted with water (15 ml) and extracted with ether. The dried (sodium sulphate) ether extract was evaporated to small volume and a drop of 70% perchloric acid added. On addition of ethanol and ethyl acetate *cis*-1,2,6-trimethylpiperidine perchlorate separated (40 mg). Crystallization from ethyl acetate afforded long colourless needles, m.p. 169–170°.

Anal. Calc. for $C_8H_{17}N.HClO_4$: C, 42·20; H, 7·97; N, 6·15. Found: C, 42·25; H, 7·92; N, 6·21.

Material from an inactive run afforded a picrate, m.p. 228–229° with decomposition, lit.[29] m.p. 224–225° with decomposition.

Kuhn-Roth Oxidation of 1,2,6-*Trimethylpiperidine*

1,2,6-Trimethylpiperidine perchlorate (112 mg) was dissolved in 6 N NaOH (5 ml) and the piperidine extracted with $CHCl_3$. The combined extracts were added to 50% phosphoric acid (10 ml) and the $CHCl_3$ removed by evaporation *in vacuo*. CrO_2 (5 g) was added and the solution refluxed for 20 min. Water (10 ml) was then added, and the mixture distilled, water being added to maintain a constant volume in the distillation flask. The distillate (60 ml) was titrated with 0·1 N NaOH, and evaporated to dryness affording sodium acetate (53·3 mg). A portion was converted to 1-acetamidonaphthalene[30] for radioactive assay. A Schmidt reaction was carried out on the remainder with sodium azide and conc. H_2SO_4 as previously described.[25]

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

[29] R. LUKES and J. JIZBA, *Chem. Listy* **46**, 622 (1952).
[30] E. LEETE, H. GREGORY and E. G. GROS, *J. Am. Chem. Soc.* **87**, 3475 (1965).