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BIOSYNTHETIC CONVERSION OF α-METHYLBUTYRIC ACID TO TIGLIC ACID IN *DATURA METELOIDES*

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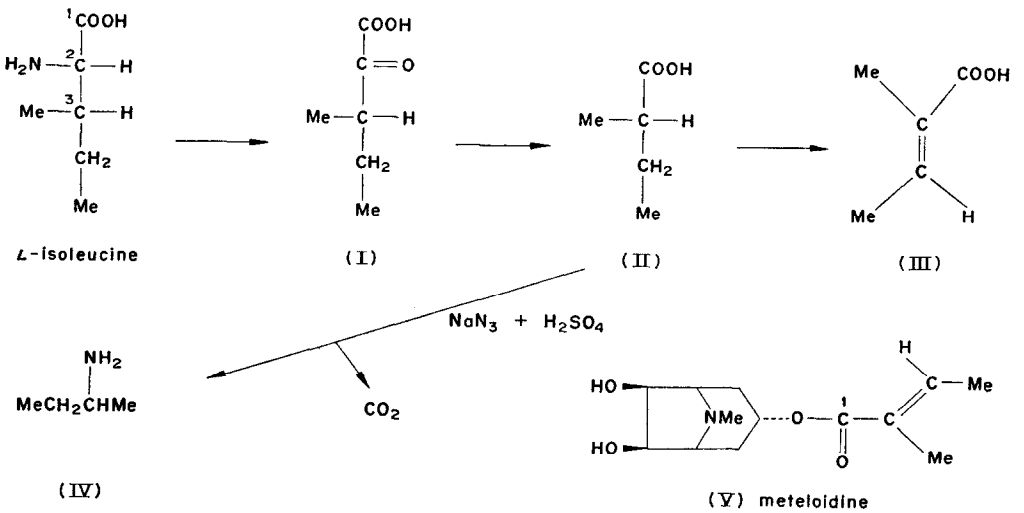
Key Word Index—*Datura meteleoides*; Solanaceae; meteloidine; tiglic acid; α-methylbutyric acid as a precursor.

Abstract—The administration of *RS*-α-methylbutyric-[1-^{14}C] acid to *Datura meteloides* plants resulted in the formation of radioactive meteloidine. A systematic degradation indicated that essentially all the activity was located at C-1 of the tiglic acid moiety of the alkaloid.

IT HAS been previously established that tiglic acid (3), the acid moiety of the ester alkaloid meteloidine (5) is derived from isoleucine in *Datura* species.[1-3] Isoleucine was also a precursor of angelic acid, the geometric isomer of tiglic acid, in *Cynoglossum officinale*.[4] Tiglic acid is also formed from isoleucine in animal tissues,[5] and it was suggested that α-keto-β-methylvaleric acid (I) and α-methylbutyric acid (II) were intermediates in this transformation, as illustrated in Scheme 1.



SCHEME 1.

* Contribution No. 124 from this Laboratory.

[1] EVANS, W. C. and WOOLLEY, J. G. (1965) *J. Pharm. Pharmacol.* **17**, Suppl., 37S.
[2] WOOLLEY, J. G. (1966) *Abhandl. Dtsch. Akad. Wiss. Berlin Kl. Chem. Geol. Biol.* **Nr. 3**, 531.
[3] LEETE, E. and MURRILL, J. B. (1967) *Tetrahedron Letters* 1233.
[4] CROUT, D. H. G. (1967) *J. Chem. Soc.* **C**, 1233.
[5] ROBINSON, W. G., BACHHAWAT, B. K. and COON, M. J. (1956) *J. Biol. Chem.* **218**, 391.

We have examined the capability of α-methylbutyric acid to serve as a precursor of tiglic acid by administering *RS*-α-methylbutyric-[1-^{14}C] acid to *D. meteloides*, feeding by the wick method. The plants were harvested after 2 weeks, and afforded radioactive meteloidine (0·14% incorporation). Hydrolysis of the alkaloid with barium hydroxide yielded teloidine (inactive), and tiglic acid, which was assayed as its α-naphthylamide. Hydrogenation of the radioactive tiglic acid yielded α-methylbutyric acid which was subjected to a Schmidt reaction affording carbon dioxide and 2-aminobutane (IV). The latter compound was collected and assayed as its *N*-benzoyl derivative. The results obtained (see Experimental) indicated that essentially all the activity of the meteloidine was located on the carboxyl carbon of tiglic acid.

α-Methylbutyric acid thus serves as a direct precursor of tiglic acid. Independently, Basey and Woolley[6] have found that α-methylbutyric acid is a precursor of tiglic acid in *D. innoxa*. In the present work racemic α-methylbutyric acid was administered to the plant. However, since this acid is being formed from L-isoleucine, having an *S*-configuration at C-3 (illustrated with a Fischer projection formula in Scheme 1), it is predicted that *S*-α-methylbutyric acid is the actual precursor of tiglic acid. This stereochemical detail is being investigated.

EXPERIMENTAL

General methods. Radioactivity measurements were carried out in a Nuclear Chicago Mark II liquid scintillation counter Radioactive compounds were dissolved in 1–2 ml EtOH H_2O, and diluted with 10 ml of a dioxane solution containing naphthalene (10%), 2,5-diphenyloxazole (0·7%), and 1,4-bis-2-(5-phenyloxazolyl)benzene (0·05%). Elementary analyses were carried out by Clark microanalytical laboratories, Urbana, Illinois.

RS-α-Methylbutyric-[1-^{14}C] *acid.* The Grignard reagent formed from 2-bromobutane (1·37 g, 10 mM) and Mg (0·12 g, 5 gatm.) in Et_2O (10 ml) was cooled to −80°. Carbon dioxide-[^{14}C] produced by adding concentrated H_2SO_4 to barium carbonate-[^{14}C] (nominal activity 2 mCi, 0·98 g, 5 mM) was admitted to the frozen reaction mixture which was allowed to warm slowly to room temp. with stirring. After 1 hr, dil. HCl was added and the mixture extracted with Et_2O. The Et_2O solution was extracted with aq. 10% Na_2CO_3 (3 × 30 ml), which was acidified with H_3PO_4 and extracted with Et_2O. Distillation (100°, 0·01 mm) of the residue obtained on evaporation of the dried (Na_2SO_4) extract yielded *RS*-α-methylbutyric-[1-^{14}C] acid as a colorless oil (0·17 g, 33%) having a specific activity of 9·5 × 10^8 dpm/mM.

Administration of RS-α-*methylbutyric-*[1-^{14}C] *acid to* Datura *meteloides and isolation of meteloidine.* α-Methylbutyric-[1-^{14}C] acid (16·4 mg, 1·54 × 10^8 dpm) was dissolved in dil. NH_3 (6 ml) and fed to three 4-month-old *D. meteloides* plants growing in soil in a greenhouse (November), by means of cotton wicks inserted into the stems of the plants near to ground level. After 14 days the plants were harvested and the alkaloids isolated as previously described.[7] Meteloidine (62 mg) was obtained having a specific activity of 9·1 × 10^5 dpm/mM (0·14% incorporation). Meteloidine hydrobromide (m.p. 255–256°) had an activity of 9·0 × 10^5 dpm/mM.

Degradation of meteloidine. The radioactive meteloidine hydrobromide and subsequent degradation products were diluted as necessary to facilitate manipulations. Activities reported are calculated for undiluted material. Meteloidine hydrobromide (140 mg) was refluxed in satd. $Ba(OH)_2$ (20 ml) for 3 hr. The cooled solution was acidified with H_2SO_4 and extracted with Et_2O continuously overnight. The solid residue obtained on evaporation of the Et_2O extract was crystallized from H_2O yielding tiglic acid (22 mg).

Tiglic acid (10 mg), α-naphthylamine (14·3 mg) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (100 mg) were stirred in H_2O (2 ml). After 1 hr the mixture was extracted with Et_2O which was then washed with 2 N HCl, 10% NaOH and H_2O. The dried ($MgSO_4$) Et_2O extract was evaporated and the residue sublimed (100°, 0·01 mm) affording a white sublimate which on crystallization from C_6H_6–hexane yielded colorless plates of tigloyl-α-naphylamide (8·2 mg, 8·9 × 10^5 dpm/mM) m.p. 120–121°. (Found: C, 80·17; H, 6·88; N, 6·34. $C_{15}H_{15}ON$ requires: C, 79·97; H, 6·71; N, 6·22%).

The aqueous solution from which the tiglic acid had been extracted was made basic with Na_2CO_3, evaporated to dryness, and the residue heated (200°, 0·01 mm) when teloidine (32 mg, < 0·02 × 10^5 dpm/mM) was obtained as a white sublimate.

[6] BASEY, K. and WOOLLEY, J. G. (1973) *Phytochemistry* **12,** in press.
[7] LEETE, E. (1972) *Phytochemistry* **11,** 1713.

Tiglic acid (40 mg) dissolved in EtOH (20 ml) was hydrogenated in the presence of Adams catalyst (20 mg) at 2 atms pressure for 12 hr. The filtered reaction mixture was neutralized with 0·1 N NaOH. The residue obtained on evaporation was crystallized from EtOH–Et_2O yielding sodium α-methylbutyrate (41 mg).

Sodium α-methylbutyrate (40 mg) was dissolved in conc. H_2SO_4 (1 ml), cooled to 0°, and NaN_3 (80 mg) added. The solution was heated to 50–60° for 1 hr, a stream of N_2 carrying the evolved CO_2 into a saturated solution of $Ba(OH)_2$ yielding $BaCO_3$ (54 mg, $9{\cdot}2 \times 10^5$ dpm/mM). The reaction mixture from the Schmidt reaction was made basic with 10% NaOH and distilled into dil. HCl. The residue obtained on evaporation of the distillate was shaken with N NaOH (10 ml) and benzoyl chloride (0·2 ml) for 3 hr. The solution was extracted with Et_2O, dried ($MgSO_4$), and evaporated to yield *N*-benzoyl-2-aminobutane (12 mg, $< 0{\cdot}05 \times 10^5$ dpm/mM) m.p. 82–83°.

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