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BIOSYNTHESIS OF THE TIGLOYL ESTERS OF *DATURA*: *CIS–TRANS* ISOMERISM*

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Key Word Index—*Datura innoxia*; Solanaceae; 3α-tigloyloxytropane; 3α,6β-ditigloyloxytropane; 3α,6β-ditigloyloxytropan-7β-ol; biosynthesis of tiglic acid; L-isoleucine, tiglic and angelic acids as precursors.

Abstract—*Datura innoxia* plants were wick fed with angelic acid-[1-^{14}C] and L-isoleucine-[U-^{14}C] to act as a positive control. After 7 days the root alkaloids 3α-tigloyloxytropane, 3α,6β-ditigloyloxytropane, and 3α,6β-ditigloyloxytropan-7β-ol were isolated and it was determined that angelic acid is not a precursor for the tigloyl moiety of these alkaloids. Tiglic acid-[1-^{14}C] which was fed via the roots to hydroponic cultures of *Datura innoxia*, was incorporated to a considerable degree after 8 days.

INTRODUCTION

THE TIGLOYL esters of *Datura*, namely 3α-tigloyloxytropane, 3α,6β-ditigloyloxytropane, meteloidine and 3α,6β-ditigloyloxytropan-7β-ol[1] are known to be derived from L(+)-isoleucine[2,3] via 2-methylbutanoic acid.[1,4] Tiglic (*cis*-2,3-dimethylacrylic) acid is apparently formed directly by dehydrogenation of 2-methylbutanoic acid, although nothing is known of the stereochemistry of this elimination. Clearly, from an examination of the stereochemistry of L(+)-isoleucine, which has the 2*S*, 3*S* configuration and (+)-2-methylbutanoic acid, which is 2*S*,[5,6] this latter enantiomer should be the immediate precursor of the tigloyl moiety. However, Crout[7] has shown that L(+)-isoleucine is also a precursor of angelic (*trans*-2,3-dimethylacrylic) acid in the ester alkaloid heliosupine in *Cynoglossum*. It is therefore possible that in *Datura* angelic acid is the first formed product from 2-methylbutanoate; tiglic acid being formed by a *cis–trans* isomerase system.

RESULTS AND DISCUSSION

The results (Table 1) clearly demonstrate that angelic acid is not a precursor of tiglic acid in *Datura* and that this plant does not possess a *cis–trans* isomerase system. From the Fischer projection formula it is almost certain that (+)-2*S*-2-methylbutanoic acid is the immediate precursor of tiglic acid. Assuming that this is so, then the subsequent dehydrogenation may proceed with removal of the C(2) proton and either the C(3) *pro S* or *pro R*

* Part IV in the series "The Biosynthesis of the Tigloyl esters in *Datura*". For part III see Ref. 1. 2117.

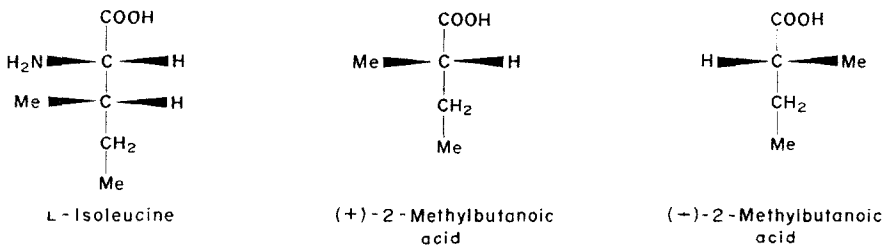
[1] BASEY, K. and WOOLLEY, J. G. (1973) *Phytochemistry* **12**, 2117.
[2] WOOLLEY, J. G. (1966) *Abhandl. Dtsch. Akad. Wiss. Berlin Kl. Chem. Geol. Biol.* Nr. 3, 531.
[3] LEETE, E. and MURRILL, J. B. (1967) *Tetrahedron Letters* 1233.
[4] LEETE, E. (1973) *Phytochemistry* **12**, 2203.
[5] GREENSTEIN, J. P. and WINITZ, M. (1961) *Chemistry of the Amino Acids*, Vol. III, p. 2043, Wiley, New York.
[6] Ref. 5, Vol. I, p. 183.

TABLE 1. COMPARATIVE FEEDING EXPERIMENTS IN *Datura*

Precursor	Isoleucine-[U-^{14}C]			Angelic acid-[1-^{14}C]		
Alkaloid	I	II	III	I	II	III
Wt. base (mg)	2·46	33·9	6·13	2·46	13·6	26
Sp.act. picrates diluted where necessary (dpm/mM × 10^{-4})	6·76	13·5	4·36	0	0	0
Calc. sp.act. original bases (dpm/mM × 10^{-4})	33·9	13·5	8·58	0	0	0
% Specific incorporation × 10^{3}*	1·8	0·7	0·44	0	0	0

* Calculated as [Sp.act. undiluted product (dpm/mM)]/[Sp.act. precursor (dpm/mM)] × 100.
I—3α-Tigloyloxytropane; II—3α,6β-ditigloyloxytropane; III—3α,6β-ditigloyloxytropan-7β-ol.

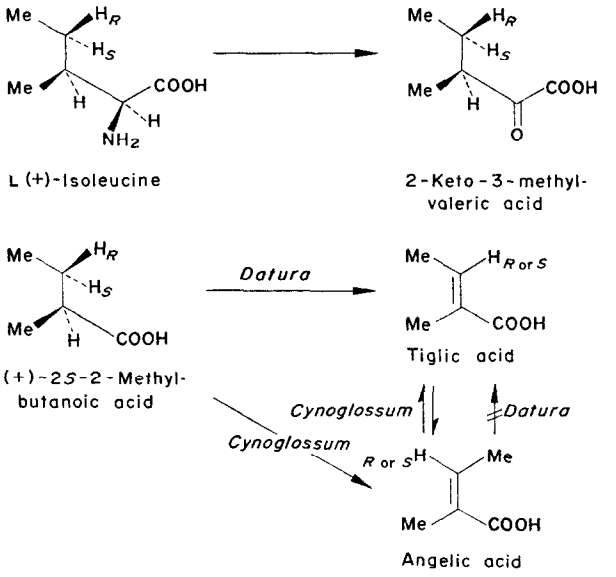
proton depending on whether the elimination mode is respectively *syn-* or *anti*-periplanar Scheme 2. L-(+)-isoleucine is also a precursor of angelic acid, the geometrical isomer of tiglic acid, in *Cynoglossum officinale* (Boraginaceae).[7] If it is assumed that this plant like *Datura* does not possess a *cis–trans* isomerase then the dehydrogenation of (+)-2*S*-2-methylbutanoic acid must proceed either with the removal of a different C(3) proton from that lost in the formation of tiglate (same elimination mode, *syn-* or *anti*-periplanar) or else the same C(3) proton is lost but a different elimination mode, *syn-* or *anti*-periplanar is used. The prochirality of the C(3) centre in 2- methylbutanoate will be the subject of a further communication.



SCHEME 1. FISCHER DIAGRAMS OF L-ISOLEUCINE AND THE ENANTIOMERS OF 2-METHYLBUTANOIC ACID.

The high specific incorporation of tiglic acid-[1-^{14}C] (cf. with the incorporation of 2-methylbutanoic acid-[1-^{14}C][1]) would suggest that the esterification takes place with the former acid, although it has not been shown whether *Datura* can dehydrogenate 2-methylbutanoyl esters. The long duration of this experiment (8 days) allows us to see how the label declines with time from 3α-tigloyloxytropane to the ditigloyl esters, perhaps indicating that the former is metabolized into the latter. This is not likely to be a reflection of the relative abundances of free tropine, tropan-3α,6β-diol and teloidine for esterification, since in *Datura* tropine is normally more abundant than the hydroxytropanes. Short term feeds with tiglic acid-[1-^{14}C] (unpublished results) reveal a decline in the specific activities in the order 3α-tigloyloxytropane > ditigloyl esters and meteloidine. The situation here is somewhat different from the isoleucine case. Since isoleucine is a precursor, as opposed to tiglic acid which is an end-product within the terms of the experiment one would expect the levels of activity to decrease from 3α-tigloyloxytropane to the ditigloyl esters, and they do (Table 1).

[7] CROUT, D. H. G. (1967) *J. Chem. Soc.* C, 1233.



SCHEME 2. THE FORMATION OF TIGLIC AND ANGELIC ACIDS FROM L(+)-ISOLEUCINE.

It would be of interest to discover whether the levels of activity in the C(3) and C(6) tigloyl moieties are the same.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY FROM TIGLIC ACID-[1-^{14}C] FEEDING EXPERIMENT IN *Datura*

Alkaloid	Wt base (mg)	% Specific incorporation*	Sp.act. diluted picrate dpm/mM × 10^{-5}	Sp.act. tiglic acid dpm/mM × 10^{-5} with % recovery in parenthesis*	Sp. act. alkamine
I	0·4	0·96	0·124	0·134 (108)	0
II	1·1	7·97	1·27	1·17 (92)	0
III	0·8	5·55	1·42	1·52 (107)	0

* Calculated as [Sp.act. undiluted product (dpm/mM)]/[Sp.act. precursor or starting material (dpm/mM)] × 100.

I—3α-Tigloyloxytropane; II—3α,6β-ditigloyloxytropane; III—3α,6β-ditigloyloxytropan-7β-ol.

EXPERIMENTAL

Counting procedures. Duplicate samples, bases as picrates, tiglic acid as the Na salt, were counted at finite thickness using a Labgear 4π gas-flow counter.[1]

Tracer compounds. Isoleucine-[U-^{14}C] and Na-[^{14}CN] were purchased from the Radiochemical Centre, Amersham.

Angelic acid-[1-^{14}C].[8,9] MeCOEt (734 mg) mixed with a solution of 500 mg Na-[^{14}CN] (500 μCi) in H_2O (2 ml) and cooled (−10°). Cooled solution of 98% H_2SO_4 (250 mg) in H_2O (*ca.* 2 ml) added dropwise with stirring followed by 1 ml N NaOH (final pH9). After 30 min vigorous stirring (at −10°) conc. HCl (2 ml)

[8] TSUYUKI, T., INAMOTO, N. and SIMAMURA, K. (1957) *Radio-Isotopes* (*Tokyo*) **6**, 93.

[9] BUCKLES, R. E. and MOCK, G. V. (1950) *J. Org. Chem.* **15**, 680.

was added and the cyanohydrin-[^{14}CN] was extracted into Et_2O (5 × 10 ml). Removal of the Et_2O afforded a colourless oil which was mixed with 98% H_2SO_4 (10 ml), cryst. $CuSO_4$ (100 mg) and heated to 125° with stirring for 1 hr. The cooled charred product was cautiously made alkaline with 50% NaOH solution (*ca.* 80 ml) and heated under reflux until the evolution of NH_3 ceased. The hydrolysate was acidified (50% H_2SO_4), steam distilled and the distillate (*ca.* 150 ml) extracted with Et_2O (5 × 20 ml). Evaporation of the solvent gave tiglic acid-[1-^{14}C], recryst. from light petrol. 254·7 mg (25% yield), sp. act. 7·3 × 10^7 dpm/mM, m.p. and m.m.p. 63° IR identical with authentic compound.

Tiglic acid-[1-^{14}C] (237·2 mg) plus 762·8 mg carrier reacted at room temp. with a solution of Br_2 (1·7 g) in CCl_4 (20 ml) for 24 hr, when the mixture was refluxed until a pale straw-yellow colour (*ca.* 1 hr).[10] The CCl_4 was then distilled off and 25% methanolic KOH (12 g) together with anhyd. K_2CO_3 (2 g) was added to the residue (the 2,3-dibromo derivative). The mixture was maintained at 55° for 2 hr, diluted with H_2O and agitated with Et_2O. The remaining aqueous solution was acidified (5 N HCl) and extracted with Et_2O distillation of which left a yellow–brown residue (3-bromoangelic acid). This was stirred with H_2O (30 ml), cooled in ice and 40 g sodium amalgam (9%) was slowly added. After 48 hr stirring, the aqueous phase was separated from the remaining Hg, acidified (5 N HCl) and extracted with Et_2O. Gentle evaporation of the Et_2O gave angelic acid-[1-^{14}C] recryst. from light petrol., 79·3 mg (8% yield) m.p. and m.m.p. 44° IR identical with authentic compound and with sp.act. 1·91 × 10^7 dpm/mM.

Tiglic acid-[1-^{14}C]. Synthesized by the method outlined above in 26% yield sp.act. 5·95 × 10^7 dpm/mM.

Feeding experiments. Angelic acid-[1-^{14}C] (52·3 mg), sp.act. 1·91 × 10^7 dpm/mM was neutralized with 0·1 M $NaHCO_3$, the soln diluted to 25 ml with H_2O and administered to five 3-month-old *Datura innoxia* plants through cotton wicks inserted into the stems close to the soil. As a positive control, three similar plants were fed with isoleucine-[U-^{14}C] (15 μCi, sp.act. 8·7 mCi/mM).

Ten *Datura innoxia* plants were grown in hydroponic culture for three months[1] then fed via the roots with a solution of sodium tiglate-[1-^{14}C] prepared by neutralizing 28·7 mg tiglic acid-[1-^{14}C] sp.act. 5·95 × 10^7 dpm/mM with 0·1 N NaOH and diluting to 25 ml with H_2O.

Isolation of alkaloids. The finely powdered roots (wts: tiglic acid-[1-^{4}C] feed, 169 g; isoleucine-[U-^{14}C], 242 g; angelic acid-[1-^{14}C], 487 g), were extracted and the bases resolved on partition columns at pH 6·8 and 5·6 as described previously.[1,11] The bases were converted to the picrates for counting and hydrolysed by boiling with 5% $Ba(OH)_2$.

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[10] BARGER, G., MARTIN, W. and MITCHELL, W. (1937) *J. Chem. Soc.* 1821.
[11] EVANS, W. C. and PARTRIDGE, M. W. (1952) *J. Pharm. Pharmacol.* **4**, 769.