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NON-REVERSIBILITY OF THE ISOLEUCINE–ACETATE PATHWAY IN *DATURA**

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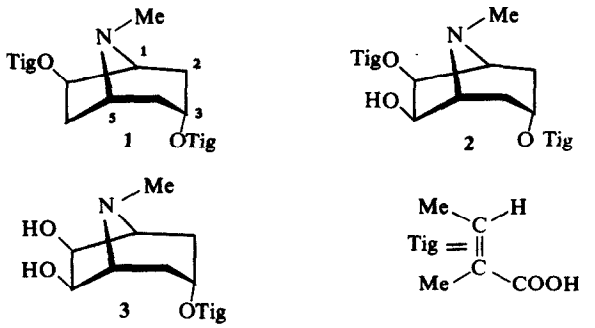
Key Word Index—*Datura meteloides*; Solanaceae; meteloidine; 3α, 6β-ditigloyloxytropane; 3α,6β-ditigloyloxytropan-7β-ol; biosynthesis of tiglic acid; isoleucine and 3-hydroxy-2-methylbutanoic acid as precursors.

Abstract—Five-month-old *Datura meteloides* plants were fed via the roots with 3-hydroxy-2-methylbutanoic acid-[1-^{14}C] and isoleucine-[U-^{14}C] as a positive control. After 5 days the plants were collected and in each case the root alkaloids 3α,6β-ditigloyloxytropane, 3α,6β-ditigloyloxytropan-7β-ol, meteloidine, hyoscine and hyoscyamine were isolated. Whereas isoleucine served as a precursor for the tiglic acid moieties 3-hydroxy-2-methylbutanoic acid did not.

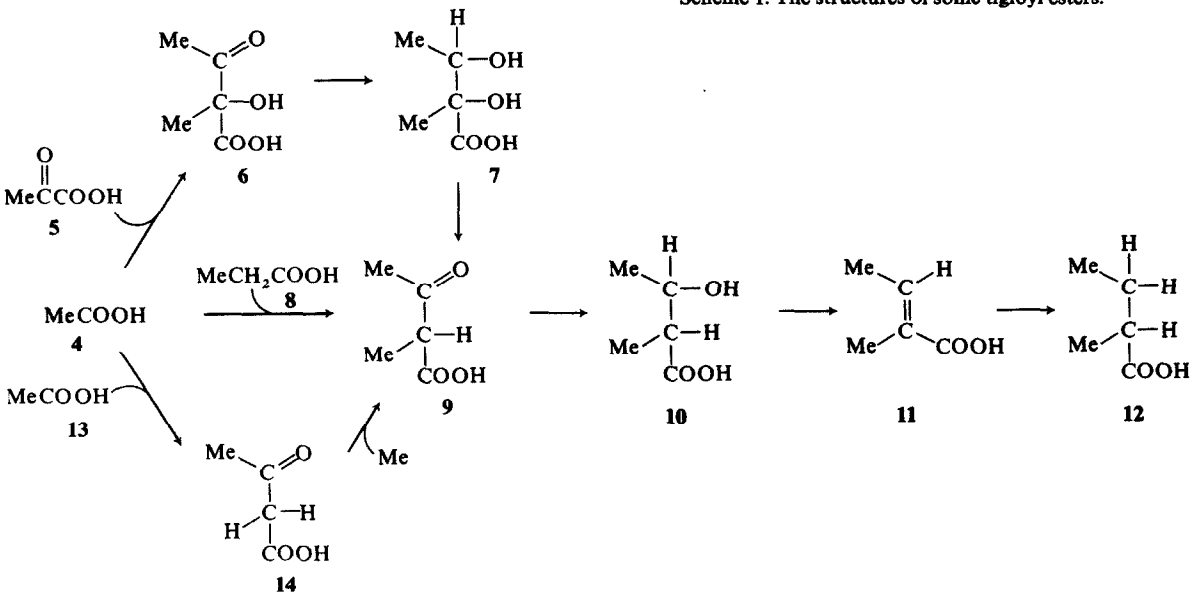
INTRODUCTION

Tiglic acid (**11**), the esterifying acid in the alkaloids 3α-tigloyloxytropane, 3α,6β-ditigloyloxytropane (**1**), 3α, 6β-ditigloyloxytropan-7β-ol (**2**) and meteloidine (**3**) is known to be derived in nature from L-isoleucine [1, 2] via 3-keto-2-methylvaleric [3–5] and 2-methylbutanoic (**12**) acids [6, 7]. However, the parasitic intestinal helminth *Ascaris lumbricoides* which contains large quantities of 2-methylbutanoic (**12**) and tiglic acids (**11**) [8] is able, under anaerobic conditions, to condense together acetate (**4**) or an active acetaldehyde and propionate (**8**) to give tiglic acid (**11**) by the exact reversal of the steps taken in the degradation of isoleucine [9, 10] (Scheme 2). In order to test the reversibility of this pathway in *Datura* 3-hydroxy-2-methylbutanoic acid-[1-^{14}C] (**10**) was synthesized and infiltrated into the roots of mature plants.



Scheme 1. The structures of some tigloyl esters.

*Part 8 in the series 'The biosynthesis of the tigloyl esters of *Datura*'. For part 7 see Beresford, P. J. and Woolley, J. G. (1975) *Phytochemistry* **14**, 2209.



Scheme 2. Three possible routes for the formation of tiglic acid from acetate.

RESULTS AND DISCUSSION

In *Ascaris lumbricoides* isolated muscle strip acetate (**4**) or 'active' acetaldehyde can condense with either propionate (**8**) to give 3-keto-2-methylbutanoic acid (**9**) [10] or pyruvate (**5**) to give 2-acetolactate (**6**) [9]. At the time it was considered that the latter acid, by a series of reductions and dehydrations, could produce tiglic acid, but the subsequent work of Saz and Weil [10] renders this suggestion very unlikely although 2-acetolactic acid is involved in the biosynthesis of the related amino acid valine. Whereas the whole organisms contain appreciable quantities of tiglic acid, the isolated muscle strips contain only the related 2-methylbutanoic acid (**12**) and Saz, Vidrine and Hubbard [9] were able to show that 3-hydroxy-2-methylbutanoic acid (**10**), formed by an enzyme specific for 3-keto-2-methylbutanoic acid (**9**) but not for acetoacetate (**14**), was used to produce 2-methylbutanoic acid (**12**), presumably via tiglic acid (**11**) although this acid was not detected.

There is another way in which acetate can be used to form tiglic acid. Leete [11] originally suggested that methylation of acetoacetate (**14**) by methionine could yield 3-keto-2-methylbutanoic acid (**9**). In the past we have fed *Datura* with acetate and propionate [1] in order to test the reversibility of the route and have failed to label the tiglic acid (**11**). However, as was pointed out by Leete [2], not only did the acetate not incorporate into the the tiglic acid, but it did not incorporate into the tropane moiety in which C(2) (3) and (4) are well known to be of acetate origin [12]. The 3 possible acetate based routes, viz. via acetoacetate (**14**), acetolactate (**6**) or 3-keto-2-methylbutanoate (**9**) have one point in common; they all give 3-hydroxy-2-methylbutanoic acid (**10**) as the immediate precursor of tiglic acid. From Table 1 it can be seen that this acid does not incorporate into tiglic acid whereas isoleucine, a more distant precursor, does.

3-Hydroxy-2-methylbutanoic acid-[1-^{14}C] was produced by the oxymercuriation of tiglic acid, a process known to proceed by *trans* addition across the double bond [15]. However, the demercuriation step with sodium borohydride is not stereospecific, in contrast to reduction with hydrogen sulphide which produces the diastereoisomer with the *threo* (Fischer nomenclature) configuration. The product contains equal proportions of *threo* and *erythro* diastereoisomers, each of which may be resolved into mirror-image forms. From the results obtained it would appear that none of the 4 isomers can serve as a precursor of tiglic acid.

Table 1. The specific activities of alkaloids and their degradation products from *Datura* plants fed with L-isoleucine-[U-^{14}C] and 3-hydroxy-2-methylbutanoic acid -[1-^{14}C]

Precursor	Alkaloid	Sp. act picrate dpm/mmol $\times 10^{-5}$	Sp. incorp. (%)*	Sp. act. re-esterified alkamine picrate dpm/mmol $\times 10^{-5}$	Sp. act. tiglic acid
3-Hydroxy-2-methylbutanoic acid-[1-^{14}C] 0.6×10^8 dpm/mmol	I	1.32	0.22	—	—
	II	0.86	0.14	—	—
	III	1.62	0.27	†	
	IV	1.95	0.32	2.16	
	V	1.29	0.21	1.17	0
L-Isoleucine-[U-^{14}C]. 1.93×10^{10} dpm/mmol	I	1.0	0.0005		
	II	1.13	0.0006		
	III	18.17	0.01		
	IV	55.26	0.03		
	V	2.13	0.001		

I—Hyoscine; II—hyoscyamine; III—3α,6β-ditigloyloxytropane IV—3α,6β-ditigloyloxytropan-7β-ol; V—meteloidine.
* Calculated as sp. act. product × 100/sp. act. precursor.
† Too little to degrade.

It is interesting to note that a good deal of radioactivity from 3-hydroxy-2-methylbutanoic acid is found in the tropane moiety of the tigloyl and the tropoyl esters. Degradation of 3-hydroxy-2-methylbutanoic acid-[1-^{14}C] along the established pathway of isoleucine metabolism, (**12**) → (**9**) → (**4**) + (**8**), gives propionate labelled at C(1) and it is difficult to understand how this label can be taken into the tropane moiety. However Saz and Weil noted that in *Ascaris* propionate-[1-^{14}C] feeds not only gave rise to 2-methylbutanoate labelled at C(1) but also to carboxyl labelled acetate (*ca* 5% of the label in 2-methylbutanoate). It is possible that acetate labelled in a similar manner, perhaps formed by carboxylation of propionate followed by cleavage of the resultant succinate between C(2) and (3), finds its way into C(3) of the tropane ring [13].

EXPERIMENTAL

Datura meteloides plants were grown on open land in Leicester from seed obtained from Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, D.D.R. The plants had all the characters previously described [14].

Counting procedures. Duplicate samples were counted in commercially available toluene or dioxane based POP/POPOP scintillators in a liquid scintillation spectrometer.

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

Synthesis of 3-RS-hydroxy-2-RS-methylbutanoic acid-[1-^{14}C]. Tiglic acid-[1-^{14}C] sp. act. 1.08×10^8 dpmm mol, mp 63°, IR identical with authentic material, was prepared in 29% yield by the method of ref. [6]. Tiglic acid-[1-^{14}C] (170 mg) plus carrier (139 mg) was added in several portions to a stirred soln of mercuric acetate (957 mg) in H_2O (4.5 ml) [15]. Stirring was continued at room temp. for 3 days when the white precipitated mercury-acetoxy addition product was collected. A further quantity was obtained by stirring for another 3 days. The mercury-acetoxy derivative was dissolved in a soln of $NaBH_4$ (45 mg) in 2N NaOH (1.5 ml) and stirred at room temp. After demercuriation was complete, the soln was acidified (dil. HCl), decanted, satd with NaCl and extracted with Et_2O (4 × 20 ml). The extract was washed with a satd soln NaCl, dried ($MgSO_4$), and evapd to give 172 mg (69%) 3-hydroxy-2-methylbutanoic acid-[1-^{14}C], $n_D^{25.5} = 1.4262$ (lit. [15] $n_D^{19} = 1.4375$), IR identical with authentic compound, sp. act. 0.6×10^8 dpm(m/mol.

Feeding Experiments. 3-Hydroxy-2-methylbutanoic acid-[1-^{14}C] (170 mg) sp. act. 0.6×10^8 dpm/mmol was neutralized (dil. NaOH) made up to 100 ml with H_2O and distributed to 5 × 5-month-old *D. meteloides* plants which had been carefully uprooted and suspended in blackened beakers containing Phostrogen soln. 4 similar plants were fed with isoleucine[U-^{14}C] (10μCi/plant). After 5 days the plants were harvested and the roots and aerial parts separately dried at 60° for 18 hr.

Isolation of alkaloids. The finely powdered roots (tiglic acid-[1-^{14}C] feed, 20 g; isoleucine-[U-^{14}C] feed, 10 g) were extracted and the bases resolved on Pi partition columns at pH 6.8 and 5.6 as described previously [6, 16]. The bases were converted to the picrates for counting and hydrolysed by boiling with 5% $Ba(OH)_2$ soln. The tiglic acid was recrystallised from petrol and sublimed. The alkamines were re-esterified with tigloyl chloride [17] and the sp. act. of the picrates determined.

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