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BIOSYNTHESIS OF 3α,6β-DITIGLOYLOXYTROPANE AND 3α,6β-DITIGLOYLOXYTROPAN-7β-OL IN *DATURA**

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Abstract—*Datura meteloides* plants were fed with tiglic acid-[1-^{14}C] via the roots and after 2 days the percentage incorporation into the alkaloids 3α-tigloyloxytropane, 3α,6β-ditigloyloxytropane, meteloidine and 3α,6β-ditigloyloxytropan-7β-ol were 15·2, 1·82, 2·2 and 1·8 respectively. 3α,6β-Ditigloyloxytropane was partially hydrolysed to 6β-hydroxy-3α-tigloyloxytropane which contained 58·1% of the radioactivity of the original base, whereas 3α,6β-ditigloyloxytropan-7β-ol gave meteloidine containing only 9·2% of the original activity. The results suggest that the di- and tri-hydroxytropanes may be formed by different routes.

INTRODUCTION

It has been shown that ornithine (**1**) is a precursor of tropane ring carbons 1, 5, 6 and 7 not only in tropine (**2**) but also in the alkamines tropan-3α,6β-diol (**3**) and teloidine (**6**) (tropan-3α,6β,7β-triol) [1–3]. The stage at which the C (6) and, when present, the C (7) β hydroxyl is introduced is uncertain but it is known that tropine is metabolized intact into the tigloyl esters of these alkamines. It is possible that tropine is itself hydroxylated but in the past we have suggested that the hydroxylation may occur at the tigloyl ester stage (i.e. with 3α-tigloyloxytropane (**7**) [4–6]). However, feeding experiments with doubly-labelled 3α-tigloyloxytropane revealed that this ester undergoes considerable hydrolysis in *Datura innoxia* and the results favoured the hydroxylation of tropine [7]. Because of the ease with which this hydrolysis takes place it was decided to approach the hydroxylation problem from a different angle and to determine whether the entering tigloyl groups equally label the 3α and 6β positions in the ditigloyl esters [6].

RESULTS AND DISCUSSION

Of the two plausible routes to the ditigloyl esters, hydroxylation of tropine (**2**) would give rise to 3α,6β-ditigloyloxytropan (**5**) and 3α,6β-ditigloyloxytropan-7β-ol (**10**) with an almost equal distribution of radioactivity between the 3α and the 6β positions during a 48 hr exposure to tiglic acid-[1-^{14}C]. On the other hand, hydroxylation of 3α-tigloyloxytropane (**7**) and subsequent tigloylation of the new hydroxyl would be more likely to label the new 6β position than the already-existing 3α group. From Table 1 it can be seen that both mechanisms occur when tiglic acid is fed to *D. meteloides*. 3α,6β-Ditigloyloxytropane (**5**) when partially hydrolysed [8, 9] gave 6β-hydroxy-3α-tigloyloxytropane (**8**) containing 58·1% of the original radioactivity. This distribution suggests that tropine (**2**) is hydroxylated and the resultant tropan-3α,6β-diol (**3**) is tigloylated at both positions simultaneously, or almost so. It is very unlikely that the sequence tropan-3α,6β-diol

* Part 6 in the series "The Biosynthesis of the Tigloyl Esters of *Datura*". For part 5 see Basey, K. and Woolley, J. G. (1975) *Phytochemistry* **14**, 2201.

Table 1. Distribution of radioactivity from tiglic acid-[1-^{14}C] feeding experiment in *Datura*

	I	*II*	*III*	*IV*
Sp. act. dpm/mmol × 10^{-5}	25·3	3·02	3·67	3·0
% sp. incorporation*	15·2	1·82	2·2	1·8
Sp. act. dilute picrate dpm/mmol × 10^{-5}		1·03		1·17
Sp. act. 6β-hydroxy-3α-tigloyl oxytropane dpm/mmol × 10^{-5}		0·599		
Sp. act. meteloidine dpm/mmol × 10^{-5}				0·108
% Radioactivity at 3α group		58·1		9·2
Calc. sp. act. of original 3α group dpm/mmol × 10^{-5}	25·3	1·75	3·67	0·28

I—3α-Tigloyloxytropane; II—3α,6β-ditigloyloxytropane; III—meteloidine; IV—3α,6β-ditigloyloxytropan-7β-ol.
* Calculated as [sp. act. base (dpm/mmol]/[sp. act. precursor (dpm/mmol)] × 100.

(**3**) → 6β-hydroxy-3α-tigloyloxytropane (**8**) → 3α,6β-ditigloyloxytropane (**5**) occurs (activity 3α tigloyl group > 6β group). Also, 6β-hydroxy-3α-tigloyloxytropane (**8**) from this source cannot be involved in the biosynthesis of meteloidine (**9**) because the specific activity of the 3α group would be too low. However, it is reasonable to expect that any 6β-hydroxy-3α-tigloyloxytropane (**8**) formed would be an intermediate and we therefore believe that it cannot be produced by the tigloylation of tropan-3α,6β-diol (**3**), but only by the hydroxylation of 3α-tigloyloxytropane (**7**). In feeding experiments with 3α,6β-ditigloyloxytropane (**5**), Evans [3] isolated the alternative intermediate 3α-hydroxy-6β-tigloyloxytropane (**4**) as a degradation product in *D. innoxia*. A small pool of this material when converted to 3α,6β-ditigloyloxytropane (**5**) would give a similar distribution to that observed and it must be considered as a possible though transient intermediate. Similarly, the results preclude the formation of 3α,6β-ditigloyloxytropan-7β-ol (**10**) from 3α,6β-ditigloyloxytropane (**5**), (radioactivity 3α group ≠ 3β group), and for the same reason the formation of teloidine (**6**) from tropan-3α,6β-diol (**3**).

3α,6β-Ditigloyloxytropan-7β-ol (**10**) when partially hydrolysed to meteloidine (**9**) was shown to have only 9·2% of the activity at the C(3) tigloyl group. The preponderance of label at the C(6) tigloyl group indicates that it must be formed from meteloidine which because of the previous arguments must itself be formed from 3α-tigloyloxytropane (**7**) probably via the 6β hydroxy derivative (**8**) (Fig. 1).

EXPERIMENTAL

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

Tiglic acid-[1-^{14}C]. Prepared as previously described [6], sp act 1·66 × 10^7 dpm/mmol.

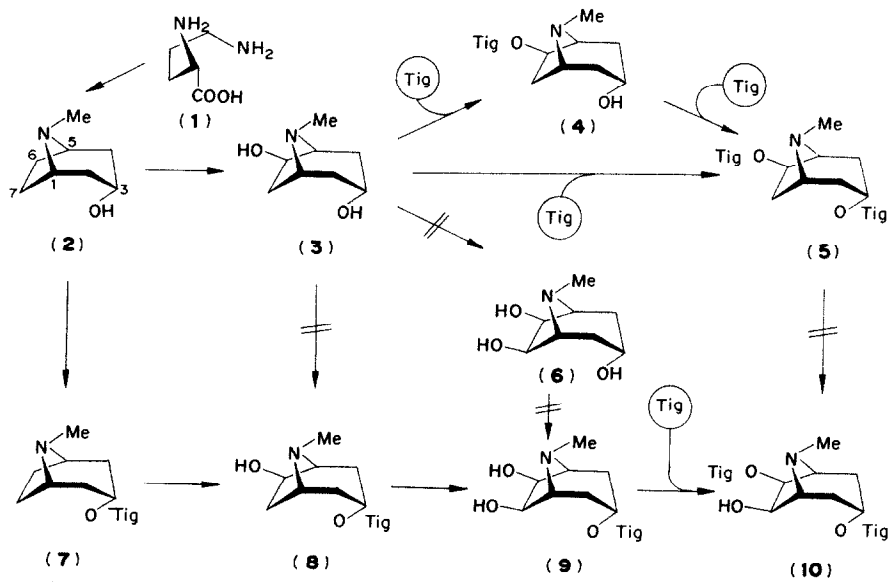

Fig. 1. Scheme for the biosynthesis of the tigloyl esters of *Datura* showing the points at which tiglic-[1-^{14}C] enters during a short-term feeding experiment.

Feeding experiments. Two 8-month-old field-grown *D. meteloides* plants were uprooted, carefully washed free from soil and allowed to stand in blackened beakers containing a soln of Na tiglate-[1-^{14}C] prepared by the neutralization of 70 mg tiglic acid-[1-^{14}C] with $NaHCO_3$ soln. After 48 hr the roots and aerial parts were separately dried at 60° for 18 hr.

Isolation of alkaloids [10]. The finely powdered roots (19·1 g) were mixed with 2 g $Ca(OH)_2$, 5 ml H_2O and after 30 min extracted with Et_2O (5 × 100 ml) over 2·5 hr. The Et_2O extract was evaporated to dryness, redissolved in petrol (5 ml) and transferred to a 10 g Kieselguhr column containing 5 ml 0·5 M Pi buffer pH 6·8. The evaporated petrol eluate (250 ml) was further chromatographed on 10 g Kieselguhr containing 5 ml Pi buffer pH 5·6, petrol eluting 3α,6β-ditigloyloxytropane (12·8 mg), picrate mp 151° (15 mg), const. sp act 2·14 × 10^5 dpm/mmol; Et_2O eluting 3α,6β-ditigloyloxytropan-7β-ol (16·8 mg), picrate mp 184° (19·8 mg) const. sp act 1·42 × 10^5 dpm/mmol. Elution of the original pH 6·8 column with Et_2O gave hyoscine (inactive) and then 3α-tigloyloxytropane (2·93 mg), diluted with 5·2 mg 3α-tigloyloxytropane picrate which gave 9·8 mg picrate mp 180°, const. sp act 1·35 × 10^6 dpm/mmol. The $CHCl_3$ eluate was collected in bulk, evaporated and chromatographed on alumina (activity II) (0·9 × 13 cm column), Et_2O–EtOH 17:3 (50 ml) eluted atropine (inactive) and the residual meteloidine was recovered from the column with $CHCl_3$ containing a few drops of NH_4OH; meteloidine picrate mp 180° (18·8 mg) const. sp act 3·67 × 10^5 dpm/mmol.

Partial hydrolysis of 3α,6β-*ditigloyloxytropane* [8, 9]. 3α,6β-Ditigloyloxytropane base was recovered from the diluted picrate (36·4 mg) sp act 1·03 × 10^5 dpm/mmol with NH_4OH – $CHCl_3$, dissolved in Me_3CO (0·75 ml) and 0·1 N NaOH (1·5 ml) and allowed to stand for 24 hr. After neutralization with dil HCl the Me_2CO was removed under red pres. The $CHCl_3$ extract (4 × 10 ml) of the basified aq residue was then chromatographed on 10 g Kieselguhr containing 5 ml 0·5 M Pi buffer pH 6·8. Elution with petrol gave residual 3α,6β-ditigloyloxytropane (6·7 mg); $CHCl_3$ gave 3·6 mg 6β-hydroxy-3α-tigloyloxytropane (yield 22·8%) which was converted to the picrate mp 154–5° sp act 5·99 × 10^4 dpm/mmol (58·1% of the radioactivity of 3α,6β-ditigloyloxytropane).

Partial hydrolysis of 3α,6β-*ditigloyloxytropan-7β-ol.* The dil picrate (47·5 mg) sp act 1·17 × 10^5 dpm/mmol was converted to the base, partially hydrolysed for 24 hr in Me_2CO (1 ml) and 0·1 N NaOH (2 ml) and (as described above) chromatographed at pH 6·8. Petrol eluted 3α,6β-ditigloyloxytropan-7β-ol (1·1 mg) and $CHCl_3$ meteloidine (16·7 mg) yield 78% which was converted to the picrate mp 176–7° sp act 1·08 × 10^4 dpm/mmol (9·2% radioactivity of the original base).

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