# BIOSYNTHESIS OF DITIGLOYL ESTERS OF DI- AND TRI-HYDROXYTROPANES IN *DATURA**

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**Abstract**—$3\alpha$-Tigloyloxytropane-[$^{14}$CO] [N-$^{14}$Me], ratio 1·6:1 and valtropine-[$^{14}$CO] [N-$^{14}$Me], ratio 1·75:1 were separately fed via cotton wicks to 4-month-old *Datura innoxia* plants. After 8 days the root alkaloids $3\alpha$-tigloyloxytropane, $3\alpha,6\beta$-ditigloyloxytropane and $3\alpha,6\beta$-ditigloyloxytropan-$7\beta$-ol were isolated and the distribution of radioactivity in the acid and alkamine moieties was determined by hydrolysis. The precursor ratios were not maintained in the isolated ditigloyl esters, a result which does not support our hypothesis that the ditigloyl esters are formed by the progressive hydroxylation of $3\alpha$-tigloyloxytropane.

## INTRODUCTION

The biosynthesis of the tigloyl moiety of the ester alkaloids $3\alpha$-tigloyloxytropane (3) $3\alpha,6\beta$-ditigloyloxytropane (5) meteloidine (6) and $3\alpha,6\beta$-ditigloyloxytropan-$7\beta$-ol (7) has been under investigation for some time [1–5]. The results of feeding experiments with tiglic acid precursors suggest that the di-hydroxy (4) and (5) and tri-hydroxytropane (teloidine) (6) and (7) esters could be formed by the progressive hydroxylation of $3\alpha$-tigloyloxytropane (3) followed by tigloylation of the introduced hydroxyls. The chief evidence for this theory is that the specific activity of the biosynthesized alkaloids usually followed the same pattern: $3\alpha$-tigloyloxytropane (3) > $3\alpha,6\beta$-ditigloyloxytropane (5) > meteloidine (6) > $3\alpha,6\beta$-ditigloyloxytropan-$7\beta$-ol (7). Moreover, Romeike has clearly demonstrated that hyoscine is formed from hyoscyamine (the tropic acid ester of tropine) via the $6\beta$-hydroxy derivative and therefore *Datura* must possess an enzyme system capable of hydroxylating tro-

pane esters [6–11]. To test our hypothesis, doubly labelled $3\alpha$-tigloyloxytropane-[$^{14}$CO] [N-$^{14}$Me] was synthesized [5,12,13] and fed by means of cotton wicks to *Datura innoxia* plants. If the alkamine:acid activity ratio of the $3\alpha$-tigloyloxytropane precursor could be shown to persist in the recovered products—$3\alpha,6\beta$-ditigloyloxytropane and $3\alpha,6\beta$-ditigloyloxytropan-$7\beta$-ol—then it would strongly favour our scheme. On the other hand, any significant deviation from the ratio could be taken to indicate that the hydroxylation of tropine was the more likely route. With hyoscyamine the hydroxylation at the $6\beta$ position only proceeds when an optically active acid [(−)-tropic acid] is present in the $3\alpha$ position and we considered that the same requirement might be necessary for the tigloyl ester series, the asymmetric acid enabling the hydroxylating enzyme to correctly identify the C (6) rather than the C (7) position. The dihydro derivative of $3\alpha$-tigloyloxytropane, valtropine (1) is known to occur naturally [14] and having (+)-2-methylbutanoic acid (8) at the $3\alpha$ position, it could be the required hydroxylation substrate. Although we know that 2-methylbutanoic acid (8) is a precursor of tiglic
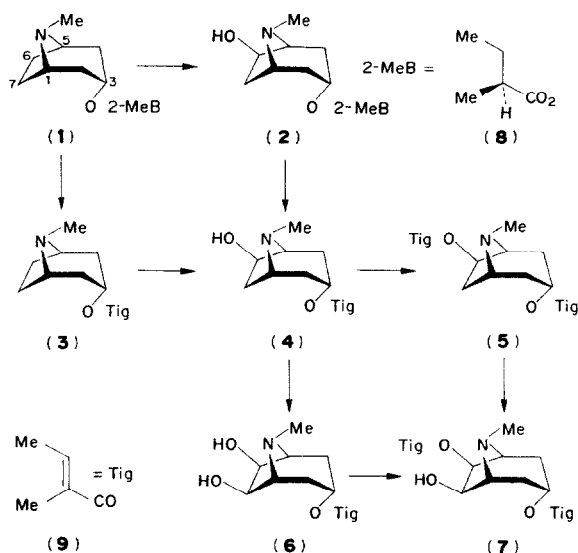
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Fig. 1. Postulated relationship between tigloyl esters in *Datura*.

acid (9) in *Datura* it is conceivable, but unlikely [3], that esterification precedes dehydrogenation. Accordingly, (±)-valtropine (3α-[2-methylbutanoyloxy] tropane-[14CO] [N-14Me]) was synthesized [3,12,13] and fed alongside the doubly labelled 3α-tigloyloxytropane to cover this possibility.

## RESULTS AND DISCUSSION

For the ester hydroxylation theory to be correct it is necessary for the acid:alkamine ratio of 3α-tigloyloxytropane-[14CO] [N-14Me] and val-

tropine-[14CO] [N-14Me] to fall to half (i.e. 0·8 and 0·87 respectively) in the ditigloyl esters since these are formed by the addition of a second non-labelled tigloyl group. From Table 1 it is clear that neither precursor is incorporated intact. 3α-Tigloyloxytropane undergoes considerable hydrolysis so that even the recovered base has a ratio of only 0·06:1 the label in the ditigloyl esters being predominantly due to the incorporation of tropine-[N-14Me]. The fact that there has been extensive hydrolysis of precursor is also borne out by the high levels of radioactivity recovered in the hyoscine and hyoscyamine. Since the valtropine-[14CO] [N-14Me] ratio is not carried over into the recovered 3α-tigloyloxytropane it would appear that *Datura* is unable to dehydrogenate this precursor and the latter base is probably formed by the direct esterification of tropine and tiglic acid. It is known that the ditigloyl esters are rapidly hydrolysed when infiltrated into *Datura* sp. and it is arguable that the introduction of 3α-tigloyloxytropane and valtropine promotes their own hydrolysis [15]. However, since the weight of tracer used was only 9·5 mg/plant and 250 g dry wt of root was obtained we believe that these esters, under undisturbed conditions, probably exchange their tigloyl groups with free tiglic acid, thereby controlling the proportion of one alkaloid to another. These results essentially duplicate earlier unpublished work with *D. meteloides* [16].

Table 1. Distribution of radioactivity from 3α-tigloyloxytropane-[-14CO] [N-14Me] and valtropine-[-14CO] [N-14Me] feeding experiments in *Datura*

| Alkaloid | I | II | III | IV | V |
|---|---|---|---|---|---|
| 3α-Tigloyloxytropane -[14CO] [N-14Me] (Ratio 1·6) Sp act (dpm/mmol × 10^{-6}) | | | | | |
| Alkaloid | 2·04 | 0·28 | 0·41 | 1·63 | 2·23 |
| Tiglic acid | 0·12 | 0·026 | 0·068 | | |
| Alkamine | 1·92 | 0·254 | 0·342 | | |
| Ratio observed | 0·06 | 0·1 | 0·2 | | |
| Valtropine-[-14CO] [N-14Me] (Ratio 1·75) Sp act (dpm/mmol × 10^{-6}) | | | | | |
| Alkaloid | 2·17 | 0·24 | 0·03 | 0·07 | 1·26 |
| Tiglic acid | 1·8 | 0·059 | 0·017 | | |
| Alkamine | 0·37 | 0·181 | 0·013 | | |
| Ratio observed | 4·9 | 0·32 | 1·3 | | |

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

## EXPERIMENTAL

*Counting procedures.* Duplicate samples, bases and alkamines as picrates, acids as Na salts, were counted at finite thickness in a gas-flow counter making appropriate corrections for self-absorption and geometry.

*Radioisotopes.* Ba-[$^{14}CO_3$], Na-[$^{14}CN$] and [$^{14}Me$]-$NH_2$-HCl were purchased from the Radiochemical Centre, Amersham.

*Tiglic acid-[1-$^{14}C$].* Synthesized by the method of Ref. 5, sp act 7·0 × $10^7$ dpm/mmol.

(±)-2-*Methylbutanoic acid-[1-$^{14}C$].* Synthesized by the method previously reported [3] sp act 8·35 × $10^7$ dpm/mmol.

*Synthesis of tropine-[N-$^{14}Me$].* Acetone dicarboxylic acid diethyl ester (1·2 g) was stirred with 20% NaOH (6 ml) until 1 phase formed, *ca* 10 min, adjusted to pH 2 with HCl and added to a soln of succindialdehyde prepared by the reaction of 0·5 ml conc HCl in $H_2O$ (5 ml) on 2,4-dimethoxytetrahydrofuran (0·8 g). The mixture was adjusted to pH 5 using a satd soln of $Na_2HPO_4$, 0·4 g [$^{14}Me$]-$NH_2$-HCl (100 μCi) in 5 ml $H_2O$ was added and the Robinson condensation was allowed to proceed in an evacuated vessel for 72 h. The mixture was then acidified with conc HCl, refluxed for 2 hr, cooled and extracted with $CHCl_3$ (3 × 50 ml). Tropinone-[N-$^{14}Me$] was extracted from the soln made alkaline with 20% NaOH using more $CHCl_3$. Evaporation of the $CHCl_3$ gave a brown, non-crystalline residue which was chromatographed on alumina (8 × 1 cm column) using petrol–$Et_2O$ 1:1 and evaporation of the eluate gave a pale yellow crystalline deposit of tropinone-[N-$^{14}Me$] (642 mg), 78% yield. Part was converted to the picrate mp and mmp 220° sp act 3·28 × $10^7$ and the remainder (500 mg) was dissolved in EtOH (20 ml) and hydrogenated at atm pres. over 0·5 g Raney nickel. The product was examined by TLC on alumina using $Et_2O$–EtOH 1:1, and tropine-[N-$^{14}Me$] was extracted from the filtered, basified (NaOH) soln with $CHCl_3$ and dried over NaOH.

*3α-Tigloyloxytropane-[N-$^{14}Me$].* Dry tropine-[N-$^{14}Me$] (300 mg) was refluxed for 4 hr with tigloyl chloride [17] (256 mg). The acidified (5 N $H_2SO_4$) product was washed with $Et_2O$ to remove the excess tiglic acid, then made alkaline (50% NaOH) and extracted with $CHCl_3$ (5 × 10 ml). The extracted 3α-tigloyloxytropane-[N-$^{14}Me$] was converted to the picrate (needles from EtOH) mp and mmp 179° const. sp act 3·28 × $10^7$ dpm/mmol, yield 372·5 mg (38%).

*Valtropine-[N-$^{14}Me$].* Dry tropine-[N-$^{14}Me$] (200 mg) and 2-methylbutanoyl chloride (160 mg) when treated as described above gave valtropine-[N-$^{14}Me$] picrate (plates from EtOH–$H_2O$) mp and mmp 222° (yield 386 mg − 60%) const. sp act 3·28 × $10^7$ dpm/mmol.

*3α-Tigloyloxytropane-[$^{14}CO$].* Tiglic acid-[1-$^{14}C$] sp act 7·0 × $10^7$ dpm/mmol (200 mg) was reacted at room temp. with $SOCl_2$ (238 mg) and $ZnCl_2$ (2 mg).[18] After the initial reaction, the mixture was kept at 30° for 15 min, when excess $SOCl_2$ was removed under vacuum. Dry tropine (282 mg) was then added and the esterification was completed by refluxing for 4 hr. The recovered 3α-tigloyloxytropane-[-$^{14}CO$] gave a picrate mp and mmp 180° sp act 7·0 × $10^7$ dpm/mmol, yield 523 mg (58%).

*Valtropine-[-$^{14}CO$].* 2-Methylbutanoic acid-[1-$^{14}C$] (204 mg) was converted to the acid chloride by means of $SOCl_2$ (238 mg) and $ZnCl_2$ (2 mg), and esterified with tropine (282 mg) as described above. Picrate yield 444 mg (48%) mp and mmp 222° sp act 8·35 × $10^7$ dpm/mmol.

*3α-Tigloyloxytropane-[N-$^{14}Me$] [-$^{14}CO$].* 3α-tigloyloxytropane-[-$^{14}CO$] picrate sp act 7·0 × $10^7$ (250 mg) was mixed with 3α-tigloyloxytropane-[N-$^{14}Me$] picrate sp act 3·28 × $10^7$ dpm/mmol (330 mg) in $H_2O$ (4 ml) and 10 ml N NaOH. Extraction with $CHCl_3$ gave the mixed base [-$^{14}CO$]/[N-$^{14}Me$] ratio 1·6/1 which was converted to the sulphate and made up to 100 ml with $H_2O$ for the feeding experiments.

*Valtropine-[N-$^{14}Me$] [-$^{14}CO$].* Valtropine-[-$^{14}CO$] picrate sp act 8·35 × $10^7$ dpm/mmol (242·2 mg) and valtropine-[N-$^{14}Me$] picrate sp act 3·28 × $10^7$ dpm/mmol (352·1 mg) were treated as described above to give the sulphate [-$^{14}CO$]/[N-$^{14}Me$] ratio 1·75/1.

*Feeding experiments.* Cotton threads were sown into the bases of the stems of 60 4-month-old *D. innoxia* plants. Half were treated with 3α-tigloyloxytropane-[N$^{14}Me$] [-$^{14}CO$] which was taken up via the cotton wicks from small vessels, and the remainder of the plants were used in the valtropine-[N-$^{14}Me$] [-$^{14}CO$] experiment. After 8 days the plants were harvested and the tops and roots were separately dried at 60° for 18 hr. The alkaloids were isolated and hydrolysed as reported previously [3,5].

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