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COMPETITIVE FEEDING EXPERIMENTS WITH TROPINE IN *DATURA**

PAMELA J. BERESFORD and JACK G. WOOLLEY

School of Pharmacy, Leicester Polytechnic, Leicester LE1 9BH, England

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Key Word Index—*Datura meteloides*; Solanaceae; biosynthesis; meteloidine; ditigloyl esters; tropine-[N-^{14}Me] precursor.

Abstract—*Datura meteloides* plants were fed with tropine-[N-^{14}Me] and the same compound together with the competitive inhibitors 3α-tigloyloxytropane: tropan-3α,6β-diol: 6β-hydroxy-3α-tigloyloxytropane: 3α,6β-ditigloyloxytropane: teloidine: meteloidine and 3α,6β-ditigloyloxytropan-7β-ol. The results obtained favour two distinct routes for the biosynthesis of the tigloyl esters of tropan-3α,6β-diol and teloidine (tropan-3α,6β,7β-triol); the first, *either* tropine → tropan-3α,6β-diol → 3α,6β-ditigloyloxytropane *or* more probably, tropine → 3α-tigloyloxytropane → 6β-hydroxy-3α-tigloyloxytropane → 3α,6β-ditigloyloxytropane; and second, tropine → 3α-tigloyloxytropane → "7β-hydroxy-3α-tigloyloxytropane" → meteloidine → 3α,6β-ditigloyloxytropan-7β-ol.

INTRODUCTION

There are two current theories concerning the biosynthesis of the hydroxytropines, tropan-3α,6β-diol and teloidine (tropan-3α,6β,7β-triol). In *Datura* these alkamines are esterified with tiglic acid and we have suggested previously that they may be formed by the progressive hydroxylation of 3α-tigloyloxytropane followed by the tigloylation of the new hydroxyls [1–3]. However, feeding experiments with this doubly-labelled alkaloid failed to support this theory, possibly because of the extensive reversible hydrolysis observed *in vivo*. [4]. In contrast, more recent feeding experiments with tiglic acid-[1-^{14}C] designed to discover the relative activities of the 3α and 6β tigloyl groups and therefore the order in which they were inserted strongly suggested that the original hypothesis viz. the hydroxylation of 3α-tigloyloxytropane (**6**) was correct for meteloidine (**8**) and 3α,6β-ditigloyloxytropan-7β-ol (**9**) but incorrect for 3α,6β-ditigloyloxytropane (**5**) (i.e. favouring the hydroxylation of tropine (**1**) [5]). Because of the problem of the hydrolysis of precursor esters which we have experienced [4,5,7], it was decided that the only other conceivable way of examining the validity of each route was to feed tropine-[N-^{14}Me], a known precursor which does not lose its label [8] alongside postulated intermediates in each of the biosynthetic schemes to act as competitive inhibitors.

RESULTS AND DISCUSSION

Tables 1 and 2, when read from left to right, show the specific activities of the bases 3α-tigloyloxytropane (**6**), 3α,6β-ditigloyloxytropane (**5**), meteloidine (**8**) and 3α,6β-ditigloyloxytropan-7β-ol (**9**) expressed as a percentage so that each feeding experiment has a "profile" which may be easily and directly compared with the profile of the control tropine-[N-^{14}Me] feeding experiment. From these results a scheme for the biosynthesis of these esters has been constructed (Fig. 1) and can be rationalized as follows. The feeding of tropine-[N-^{14}Me] with 3α,6β-ditigloyloxytropan-7β-ol (**9**), which is an end-product causes a build

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Table 1. Competitive feeding experiments with tropine-[N-^{14}Me] and various inhibitors in *Datura* (1973)

Compounds fed	Sp act profiles, all as dpm/mmol × 10^{-5} with distribution as a percentage in parenthesis I	II	III	IV
Tropine-[N-^{14}Me] (**1**)	19·8	0·19	5·28	1·43
	(74·2)	(0·71)	(19·77)	(5·3)
(**1**) plus	31·9	5·74	13·5	4·27
3α-Tigloyloxytropane	(57)	(10·36)	(24·36)	(7·71)
(**1**) plus	3·28	1·43	4·88	1·08
(+)-Tropan-3α,6β-diol	(30·74)	(13·4)	(45·74)	(10·12)
(**1**) plus	12·5	1·4	13·8	0·99
(+)-6β-Hydroxy-3α-tigloyloxytropane	(43·57)	(4·88)	(48·1)	(3·45)
(**1**) plus	2·21	2·48	9·57	3·14
(−)-3α,6β-Ditigloyloxytropane	(12·7)	(14·25)	(55·0)	(18·04)
(**1**) plus	6·04	1·34	7·46	1·12
Teloidine	(37·84)	(8·39)	(46·74)	(7·02)
(**1**) plus	46·9	4·76	9·57	2·90
Meteloidine	(73·13)	(7·46)	(14·92)	(4·52)
(**1**) plus	6·42	2·31	15·2	1·33
3α,6β-ditigloyloxytropan-7β-ol	(25·31)	(9·12)	(59·94)	(5·24)

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

up in the specific activity of meteloidine (**8**) and, presumably because there is a feed-back mechanism, depresses the esterification of tropine (**1**) with tiglic acid to produce 3α-tigloyloxytropane (**6**), but promotes the alternative biosynthetic route with the formation of 3α,6β-ditigloyloxytropane (**5**) possibly via tropan-3α,6β-diol (**2**). Teloidine (**10**) behaves in a similar fashion and there is the suggestion that its presence promotes the synthesis of 3α,6β-ditigloyloxytropan-7β-ol (**9**) from meteloidine (**8**) and for this reason we believe that the biosynthetic route branches at this point, teloidine (**10**) and 3α,6β-ditigloyloxytropan-7β-ol (**9**) both being considered as end-products and having a feed-back control on the formation of 3α-tigloyloxytropane (**6**) from tropine (**1**). The latter inhibitory effect is not exhibited by meteloidine (**8**) (presumably because it is not an end-product), nor does it cause any appreciable depression of the specific activity of 3α,6β-ditigloyloxytropan-7β-ol (**9**) probably because there is the alternative degradation route

Table 2. Competitive feeding experiments with tropine-[N-^{14}Me] and various inhibitors in *Datura* (1974)

Compounds fed	Sp act profiles, all as dpm/mmol × 10^{-5} with distribution as a percentage in parenthesis I	II	III	IV
Tropine-[N-^{14}Me] (**1**)	31·8	2·11	3·15	1·41
	(82·8)	(5·49)	(8·2)	(3·67)
(**1**) plus	45·7	1·28	4·16	1·99
3α-Tigloyloxytropane	(85·9)	(2·4)	(7·82)	(3·74)
(**1**) plus	8·65	0·715	0·668	0·789
(±)-Tropan-3α,6β-diol	(80)	(6·6)	(6·1)	(7·2)
(**1**) plus	6·17	1·53	11·7	1·32
(+)-6β-Hydroxy-3α-tigloyloxytropane	(29·8)	(7·39)	(56·5)	(6·3)
(**1**) plus	1·13	0·918	2·78	1·50
(−)-3α,6β-Ditigloyloxytropane	(17·85)	(14·5)	(43·9)	(23·7)
(**1**) plus	11·0	0·979	5·65	1·22
Teloidine	(58·3)	(5·18)	(29·9)	(6·46)
(**1**) plus	50·1	3·91	13·3	3·34
Meteloidine	(71·1)	(5·5)	(18·9)	(4·74)
(**1**) plus	9·1	3·22	4·99	1·47
3α,6β-Ditigloyloxytropan-7β-ol	(47·9)	(16·97)	(26·3)	(7·75)

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

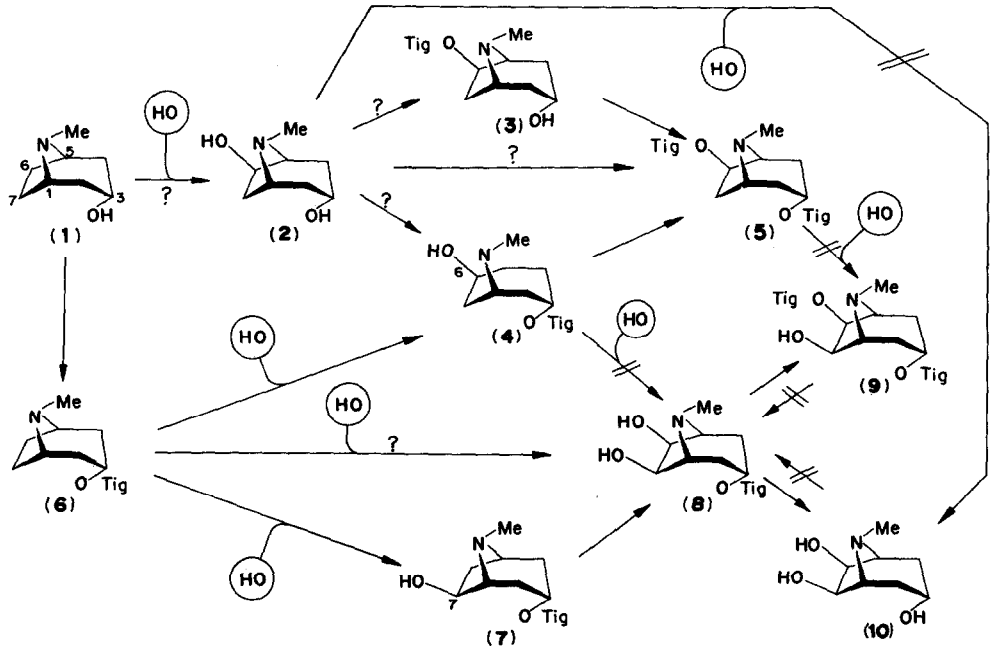

Fig. 1. Proposed scheme for the biosynthesis of the tigloyl esters in *Datura*.

available, the formation of teloidine (**10**). The feeding of these teloidine derivatives does not have a dramatic effect on the synthesis of dihydroxytropane derivatives as one might have anticipated. But 3α,6β-ditigloyloxytropan-7β-ol (**9**) which produces the maximal depression of the esterification of tropine (**1**) and tiglic acid, stimulates the formation of 3α,6β-ditigloyloxytropane (**5**) most of all, which agrees with the proposed biosynthetic scheme.

6β-Hydroxy-3α-tigloyloxytropane (**4**) which can be considered as an intermediate in the formation of both meteloidine (**8**) and 3α,6β-ditigloyloxytropane (**5**) markedly stimulates the formation of the former but not the latter, and we therefore feel that it is more likely to be involved in the biosynthesis of 3α,6β-ditigloyloxytropane (**5**) *cf* [5]. This raises an interesting problem with the formation of meteloidine (**8**) because it must be formed either by the introduction of the two β hydroxyls simultaneously (which we feel is unlikely) or possibly that it is formed from the "other" 6β-hydroxy-3α-tigloyloxytropane (**7**) (i.e. that based on (−)-tropan-3α,6β-diol which has the 3*S* 6*S* configuration [9] and in structure (**7**) in Fig. 1 is designated 7β-hydroxy-3α-tigloyloxytropane). This compound as far as we know has not been isolated from natural sources, but in any case it would chromatograph and isolate in the same way as its enantiomer, which is known, and would be inseparable from it. However, 6β-hydroxy-3α-tigloyloxytropane (**4**) does strongly suppress the production of 3α-tigloyloxytropane (**6**) and we must, therefore, consider that 3α-tigloyloxytropane (**6**) does give both 6β and '7β' hydroxy derivatives.

3α,6β-Ditigloyloxytropane (**5**) also strongly inhibits the formation of 3α-tigloyloxytropane (**6**), even more strongly than 6β-hydroxy-3α-tigloyloxytropane (**4**) and we consider that once again the feed-back control is exerted by the end-product rather than the intermediate. This result in itself would suggest that 3α,6β-ditigloyloxytropane (**5**) is formed from 3α-tigloyloxytropane (**6**) via 6β-hydroxy-3α-tigloyloxytropane (**4**) but some doubt has previously been cast on this idea [5]. On the other hand, tropan-3α,6β-diol (**2**) does not appear to inhibit the production of 3α,6β-ditigloyloxytropane (**5**) as expected, and so on balance the formation of 3α,6β-ditigloyloxytropane (**5**) from 3α-tigloyloxytropane (**6**) is still a more than tenable theory. Inhibition of this route by 3α,6β-ditigloy-

loxytropane (**5**) clearly allows more 3α-tigloyloxytropane (**6**) to be introduced into the biosynthesis of meteloidine (**8**) and 3α,6β-ditigloyloxytropan-7β-ol (**9**).

In one experiment only, tropan-3α,6β-diol (**2**) inhibited the formation of 3α-tigloyloxytropane (**6**) and we believe that this is because the inhibiting alkamine was different in each experiment. In Table 1 (+)-tropan-3α,6β-diol (**2**), obtained by hydrolysis of the natural base (−)-3α,6β-ditigloyloxytropane (**5**), was used, but in Table 2 (±) alkamine produced by the reduction of (±)-6β-hydroxytropan-3-one was fed. Therefore the results may be easily rationalized. In experiment 1 (+)-tropan-3α,6β-diol quite correctly promotes the synthesis of meteloidine (**8**) and 3α,6β-ditigloyloxytropan-7β-ol (**9**) since we believe that the laevo alkamine (with the 3*S* 6*S* configuration [9]) i.e. that forming 7β-hydroxy-3α-tigloyloxytropane (**7**) is used in this route. In Table 2 the (−)-tropan-3α,6β-diol content of the (±) alkamine would be expected to depress the formation of meteloidine (**8**) as the results demonstrate. Tropan-3α,6β-diol (**2**) appears to have little effect on the biosynthesis of 3α,6β-ditigloyloxytropane (**5**) and we therefore have some very serious doubts about its role as an intermediate.

EXPERIMENTAL

D. meteloides seeds were obtained from Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, D.D.R. The plants were grown on open land in Leicester and had all the characters previously described [10].

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

Inhibitor alkaloids. These were obtained from *D. meteloides* or *D. innoxia* by the partition column technique previously described [11] and were authenticated by picrate mp and mmp and IR spectra. (+)-Tropan-3α,6β-diol was prepared by hydrolysis of (−)-3α,6β-ditigloyloxytropane in boiling $Ba(OH)_2$ [2] and the (±) alkamine by the reduction of (±)-6β-hydroxytropan-3-one with H_2 in the presence of Raney nickel [12]. 6β-Hydroxy-3α-tigloyloxytropane was prepared by the partial hydrolysis of (−)-3α,6β-ditigloyloxytropane [13] in dil alkali. Teloidine was produced by the hydrolysis of 3α,6β-ditigloyloxytropan-7β-ol in boiling $Ba(OH)_2$. 3α-Tigloyloxytropane was synthesised from tropine and tigloyl chloride [14].

Tropine-[N-^{14}Me]. In the first experiment tropine-[N-^{14}Me] sp act $3{\cdot}28 \times 10^7$ dpm/mmol, previously prepared was used [4] and in the second, similarly prepared tropine-[N-^{14}Me] (yield 57%), sp act $1{\cdot}86 \times 10^8$ dpm/mmol was infiltrated into the plants.

Feeding expts. Six-month-old *D. meteloides* plants were carefully uprooted, washed free from soil and allowed to stand in blackened beakers containing tropine-[N-^{14}Me] and 2·5 mg of each inhibitor per plant. In expt 1 each plant received 0·62 mg labelled tropine and 4·9 mg each in expt 2. Expt 1 was terminated after 6 days, expt 2 after 4 days, when the aerial parts and roots were separately dried at 60° for 18 hr. The alkaloids were isolated from the roots of each inhibition test group of plants as described previously [11], characterised as the picrates (mp and mmp) and recrystallised to constant sp act.

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REFERENCES

1. Woolley, J. G. (1966) *Abhand. Deut. Akad. Wiss. Berlin Kl. Chem. Geol. Biol* (3) 531.
2. Basey, K. and Woolley, J. G. (1973) *Phytochemistry* **12**, 2197.
3. Basey, K. and Woolley, J. G. (1973) *Phytochemistry* **12**, 2883.
4. Basey, K. and Woolley, J. G. *Phytochemistry* **14**, Ms 691.
5. Beresford, P. J. and Woolley, J. G. (1975) *Phytochemistry* **14**, 2205.
6. Evans, W. C. and Griffin, W. J. (1964) *Phytochemistry* **3**, 503.
7. Woolley, J. G. (1966) Ph.D. Thesis, University of Nottingham.
8. Leete, E. (1972) *Phytochemistry* **11**, 1713.
9. Fodor, G. and Soti, F. (1965) *J. Chem. Soc.* 6830.
10. Avery, A. G., Satina, S. and Rietsema, J. (1959) in *Blakeslee: The Genus Datura* p. 16, Ronald Press, New York.
11. Evans, W. C. and Woolley, J. G. (1965) *J. Chem. Soc.* 4936.
12. Beresford, P. J. and Woolley, J. G. (1974) *Phytochemistry* **13**, 2511.
13. Beresford, P. J. and Woolley, J. G. (1974) *Phytochemistry* **13**, 1249.
14. Barger, G., Mitchell, W. and Martin, W. F. (1937) *J. Chem. Soc.* 1820.