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BIOSYNTHESIS OF METELOIDINE FROM 3α-TIGLOYLOXYTROPANE IN *DATURA INNOXIA*

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Key Word Index—*Datura innoxia*; Solanaceae; 3α-tigloyloxytropane; meteloidine; alkaloid biosynthesis.

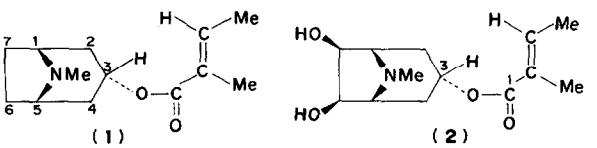
Abstract—The administration of 3α-tigloyl-[1-^{14}C]-oxytropane-[3β-^{3}H] (^{3}H/^{14}C = 11·0) to *Datura innoxia* plants for 7 days led to the formation of radioactive meteloidine (^{3}H/^{14}C = 11·6). Degradation of the meteloidine indicated that the alkaloid was labeled specifically with ^{3}H at C-3 of its teloidine moiety, and on the carbonyl group of its tigloyl residue with ^{14}C. These results strongly favor the hypothesis that hydroxylation of tropine occurs after formation of its tigloyl ester.

INTRODUCTION

We have previously shown [1] that the teloidine moiety of meteloidine (**2**) is derived from tropine, hydroxylation occurring at C-6 and C-7. The work of Evans and Woolley [2,3] suggested that this hydroxylation may occur after the formation of the tigloyl ester of tropine. We have now tested this hypothesis by feeding 3α-tigloyl-[1-^{14}C]-oxytropane-[3β-^{3}H] (**1**) to *Datura innoxia* plants.

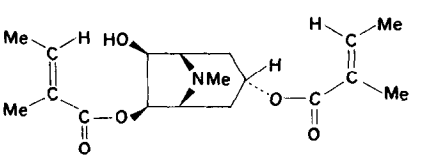
RESULTS AND DISCUSSION

Tiglic-[1-^{14}C] acid [4] and tropine-[3β-^{3}H] [1] were prepared as previously described. 3α-Tigloyloxytropane was obtained by heating tigloyl chloride with tropine hydrochloride at 100° [5,6]. 3α-Tigloyl-[1-^{14}C]-oxytropane hydrochloride and 3α-tigloyloxytropane-[3β-^{3}H] hydrochloride were assayed for radioactivity, mixed and fed to 4-month-old *Datura innoxia* plants by the wick method. The plants were harvested after 7 days. TLC on the crude alkaloids afforded radioactive meteloidine, 7β-hydroxy-3α,6β-ditigloyloxytropane (**3**), and 3α-tigloyloxytropane, activities of these alkaloids being recorded in the Table. The meteloidine was degraded as previously described [1] (see Experimental) and it was established that essentially all the ^{3}H was located at the 3β-position of the teloidine half of the molecule. The tiglic acid contained all the ^{14}C activity and this was located at the carbonyl group. Since the ^{3}H/^{14}C ratio in the isolated meteloidine was essentially the same as in the administered 3α-tigloyloxytropane it seems probable that hydroxylation at C-6 and C-7 occurs on the ester. The recovered 3α-tigloyloxytropane had low specific activity indicating that it undergoes rapid metabolism in the plant. It contained very little ^{14}C and this result can be rationalized by postulating a reversible hydrolysis of this ester to tiglic acid and tropine, the latter being then metabolized at a slower rate than tiglic acid.



(1)

(2)



(3)

The incorporation of activity into 7β-hydroxy-3α,6β-ditigloyloxytropane was lower than that into meteloidine, and the ^{3}H/^{14}C ratio was much

* Contribution No. 136 from this Laboratory.

Table 1. Activities of the 3α-Tigloyl-[1-^{14}C]-oxytropane-[3β-^{3}H] fed, the isolated alkaloids, and the degradation products of meteloidine

Alkaloids (wt)	Specific activity (dpm/mM) ^{3}H	^{14}C	^{3}H/^{14}C	Specific incorporation (^{3}H, %)
Alkaloid fed				
3α-Tigloyl-[1-^{14}C]-oxytropane-[3β-^{3}H]–HCl (20 mg)	$1{\cdot}34 \times 10^{9}$	$1{\cdot}22 \times 10^{8}$	11·0	
Alkaloids isolated				
Meteloidine (38 mg)	$4{\cdot}75 \times 10^{6}$	$4{\cdot}10 \times 10^{5}$	11·6	0·35
3α-Tigloyloxytropane (26 mg)	$2{\cdot}27 \times 10^{4}$	$<2{\cdot}0 \times 10^{2}$	>100	0·0017
7β-Hydroxy-3α,6β-ditigloyl-oxytropane (18 mg)	$1{\cdot}17 \times 10^{6}$	$3{\cdot}31 \times 10^{4}$	35	0·09
Degradation products of the meteloidine				
Isopropylideneteloidine	$4{\cdot}10 \times 10^{6}$	negligible		
Isopropylideneteloidinone	$<0{\cdot}1 \times 10^{6}$	–		
Tigloyl-α-naphthylamide	$6{\cdot}0 \times 10^{3}$	$3{\cdot}59 \times 10^{5}$		
Barium carbonate (from the COOH group of tiglic acid)	—	$3{\cdot}3 \times 10^{5}$		

higher than the administered 3α-tigloyloxytropane. This result suggests that one or both of the tigloyl residues of this alkaloid are labile, reversible hydrolysis and esterification with non-radioactive tiglic acid occurring during the feeding experiment.

EXPERIMENTAL

General methods. A Nuclear Chicago Mark II Liquid scintillation Counter was used for assay of the radioactive compounds using dioxane–EtOH with the usual scintillators [7].

3α-Tigloyloxytropane-[3β-^{3}H] hydrochloride. Tigloyl chloride (236 mg, 2 mM) and tropine-[3β-^{3}H] hydrochloride [1] (190 mg, $3{\cdot}0 \times 10^{9}$ dpm, 1·1 mM) were heated on a steam bath for 1·5 hr. Dil. K_2CO_3 (5%) was added to cooled reaction mixture which was then extracted with Et_2O. Residue obtained on evaporation of dried ($MgSO_4$) extract was treated with a soln of HCl in Et_2O. The resultant solid was crystallized to constant activity from EtOAc affording 3α-tigloyloxytropane-[3β-^{3}H] hydrochloride (50 mg), mp 215–216° (lit. [6] mp 214·5–217·5°), having an activity of $2{\cdot}68 \times 10^{9}$ dpm/mM.

3α-Tigloyl-[1-^{14}C]-oxytropane hydrochloride. Tiglic-[1-^{14}C]-acid [4] (460 mg, $1{\cdot}1 \times 10^{9}$ dpm, 4·6 mM) and $SOCl_2$ (6 ml) were stirred at room temperature for 30 min and then refluxed for 2 hr. Excess $SOCl_2$ was removed at red pres and the residual tigloyl chloride heated with tropine hydrochloride (950 mg, 5·53 mM) on a steam bath for 1·5 hr. The 3α-tigloyl-[1-^{14}C]-oxytropane hydrochloride was isolated as described in the previous section and had an activity of $2{\cdot}44 \times 10^{8}$ dpm/mM.

Administration of 3α-tigloyl-[1-^{14}C]-oxytropane-[3β-^{3}H] hydrochloride to D. innoxia *and isolation of the alkaloids.* The previously described ^{3}H and ^{14}C labeled 3α-tigloyloxytropane hydrochloride (10 mg of each) were dissolved in H_2O and fed to 8 4-month-old *D. innoxia* plants growing in soil in a greenhouse, by means of cotton wicks inserted into the stems of plants near to ground level. After 1 week the plants were harvested (fr. wt 745 g) and macerated in a Waring blendor with a mixture of $CHCl_3$ (1 liter), Et_2O (1 liter), and conc NH_4OH (250 ml). The organic layer was evaporated to 600 ml, and extracted with 2 N H_2SO_4 (6 × 200 ml). This acid extract was made basic with K_2CO_3 and extracted with $CHCl_3$. Dried ($MgSO_4$) extract on evaporation yielded crude alkaloids (215 mg, ^{3}H: $4{\cdot}63 \times 10^{6}$ dpm, ^{14}C: $2{\cdot}1 \times 10^{5}$ dpm). Alkaloids were separated by preparative TLC on Si gel PF-254 (Merck) as previously described [1].

Degradation of the meteloidine. The meteloidine isolated from the plant was converted to isopropylidineteloidine and tiglic acid (assayed as tigloyl-α-naphthylamide [8] as previously described [9]. The isopropylideneteloidine was oxidized with CrO_3 in C_5H_5N affording isopropylideneteloidinone. The tiglic acid was subjected to a Schmidt reaction affording CO_2 which was collected and assayed as $BaCO_3$. The activities of these degradation products are recorded in the Table.

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