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METABOLISM OF TIGLOYL ESTERS IN THE AERIAL PARTS OF *DATURA*

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Abstract—Mature *Datura innoxia* plants incorporate $^{14}CO_2$ more rapidly into the tigloyl groups of the ditigloyl ester alkaloids than into the hydroxytropane moiety of the molecule. 3,6-Ditigloyloxytropane fed to the leaves of *D. innoxia* and *D. cornigera* undergoes a stepwise hydrolysis by which 3-hydroxy-6-tigloyloxytropane is formed in both species; in addition, 3,6-dihydroxytropane is produced in *D. innoxia* and 6-hydroxy-3-tigloyloxytropane in *D. cornigera*. 7-Hydroxy-3,6-ditigloyloxytropane undergoes a similar hydrolysis in the aerial organs of *Datura* and gives rise to meteloidine in *D. innoxia* and *D. ferox*; another monoester and teloidine have also been shown to be derived from it in *D. innoxia*. 3,6,7-Tritigloyloxytropane appears to undergo a similar degradation in the leaves of *D. innoxia*, 7-hydroxy-3,6-ditigloyloxytropane being an intermediate in its breakdown. This, in conjunction with other evidence[1] suggests that meteloidine and 3-hydroxy-6-tigloyloxytropane are formed by this method in the leaves of normal plants.

INTRODUCTION

THE mono- and ditigloyl esters of the hydroxytropanes are common minor components of the alkaloidal mixture obtained from a number of species of *Datura*. 3, 6-Ditigloyloxytropane and 7-hydroxy-3,6-ditigloyloxytropane appear to occur principally in the roots;[2-4] they have not been isolated from the aerial parts of normal plants although Verzár-Petri and Sárkány[5] have suggested, on the grounds of paper chromatographic evidence, the occurrence of 7-hydroxy-3,6-ditigloyloxytropane in the leaves of *D. innoxia*. Meteloidine (6,7-dihydroxy-3-tigloyloxytropane) has been isolated from the leaves of *D. meteloides*,[6] *D. ferox*[7] and *D. innoxia*[8] and from the roots of *D. innoxia*,[2] *D. ferox*,[2,9] *D. leichhardtii*[4] and *D. stramonium* (paper chromatography).[9] 3-Hydroxy-6-tigloyloxytropane is a minor component of the leaves of *D. cornigera*,[10] tigloidine has been obtained from the roots of *D. innoxia*[2] and 3-tigloyloxytropane from the roots of *D. ferox*[2] and *D. leichhardtii*.[4] 3,6,7-Tritigloyloxytropane has not been recorded as a naturally occurring alkaloid but the free hydroxytropanes are found, particularly in the roots[2,3] of a number of species.

Romeike[9] has demonstrated the presence of meteloidine in the rising sap of *D. ferox* and *D. stramonium* and, that the ditigloyl esters are translocated from the roots into the leaves has recently been shown[1] by the isolation of these alkaloids from the exuding sap of decapitated plants and by the accumulation of 3,6-ditigloyloxytropane in the scions of grafted

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plants. We now report the results of an investigation into some aspects of the metabolism of the tigloyl esters in the aerial parts of *Datura*.

RESULTS

Production of Labelled Alkaloids

As a preliminary to the main investigation, generally labelled alkaloids were produced by the incorporation of $^{14}CO_2$ into *D. innoxia* plants. The activities of the alkaloids subsequently isolated are shown in Table 1.

TABLE 1. PRODUCTION OF ^{14}C—GENERALLY LABELLED ALKALOIDS IN *D. innoxia*

No. of Plants and $^{14}CO_2$ doses	Time between initial treatment and harvest (days)	Isolated alkaloids			
		Compound	Wt. (mg)	Specific activity (cpm/mM) $\times 10^{-5}$	% Total activity in alkamine moiety
10 Plants. 0·36 mc in a single dose	5	3,6-Ditigloyl-oxytropane	94	1·8	18·8
		Hyoscine (Roots)	33	1·2	21·6
		Hyoscine (aerial parts)	35	0·5	
10 Plants. 0·36 mc in a single dose	28	3,6-Ditigloyl-oxytropane	75	4·8	12·7
		7-Hydroxy-3,6-ditigloyl-oxytropane	34	3·1	21·8
		Hyoscine (roots)	45	3·0	17·9
		Hyoscine (aerial parts)	45	2·8	
16 Plants. 1·0 mc administered during 4 weeks, on alternate days	42	3,6-Ditigloyl-oxytropane	96	4·0	20·7
		7-Hydroxy-3,6-ditigloyl-oxytropane	42	2·5	23·7
		Hyoscine (roots)	35	3·1	26·6
		Hyoscine (aerial parts)	91	3·2	

The activities (cpm/mM) of the alkaloids isolated from eight *D. innoxia* plants (20 weeks old) grown in sand culture and fed with ornithine-2-^{14}C (50 μc in 100 mg) were 3,6-ditigloyloxytropane $2{\cdot}1 \times 10^3$, 7-hydroxy-3,6-ditigloyloxytropane $0{\cdot}7 \times 10^3$, hyoscine (roots) $12{\cdot}8 \times 10^3$, hyoscyamine (roots) $6{\cdot}9 \times 10^3$ and hyoscine (aerial parts) $6{\cdot}3 \times 10^3$. Hydrolysis of the 3,6-ditigloyl ester and of the hyoscine isolated from the aerial parts and activity measurements on the products showed the ^{14}C labelling to be distributed in both parts of the molecule.

Metabolism of 3,6-*Ditigloyloxytropane in the Aerial Parts of* D. innoxia *and* D. cornigera

To investigate the fate of 3,6-ditigloyloxytropane when introduced into the aerial parts of *D. innoxia* and *D. cornigera*, the alkaloid, in some instances ^{14}C generally labelled, was

infiltrated into either normal or alkaloid-free shoots of plants which were subsequently analysed for alkaloids by chromatography. The results are recorded in Table 2. In a similar

TABLE 2. ISOLATION OF ALKALOIDS FROM *D. Innoxia* AND *D. Cornigera* AFTER INFILTRATION OF 3,6-DITIGLOYLOXYTROPANE

Plant material	Wt. of 3,6-ditigloyl-oxytropane (mg of base) and sp. act. where relevant	Time before harvesting (hr)	Dry wt. (g)	Isolated alkaloids		
				Compound	Wt. (mg)	Specific activity (cpm/mM)
Alkaloid-free scions of *D. innoxia* detached from *Cyphomandra* stocks. 32 weeks old (2 scions)	200	96	8·0	3,6-Ditigloyloxy-tropane	40	
				3-Hydroxy-6-tigloyloxytropane	30	
Ditto (1 scion)	200	72	5·3	3,6-Ditigloyloxy-tropane	119	
				3-Hydroxy-6-tigloyloxytropane	29	
Ditto (3 scions)	196 $0·8 \times 10^5$ cpm/mM (picrate)	72	9·5	3,6-Ditigloyloxy-tropane	29	$0·8 \times 10^5$ (picrate)
				3-Hydroxy-6-tigloyloxytropane	31	$0·5 \times 10^5$ (hydro-bromide) ($0·5 \times 10^5$)*
				3,6-Dihydroxy-tropane (as ditigloyl ester)	0·5	$0·2 \times 10^5$ ($0·2 \times 10^5$)*
Ditto (2 scions)	300	48	8·9	3,6-Ditigloyloxy-tropane	150	
				3-Hydroxy-6-tigloyloxytropane	12	
Normal detached *D. cornigera* shoots (2) containing hyoscine and noratropine	118 $7·3 \times 10^4$ cpm/mM (picrate)	24	19·0	3,6-Ditigloyloxy-tropane	19·3	$7·6 \times 10^4$ (picrate)
				Hyoscine	47	Inactive
				3-Hydroxy-6-tigloyloxytropane	10	$4·3 \times 10^4$ (picrate) ($4·5 \times 10^4$)*
				6-Hydroxy-3-tigloyloxytropane	4	$2·7 \times 10^4$ (picrate) ($4·5 \times 10^4$)*
				Noratropine	21	Inactive
Powdered *D. innoxia* leaves with 11·5 mg 3,6-ditigloyloxy-tropane added			5·0	3,6-Ditigloyloxy-tropane	10·8	

* Calculated activity if product derived directly from 3,6-ditigloyloxytropane (20·7% of activity in the tropane moiety).

experiment to these, (+)-3,6-dihydroxytropane tartrate (43 mg base) was fed to a single, detached alkaloid-free scion of *D. innoxia*. The shoot was dried after 42 hr and the unchanged dihydroxytropane recovered from it in 49% yield.

Biogenesis of Meteloidine in D. innoxia *and* D. ferox

Similar experiments to the above involved the infiltration of 7-hydroxy-3,6-ditigloyloxytropane, meteloidine and 3,6,7-tritigloyloxytropane into *D. innoxia* and *D. ferox*. The results are recorded in Table 3.

TABLE 3. ISOLATION OF ALKALOIDS FROM *D. innoxia* AND *D. ferox* AFTER INFILTRATION OF 3,6,7-TRIHYDROXYTROPANE DERIVATIVES

Plant material	Infiltrated alkaloid	Time before harvesting (hr)	Dry wt. (g)	Isolated alkaloids: Compound	Wt. (mg)	Specific activity (cpm/mM)
Alkaloid-free scions (2) of *D. innoxia* detached from *Cyphomandra* stocks. (32 weeks)	7-Hydroxy-3,6-ditigloyloxytropane hydrochloride (222 mg base)	96	9·2	7-Hydroxy-3,6-ditigloyloxytropane	58	
				Meteloidine	15·2	
Ditto (1 scion)	Ditto	72	6·0	7-Hydroxy-3,6-ditigloyloxytropane	128	
				Meteloidine	5	
				Unidentified alkaloid (?3,6-dihydroxy-7-tigloyloxytropane)	8	
Ditto (3 scions, 30 weeks old)	Ditto. (198 mg base, sp. act. $1·0 \times 10^4$ cpm/mM)	72	11·3	7-Hydroxy-3,6-ditigloyloxytropane	75	$1·0 \times 10^4$
				Meteloidine	3·2	$0·7 \times 10^4$ ($0·7 \times 10^4$)*
				3,6,7-Trihydroxytropane (as tritigloyl ester)	5	$0·2 \times 10^4$ ($0·2 \times 10^4$)*
Ditto (1 scion, 32 weeks old)	Meteloidine sulphate (20 mg base)	96	4·0	Meteloidine	4	
				3,6,7-Trihydroxytropane (as tritigloyl ester)	10·5	
Powdered *D. innoxia* shoots with 7-hydroxy-3,6-ditigloyloxytropane (10 mg) added			5·0	7-Hydroxy-3,6-ditigloyloxytropane. Other bases not isolated	9·7	
Normal detached *D. ferox* shoots containing hyoscine and meteloidine (3 shoots, 34 weeks old)	7-Hydroxy-3,6-ditigloyloxytropane sulphate (90 mg base, sp. act. $1·0 \times 10^4$ cpm/mM)	96	6·8	7-Hydroxy-3,6-ditigloyloxytropane	4·3	$1·0 \times 10^4$
				Hyoscine	11	Inactive
				Meteloidine	8·4	$0·2 \times 10^4$ ($0·7 \times 10^4$)*
Ditto (5 shoots, 25 weeks old)	Meteloidine sulphate (50 mg base)	96	5·3	Hyoscine	4·8	
				Meteloidine	38	
Ditto	None	96	5·3	Hyoscine	4·8	
				Meteloidine	3·0	

TABLE 3 (continued)

Plant Material	Infiltrated alkaloid	Time before harvesting (hr)	Dry wt. (g)	Isolated alkaloids: Compound	Isolated alkaloids: Wt. (mg)	Isolated alkaloids: Specific activity (cpm/mM)
Ditto	Meteloidine sulphate (50 mg base)	3	6·2	Hyoscine	5·0	
				Meteloidine	39	
Powdered *D. ferox* shoots with meteloidine (50 mg) added			5·0	Hyoscine	7·7	
				Meteloidine	43	
Ditto, no meteloidine added			5·0	Hyoscine	7·0	
				Meteloidine	5·0	
Alkaloid-free scions (4) of *D. innoxia* detached from *Cypho-mandra* stocks	3,6,7-Tritigloyl-oxytropane sulphate (100 mg base)	32	13·0	3,6,7-Tritigloyl-oxytropane	59	
				7-Hydroxy-3,6-ditigloyloxy-tropane	4·0	
				Meteloidine?	<1·0	

* Calculated activity if product all derived directly from 7-hydroxy-3,6-ditigloyloxytropane (23·7% of activity in the tropane moiety).

Whereas the administration of the naturally occurring tigloyl esters to shoots does not produce any noticeable toxic symptoms in the tissues, 3,6,7-tritigloyloxytropane (88 mg), infiltrated as the sulphate into two detached alkaloid-free scions of *D. innoxia*, gave after 5 hr, translucent areas around the veins and a complete collapse of the petiole similar to those resulting from the injection of sodium tiglate.[11] With a reduced dose (25 mg base per scion) no ill-effects were noted after 24 hr but the toxic symptoms began to appear after 32 hr. The scions were immediately harvested and subsequently analysed (Table 3).

DISCUSSION

D. innoxia plants subjected to the doses of $^{14}CO_2$ indicated in Table 1 give generally labelled ditigloyl ester alkaloids of sufficient activity for subsequent use in metabolic studies. There appears to be little difference in the activities of alkaloids produced by the administration of successive small doses of $^{14}CO_2$ to plants and by a single larger dose. However, the activity of hyoscine of the roots builds up much more rapidly than that of hyoscine in the aerial parts and supports the concept of hyoscine formation in the roots of *D. innoxia*. In *ad-hoc* experiments which involved the exposure of a few plants to $^{14}CO_2$ over short periods of time it appeared that the tiglic acid moieties of 3,6-ditigloyloxytropane and 7-hydroxy-3,6-ditigloyloxytropane became labelled more rapidly than the alkamine; thus 7 hr after exposure of plants to $^{14}CO_2$ the activities of the alkaloids were almost entirely confined to the tiglic acid residues. Even in the longer term experiments (Table 1) the alkaloids were still labelled predominantly in the tiglic acid moieties.

Previous work[12] with young plants (12 weeks) of *D. stramonium* has shown that ornithine-2-^{14}C gives rise to labelled hyoscine and hyoscyamine, the latter being specifically labelled at a bridgehead carbon. In the present instance, with *D. innoxia*, only a very low incorporation

[11] M. WELLENDORF, Thesis, University of Nottingham, 1959.
[12] E. LEETE, *J. Amer. Chem. Soc.* **84**, 55 (1962).

of ornithine into both hyoscine and the tigloyl esters was obtained, and this in a non-specific manner. The results of the experiments with both $^{14}CO_2$ and ornithine would support the hypothesis that in *D. innoxia* plants of the age employed (about 20-30 weeks), the tropane moiety, if still being formed is derived to a considerable extent from a source already established in the plant. $^{14}CO_2$ is still, in plants of this age, rapidly incorporated into the tiglic acid portion of the molecule.

The ditigloyl ester alkaloids are rapidly degraded after entering the detached aerial parts of *D. innoxia*, *D. ferox* and *D. cornigera* (Table 2) and, as they are normally present in the ascending sap, this accounts for their apparent absence from normal leaves. The isolation of 3-hydroxy-6-tigloyloxytropane from shoots infiltrated with 3,6-ditigloyloxytropane indicates one pathway involved in the phytochemical hydrolysis of the ditigloyl ester and also suggests the origin of the mono ester as found in normal *D. cornigera* leaves.[10] That the removal of the acids does not occur in a specific sequence is shown by the isolation of both 6-hydroxy-3-tigloyloxytropane and the 6-tigloyl ester from *D. cornigera* shoots injected with labelled 3,6-ditigloyloxytropane. The specific activities of both monotigloyl esters derived from labelled 3,6-ditigloyloxytropane showed them to be formed directly by the elimination of one ester group. Neither of the monotigloyl esters accumulates to any extent in the plant and hydrolysis proceeds to 3,6-dihydroxytropane. The low overall recoveries of the injected alkaloid, including that of injected dihydroxytropane itself, could arise through degradation of the tropane ring but this has not been shown experimentally. A control estimation involving dried *D. innoxia* leaves and 3,6-ditigloyloxytropane showed that no hydrolysis of the alkaloid occurred as a result of the extraction procedure used.

Meteloidine is shown to arise in both *D. innoxia* and *D. ferox* by the partial hydrolysis of 7-hydroxy-3,6-ditigloyloxytropane (Table 3). The use of labelled alkaloid and the specific activity of the isolated meteloidine confirms the direct derivation. As meteloidine accumulates to some extent (about 0·03%) in the normal leaves of *D. ferox*, it may not be the principal intermediate in the complete rapid breakdown of 7-hydroxy-3,6-ditigloyloxytropane in the plant. This is supported by the results of the infiltration of labelled 7-hydroxy-3,6-ditigloyloxytropane into *D. ferox* shoots containing a normal complement of inactive meteloidine. At the end of the experiment 53% of the injected alkaloid had been metabolized but the activity of the isolated meteloidine was only 30% of that expected had it been derived entirely from the injected alkaloid. Its dilution with considerable inactive meteloidine suggests a relatively slow accumulation and turnover of the alkaloid. The injection of meteloidine into *D. ferox* scions and its theoretical recovery compared with a control experiment is further evidence that meteloidine is not necessarily involved in the main pathway of the complete hydrolysis of the ditigloyl ester. With *D. innoxia* however, in which meteloidine does not accumulate to any extent in the leaves, the recovery of meteloidine from shoots injected with meteloidine sulphate was less than with *D. ferox* and 3,6,7-trihydroxytropane was also isolated from the injected leaves. The existence of an alternative pathway for the hydrolysis of the ditigloyl alkaloid was afforded in one experiment with *D. innoxia* scions infiltrated with the alkaloid by the isolation of another ester alkaloid, in addition to meteloidine. This alkaloid, picrate m.p. 181° gave 3,6,7-tritigloyloxytropane on treatment with tigloyl chloride and was probably 3,6-dihydroxy-7-tigloyloxytropane.

3,6,7-Tritigloyloxytropane has not been reported as a normal constituent of *D. innoxia* roots and the results of the infiltration of it into detached scions show it to be far more toxic to the aerial organs than the ditigloyl esters. The degradation of the alkaloid in *D. innoxia* leaves appears to follow the same pattern as for the other ester alkaloids.

EXPERIMENTAL

Radioactivity Measurements

The alkaloid picrate or other salt (1 mg) in a dimpled planchet was dissolved in acetone (0·2 ml for picrates) and the solvent removed with an i.r. lamp. This left the salt as a thin film over the dimpled surface and its activity was determined in a Geiger 2π gas-flow counter (Labgear Type D1426).

Cultivation of Plants

Alkaloid-free stems and leaves of *D. innoxia* were obtained by grafting the aerial parts of young *D. innoxia* seedlings onto the stocks of *Cyphomandra betacea* by the approach graft method. Successful grafts resembled normal *D. innoxia* plants in their growth and form. Plants were individually tested for absence of alkaloids by paper chromatography before use as, particularly with older plants (6 months), there was a tendency towards internal root formation by the scion at the graft union, which resulted in the appearance of alkaloids in the leaves. The *D. ferox* plants were raised in pots in a temperate greenhouse and were used at the flowering stage. The shoots of *D. cornigera* were obtained on 15 October from perennial plants which had been growing in the field throughout the summer months.

Production of ^{14}C-labelled Alkaloids

For treatment with $^{14}CO_2$, *D. innoxia* plants (20 weeks old) which had been once pruned during the season were placed in a growing chamber (430 l. capacity) provided with $^{14}CO_2$ monitoring, circulatory and water condensing systems. $^{14}CO_2$ was liberated from $Ba^{14}CO_3$ mixed with inactive barium carbonate by treatment with lactic acid (50%). The carbon dioxide level in the chamber was maintained at approximately 0·1% by the addition of calculated amounts of inactive carbon dioxide.

The roots and aerial portions were subsequently dried separately, powdered, each moistened with water, mixed with 10 g calcium hydroxide and exhausted with ether. The ether extracts, after evaporation, were redissolved in ether and submitted to column chromatography [for the roots—kieselguhr (20 g) mixed with 0·25 M phosphate buffer solution (10 ml), pH 6·1; for the aerial parts—kieselguhr (15 g) mixed with 0·25 M phosphate buffer solution (6 ml), pH 6·1] using petrol (b.p. 40–60°), ether, and chloroform as eluants. With root extracts, 3,6-ditigloyloxytropane was eluted from the column with petrol, 7-hydroxy-3,6-ditigloyloxytropane in the first portion of the ether eluate (50 ml) and hyoscine in the second ether eluate (150 ml). The basic mixture obtained from the chloroform was shown by paper chromatography to contain hyoscyamine. With extracts of the aerial parts, the petrol eluate contained pigments, ether eluted hyoscine (the principal alkaloid) and the chloroform eluate contained hyoscyamine. The ditigloyl esters and hyoscine were recovered as picrates[2] which were recrystallized to a constant specific activity and constant m.p. (see below).

For hydrolysis, the bases were liberated from samples of the alkaloid picrates (about 3 mg) and heated in a sealed tube with 5% $Ba(OH)_2$ (2 ml) for 2 hr at 100°. From the mixture, acidified with dil. H_2SO_4, tiglic acid was extracted with ether (3 × 5 ml). The ether extract was neutralized with alcoholic KOH and the solvent removed. The residue, in water, was transferred to a dimpled planchet, evaporated and the activity of the residue determined. The alkamine was obtained from the acidic aqueous solution by treating the mixture with $BaCO_3$, filtering, and evaporation of the filtrate under reduced pressure. The activity of the solid residue was measured.

The weight and activities of the isolated alkaloids are given in Table 1.

For the feeding experiment involving ornithine-2-^{14}C, eight *D. innoxia* plants, 20 weeks old, growing in sand culture[13] were employed. Fifty μc ornithine hydrochloride-2-^{14}C in 100 mg was added to the culture solution. After 72 hr the activity of the nutrient solution was negligible and the plants were collected. The aerial parts and roots were separately dried at 50° for 12 hr and the alkaloids extracted, fractionated and examined in a manner similar to that described above.

Administration of Inactive and ^{14}C-labelled Alkaloids

The labelled alkaloid picrates, prepared as indicated above, were suitably diluted with inactive picrate, the bases recovered and neutralized with dilute sulphuric or hydrochloric acid. All alkaloids were administered to the aerial shoots by placing the cut ends of scions in an aqueous solution (usually 10 ml) containing the alkaloid sulphate or hydrochloride. The uptake of the solution varied with different plants but it was usually complete in 9–12 hr. Small quantities of distilled water (about 10 ml) were then added as necessary and, before harvesting, all the liquid was allowed to be absorbed into the shoot.

Extraction and Fractionation of Alkaloids

The shoots were dried and powdered after harvesting, and before extraction were moistened with 20% w/w of water with a similar quantity of $Ca(OH)_2$ and, after 30 min, exhausted with ether. The evaporated ether extract was submitted to partition chromatography on columns as described above but using pH 6·8 buffer. Pigments and 3,6-ditigloyloxytropane or 7-hydroxy-3,6-ditigloyloxytropane were eluted with petrol (b.p. 40–60°), hyoscine with ether and 3-hydroxy-6-tigloyloxytropane or meteloidine with chloroform. The alkaloids recovered were characterized by R_f values, the preparation of picrates, and i.r. spectra. Analyses of 3-hydroxy-6-tigloyloxytropane isolated from *D. innoxia* alkaloid-free scions fed with 3,6-ditigloyloxytropane (Found: C, 48·7; H, 6·6. Calc. for $C_{13}H_{21}O_3N.HBr$: C, 48·75; H, 6·9%). For the meteloidine obtained by treatment of alkaloid-free scions of *D. innoxia* with 7-hydroxy-3:6-ditigloyloxytropane hydrochloride and isolated as the picrate (Found: C, 47·4; H, 5·2. Calc. for $C_{13}H_{21}O_4N.C_6H_3O_7N_3$: C, 47·1; H, 4·95%).

3,6-Dihydroxytropane was recovered from the original dried marc by percolation with dilute solution of ammonia (400 ml, 2 N). The residue obtained after removal of the solvent was submitted to continuous extraction (Soxhlet) with acetone as solvent. Evaporation of the solution afforded a solid which was tested by paper chromatography and then used for the preparation of the ditigloyl derivative for greater ease of isolation. 3,6,7-Trihydroxytropane was extracted from marcs in a similar manner and the entire dried extract treated with tigloyl chloride. The esterified base was extracted in chloroform from the mixture and purified by chromatography on alumina (30 g) with petrol (b.p. 40–60°)–ether (1:1) as developing solvent. The eluted ester was characterized as the picrate—see below.

The alkaloids contained in a shoot of *D. cornigera* fed with ^{14}C-labelled 3,6-ditigloyloxytropane (118 mg) were fractionated on kieselguhr (15 g) loaded with 0·5 M phosphate buffer solution (7·5 ml), pH 6·8. Pigments and 3,6-ditigloyloxytropane were eluted with petrol (b.p. 40–60°), hyoscine with the first fraction (100 ml) of ether and 3-hydroxy-6-tigloyloxytropane by the second fraction (300 ml). The monotigloyl ester was isolated as the hydrochloride, and characterized by m.p. and mixed m.p. 185° and its i.r. spectrum. The initial

[13] W. C. EVANS and W. J. GRIFFIN, *Lloydia*, **25**, 139 (1962).

chloroform eluate (60 ml) was shown by thin layer chromatography to contain a mixture of bases, and the second (200 ml) contained noratropine, characterized as the picrate m.p. and mixed m.p. 227°. The mixture of bases in the first chloroform eluate was separated on alumina (20 g); development with ether–ethanol (92·5:7·5) gave 3-hydroxy-6-tigloyloxytropane, and ether–ethanol (90:10) eluted a base which on neutralization with dilute H_2SO_4 and treatment with sodium picrate gave 6-hydroxy-3-tigloyloxytropane picrate, m.p. 150°, mixed m.p. with authentic material (m.p. 156°) 150°. R_f values, on thin layer chromatography, of the isolated and reference compound were identical but lack of material prevented the complete identification of the isolated alkaloid. Noratropine was finally eluted from the alumina column by alcohol.

For the fractionation of the alkaloid mixtures obtained from scions injected with meteloidine, columns of kieselguhr (10 g) loaded with 0·15 M phosphate buffer solution (3·2 ml), pH 6·1 were used. The alkaloids were eluted as above.

Preparation of 3,6,7:*Tritigloyloxytropane and its Derivatives*

Meteloidine (0·25 g) was added to freshly distilled tigloyl chloride (0·3 g), the mixture heated gently under reflux for 5 min and then maintained at 90° for 4 hr. It was treated with water (10 ml), made alkaline with 2 N NaOH (10 ml), and shaken with chloroform (3 × 100 ml). Removal of the solvent gave a gum (0·45 g) which did not crystallize on standing. The base, neutralized with 0·1 N hydrochloric acid and treated with sodium picrate solution afforded 3,6,7-tritigloyloxytropane picrate, short needles from aqueous ethanol, m.p. 148° (Found: C, 53·7; H, 5·6; N, 8·9. $C_{23}H_{33}O_6N.C_6H_3O_7N_3$ required: C, 53·7; H, 5·6; N, 8·6%). Chloroplatinic acid solution (5%) added to the hydrochloride solution gave 3,6,7-tritigloyloxytropane chloroplatinate, feathery needles from aqueous acetone, m.p. 227° (Found: C, 44·3; H, 5·5. $(C_{23}H_{33}O_6N)_2.H_2PtCl_6$ required: C, 44·2; 5·5%). A solution of the base in ethanol, neutralized with alcoholic HBr solution and diluted with petrol afforded the hydrobromide, long white needles, m.p. 189°.

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