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BIOSYNTHESIS OF TROPANE ESTER ALKALOIDS IN *DATURA*

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Key Word Index—*Datura meteloides*; Solanaceae; biosynthesis; tropane alkaloids; labelled hygrine; tigloyl hygroline.

Abstract—*Datura meteloides* plants were fed via the roots with [1″,2′-^{14}C]tigloyl hygroline and as a control, [2′-^{14}C]hygrine. After a week the alkaloids were isolated and degraded. Despite hydrolysis of the putative precursor it was possible, by label ratio, to show that esterification occurs after, and not before, the tropane ring has been synthesized. Hygroline is proposed as a possible intermediate.

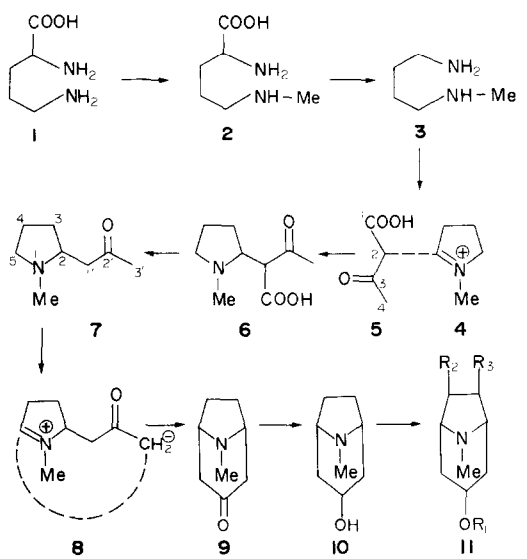
INTRODUCTION

Hygrine (**7**) is an established precursor of the tropane alkaloids [1] (Scheme 1). It has been shown [2, 3] that *Datura* is stereoselective and uses only the (+)-(2*R*)-hygrine enantiomer. This was not the case for three other solanaceous genera (*Atropa*, *Hyoscyamus* and *Physalis*) investigated [4].

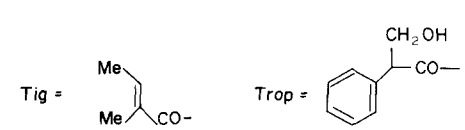
The biosynthesis of hygrine (**7**) is known to involve ornithine (**1**) and acetoacetate (**5**) [1,5]. It is thought that this proceeds by a Mannich type condensation between the ornithine derived *N*-methyl-Δ^1-pyrrolinium cation (**4**) [6] and C-2 of acetoacetate (**5**) [7]. The resulting hygrine-α-carboxylic acid (**6**) is decarboxylated giving hygrine (**7**). The final cyclization in the pathway to the tropane moiety (**9**) is thought to proceed via dehydrohygrine (**8**); a second intramolecular Mannich type reaction involving the quaternary Schiffs base on the pyrrolidine ring and the terminal methyl of the side chain (C-3) yielding tropinone (**9**) [8]. Stereospecific reduction of the tropinone (**9**) gives tropine (**10**) which is assumed to esterify to give the various tropane ester alkaloids commonly found in *Datura* (**11a–e**) [9, 10].

There are other possible routes from hygrine (**7**) to the tropane esters (**11a–e**) that, hitherto, have not been considered (Scheme 2). It is possible for instance that hygrine (**7**) is reduced to hygroline (**12**) prior to cyclization. This compound could then cyclize to give tropine (**10**). It is also possible that esterification is a pre-cyclization event and that the hygroline esters [tigloyl or tropoyl hygroline (**13**)] are the immediate precursors of the tropane moiety.

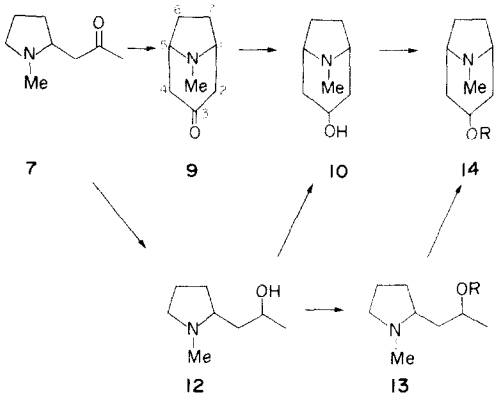
In order to investigate this problem [1″,2′-^{14}C]tigloyl hygroline (**15**) was fed to *Datura* plants. The compound was synthesized by platinum oxide reduction of DL-[2′-^{14}C]hygrine followed by esterification with [1-^{14}C]tigloyl chloride. No attempt was made to resolve the stereoisomers of the tigloyl hygroline. The rationale for using this doubly labelled material was so that ester hydrolysis to hygrine and the subsequent incorporation of this known precursor would be visible due to alterations in the label ratio between the acid (**17**) and the alkamine (**16**) components of the derived tropane esters.



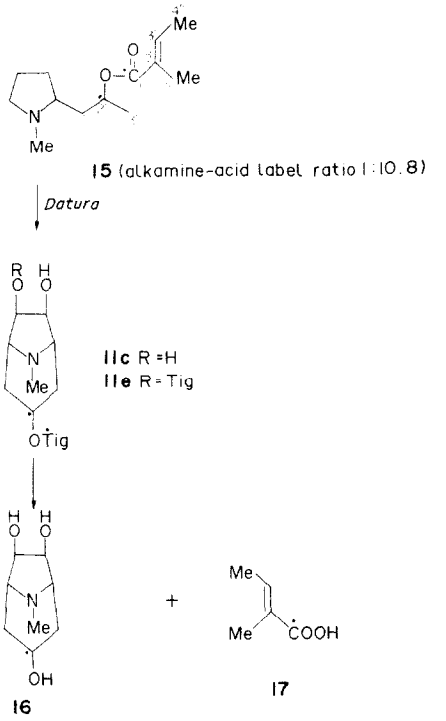
IIa Hyoscine R_1 = Trop, R_2 + R_3 = O
IIb Hyoscyamine R_1 = Trop, R_2 = R_3 = H
IIc Meteloidine R_1 = Tig, R_2 = R_3 = OH
IId 3α,6β-Ditigloyloxytropane R_1 = Tig, R_2 = OTig, R_3 = H
IIe 3α,6β-Ditigloyloxytropan-7β-ol R_1 = Tig, R_2 = OTig, R_3 = OH



Scheme 1. Formation of tropane alkaloids from ornithine.



Scheme 2. Possible routes to the tropane alkaloids from hygrine. For simplicity only the C-3 derivatives are shown. R = Tig or Trop.



Scheme 3. The degradation of 3α,3β-ditigloyloxytropan-7β-ol (**11e**) and metaloidine (**11c**) from plants fed [1″,2′-^{14}C]tigloyl hygroline (**15**). The dots indicate labelled carbons.

RESULTS AND DISCUSSION

The labelled tigloyl hygroline (**15**) was fed via the roots to seven 5-month-old *Datura meteloides* Dun. plants. After 7 days the plants were harvested and the alkaloids isolated. The tigloyl ester alkaloids 3α,6β-ditigloyloxytropane (**11d**), meteloidine (**11c**) and 3α,6β-ditigloyloxytropan-7β-ol (**11e**) were purified to constant specific activity as their picrate derivatives. The latter two were then hydrolysed in barium hydroxide to give the constituent alkamine (teloidine) (**16**) and tiglic acid (**17**) (Scheme 3). The tropoyl ester alkaloids hyoscine (**11a**) and hyoscyamine (**11b**) were merely purified to constant specific activity. These data are shown in Table 1.

In the control experiment, five 5-month-old *D. meteloides* plants were fed with DL-[2′-^{14}C]hygrine. After 7 days the alkaloids were isolated and purified. The meteloidine (**11c**), which has not hitherto been demonstrated to be a metabolite of hygrine, was converted to isopropylideneteloidinone (**19**). This was coupled with phenyl magnesium bromide to give the carbinol (**20**) which was oxidized with permanganate to yield benzoic acid (21) (Scheme 4). In this way hygrine (**7**) was shown to have been incorporated in the expected manner, i.e. C-2′ of hygrine (**7**) giving rise to the C-3 of the tropane moiety. These data are shown in Table 2.

From Table 1 it is clear that the tigloyl hygroline (**15**) has undergone extensive hydrolysis since both hyoscine (**11a**) and hyoscyamine (**11b**) are labelled. It was not unexpected, therefore, to find that the label ratios for the 3α,6β-ditigloyloxytropan-7β-ol (**11e**) and the meteloidine (**11c**) were considerably altered (the similarity between the label ratios for these alkaloids is misleading since the former possesses two tiglate residues and the latter only one). The lowering of the ratios probably indicates a larger 'pool' size of the tiglate than the alkamine.

Table 2 shows that the hygrine (**7**) control follows roughly the same pattern of incorporation with the tropoyl esters being the most active. The hygrine component of the tigloyl hygroline molecule (**15**), which has the same specific activity as the control,

Table 1. The specific radioactivities and ratio of ^{14}C-alkamine–^{14}C acid of the root alkaloids of *D. meteloides* after feeding with [1″,2′-^{14}C]tigloyl hygroline (ratio 1:10.8)

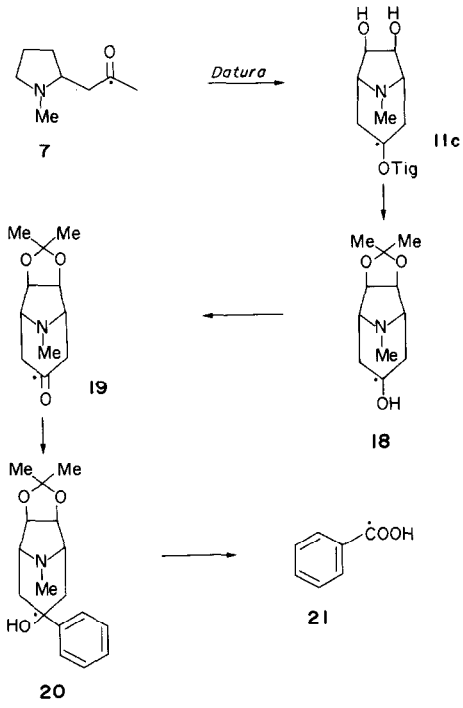
Alkaloid	Sp. act. (10^{-4} dpm/mmol)			Ratio of ^{14}C-alkamine–^{14}C-acid
	Alkaloid	Alkamine	Tiglic acid	
Hyoscine	6.98	—	—	—
Hyoscyamine	10.06	—	—	—
3α,6β-Ditigloyloxytropane	4.60	*	*	*
3α,6β-Ditigloyloxytropan-7β-ol	2.04	1.191	0.625	1:0.525
Meteloidine	2.48	1.761	0.949	1:0.539

*Too little to degrade.

Table 2. The specific activities of the root alkaloids isolated from *Datura meteloides* after feeding with DL-[2'-^{14}C]hygrine

Alkaloid	Specific incorporation (%)*	Sp. act. (10^{-3} dpm/mmol)			
		Alkaloid	Alkaloid + carrier	Benzoic acid	Tiglic acid
Hyoscine	0.073	7.3	—	—	—
Hyoscyamine	0.181	18.1	—	—	—
3α,6β-Ditigloyloxytropane	0.059	5.9	—	—	—
3α,6β-Ditigloyloxytropan-7β-ol	0.029	2.9	—	—	—
Meteloidine	0.088	8.8	5.09	4.42	0

*(Sp. act. base sp. act. precursor) × 100.



Scheme 4. The degradation of meteloidine to demonstrate the incorporation of [2'-^{14}C]hygrine into the tropane moiety. The dots indicate labelled carbons.

gave higher specific incorporation values than the control (cf. column 2 Table 1 with column 1 Table 2). Since the tigloyl hygroline (**15**) has been hydrolysed *Datura* must have the ability to convert hygroline (**12**) to hygrine (**7**) so that this can be incorporated into the tropoyl and tigloyl esters (**11a–e**). Alternatively, in view of the greater incorporation into the tropane moiety by tigloyl hygroline (**15**) when compared with hygrine (**7**), perhaps hygroline (**12**) is the immediate tropane ring precursor. Hygroline (**12**), like hygrine (**7**), has been found in tropane-containing species. (−)-Hygroline has been isolated from *Cochlearia artica* (Cruciferae) [11] and *Erythroxylon coca* (Erythroxylaceae) [12]. Recently both diastereoisomers of hygroline (**12**) have been found in *Schizanthus hookeri* (Solanaceae) [13].

Tigloyl hygroline (**15**) is not a direct precursor of the tropane ring, therefore esterification of the tropane ring must be a post-cyclization event. This does not, however, rule out the possibility that hygrine (**7**) is reduced to hygroline (**12**) prior to tropane formation.

EXPERIMENTAL

Plant material. D. meteloides Dun. plants were grown on open land in Leicester U.K. from seed obtained from the Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, East Germany. The plants had all the characters previously described [14].

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

Tracer material. Na^{14}CN for the synthesis of tiglic acid and sodium [3-^{14}C]acetoacetate for the synthesis of hygrine were purchased from the Radiochemical Centre, Amersham, U.K.

Synthesis of [1-^{14}C]*tigloyl chloride.* [1-^{14}C]Tiglic acid (1.08×10^8 dpm/mmol), mp and mmp 63°, was synthesized in 29% yield by the previously reported method [15, 16]. The acid (190 mg) was mixed at room temp. with $SOCl_2$ (240 mg) and $ZnCl_2$ (2 mg). When the reaction subsided the vessel was heated (30°) for 15 min. Excess $SOCl_2$ was removed by evacuation at red. pres. The [1-^{14}C]tigloyl chloride was used immediately for the synthesis of [1",2'-^{14}C]tigloyl hygroline.

Synthesis of DL-[2'-^{14}C]*hygrine* [17]. Ethyl [3-^{14}C] acetoacetate (10 g, 350 μCi) was stirred at room temp. for 24 hr with 2.5% KOH (300 ml) and then added to a soln which had been prepared by reducing freshly distilled *N*-methylpyrrolidone (4 g) in Et_2O (8 ml) by dropwise addition of $LiAlH_4$ (0.98 g) in Et_2O over a 1 hr period. The mixture was adjusted to pH 7 with 0.1 M HCl, stirred at room temp. for a further 40 hr and then reduced to a vol. of *ca* 60 ml under red. pres. The soln was basified (NaOH) and continuously extracted with Et_2O for 24 hr. Evaporation of the dried (Na_2SO_4) Et_2O extract gave DL-[2'-^{14}C]hygrine (3 g), IR (film) identical to authentic compound, sp. act. 1×10^7 dpm/mmol. The labelled hygrine (2 g) in MeOH (10 ml) was

treated with (+)-tartaric acid (2.2 g) in MeOH (20 ml) and then evaporated to give the tartrate salt which was recrystallized from MeOH, yield quantitative, mp 129–130°.

Synthesis of (2-RS, 2′-RS)-[1″-2′-^{14}C]tigloyl hygroline. DL-[2′-^{14}C]Hygrine-(+)-tartrate (1 g 1×10^7 dpm/mmol) in H_2O (5 ml) was shaken vigorously at room temp. with PtO_2 (100 mg) in a H_2 atmosphere for 4 hr. The soln was filtered, basified (20% NaOH) and extracted with $CHCl_3$ (3×10 ml). The extract was dried (Na_2SO_4), filtered and evaporated to give (2-*RS*, 2′-*RS*)-hygroline (250 mg), picrate mp 128–129° (identical with lit. [18]).

The hygroline (200 mg) was dried (NaOH) and added slowly to a slight excess of [1-^{14}C]tigloyl chloride (1.08×10^8 dpm/mmol) [19]. The resulting red oil was refluxed (3 hr), acidified (dil. H_2SO_4) and partitioned with Et_2O (3×10 ml) to remove excess tiglic acid. The aq. phase was basified (conc. NaOH) and extracted into Et_2O (5×10 ml) giving [1″-2′-^{14}C]tigloyl hygroline (1.18×10^8 dpm/mmol; ^{14}C-acid–^{14}C-alkamine, 1:10.8). No traces of residual hygroline were detected by TLC. ^{1}H NMR (100 MHz, $CDCl_3$): δ 1.2 (3H, *d*, H-3′), 1.7 (3H, *d*, H-4″), 1.8 (3H, *s*, H-5″), 1.6–2.2 (8H, unresolved *m*, H-3, H-4, H-5, H-1′), 2.3 (3H, *s*, H-6), 3.0 (1H, *m*, H-2), 5.0 (1 H, *m*, H-2′), 6.8 (1 H, *m*, H-3″); EIMS (probe) 70 eV, *m/z* (rel. int.): 225 $[M]^+$ (3.2), 142 (4.7), 126 (7.4), 99 (6.3), 97 (20), 85 (32), 84 (100), 83 (41), 82 (36), 71 (4.7), 126 (7.4), 99 (6.3), 97 (20), 85 (32), 84 (100), 83 (41), 82 (36), 71 (20), 69 (21), 57 (34), 55 (62), 43 (28), 42 (32), 41 (31). Picrate (rhomboid crystals from EtOH–H_2O) mp 116°. (Found: C, 50.33; H, 5.73; N, 12.29. $C_{19}H_{26}N_4O_9$ requires: C, 50.33; H, 5.52; N, 12.36%.)

Feeding experiments. The [1″,2′-^{14}C]tigloyl hygroline (35 mg) was neutralized (dil. H_2SO_4), made up to 35 ml with H_2O and administered to seven 5-month-old *D. meteloides* plants which had been carefully uprooted and suspended in blackened beakers containing Phostrogen soln. Five similar plants were each fed with DL-[2′-^{14}C]hygrine tartrate (2.0 mg). After 6 days the plants were harvested and the roots and aerial parts separately dried at 60° for 24 hr.

Isolation of the alkaloids. The finely powdered roots (tigloyl hygroline feed, 35 g; hygrine feed, 33 g) were extracted and the bases resolved on Pi partition columns at pH 6.8 and 5.6 as described previously [20, 21]. The bases were converted to their picrate salts for counting.

Degradation of 3α,6β-ditigloyloxytropan-7β-ol and meteloidine from the tigloyl hygroline feed. 3α,6β-Ditigloyloxytropan-7β-ol (2.040×10^4 dpm/mmol) was regenerated from its picrate (50 mg), dissolved in EtOH (1 ml) and transfered to a tube containing 5% $Ba(OH)_2$ soln and sealed. The tube was heated in steam (3 hr) following which the contents were acidified (50% H_2SO_4) and extracted with Et_2O (5×5 ml). The Et_2O was dried (Na_2SO_4), filtered and then carefully evaporated. The resultant tiglic acid was recrystallized from petrol and sublimed yielding 2.3 mg, mp 62°, sp. act. 0.625×10^4 dpm/mmol. The hydrolysis mixture was neutralized ($BaCO_3$), filtered and then evaporated to dryness and desiccated. The dry residue was steam heated (2 hr) with an excess of freshly distilled tigloyl chloride (20 mg), acidified (50% H_2SO_4) and extacted with Et_2O (3×15 ml) to remove excess tiglic acid. The remaining soln was basified (NaOH) and the re-esterified alkamines extracted into $CHCl_3$ (5×10 ml). The resultant 3α,6β,7β-tritigloyloxytropane (identical R_f on TLC and IR to authentic sample) was converted to its picrate (40 mg), mp 147°, sp. act. 1.191×10^4 dpm/mmol. The combined activity of alkamine and acid was 1.816×10^4 dpm/mmol (89% recovery of original label) and their label ratio was 1:0.525.

The active meteloidine (2.48×10^4 dpm/mmol) was recovered from its picrate (100 mg) and hydrolysed as above with 5% $Ba(OH)_2$ (15 ml) giving tiglic acid (5.0 mg) mp 61–62°, sp. act. 9.49×10^3 dpm/mmol. The alkamine was re-esterified with tigloyl chloride (40 mg) and the resultant 3α,6β,7β-tritigloyloxytropane (7.5 mg), mp 148°, had sp. act. 1.761×10^4 dpm/mmol. The combined activity of the alkamine and the acid was 2.71×10^4 dpm/mmol (109% recovery of label) and the label ratio was 1:0.539.

Degradation of meteloidine from the DL-*hygrine control feed.* [22] Diluted meteloidine picrate (41 mg active + 30 mg cold; 5.09×10^3 dpm/mmol was regenerated from its picrate (71 mg) and stirred in Me_2CO (2.5 ml) containing conc. HCl (0.1 ml) for 5 hr at room temp. KOH (0.3 g) in H_2O (0.5 ml) was then added and the Me_2CO removed *in vacuo*. The residue was redissolved in MeOH (2.5 ml), refluxed for 20 hr, evaporated and then satd with K_2CO_3 (2.5 ml). The mixture was continuously extracted with Et_2O for 4 days. The Et_2O extract was dried (Na_2SO_4) and evaporated to yield isopropylideneteloidine which was recrystallized from EtOAc and iso-octane giving colourless needles (23 mg), mp 128–129° (lit. value 131–133° [22]). The Et_2O extracted aq. phase was acidified (dil. HCl) and extracted with Et_2O (5×2 ml) yielding tiglic acid which was recrystallized from hot petrol giving 5.5 mg, mp 64°, of no detectable sp. act.

The isopropylideneteloidine (23 mg) in pyridine (0.5 ml) was added to a soln of CrO_3 (23 mg) in pyridine (0.7 ml) that had been stirring for 15 min. The combined mixtures were stirred (24 hr) and then Et_2O (5 ml) was added and the ppted salts removed. The soln was evaporated to dryness, the residue redissolved in Et_2O (5 ml) and then treated with an excess of PhMgBr. The mixture was stirred under N_2 (48 hr) and 5% HCl (10 ml) added. The Et_2O layer was discarded and the aq. phase was washed with Et_2O (3×5 ml) before being basified (10% NaOH) and extracted with $CHCl_3$. The $CHCl_3$ extract was dried (Na_2SO_4), filtered, evaporated to dryness and then refluxed with $KMnO_4$ (200 mg) in H_2O (20 ml) for 4 hr. The reaction mixture was stirred with EtOH (20 ml), filtered and then extracted with Et_2O (5×20 ml) following acidification (10% HCl). After drying (Na_2SO_4) the Et_2O was evaporated and the residue sublimed to give benzoic acid (5.4 mg), mp 120° sp. act. 4.42×10^3 dpm/mmol (89% recovery of label).

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REFERENCES

1. O'Donovan, D. G. and Keogh, M. F. (1969) *J. Chem. Soc. C*, 223.
2. McGaw, B. A. and Woolley, J. G. (1977) *J. Pharm. Pharmacol.* **29**, 16.
3. McGaw, B. A. and Woolley, J. G. (1978) *Phytochemistry* **17**, 257.
4. McGaw, B. A. and Woolley, J. G. (1979) *Phytochemistry* **18**, 189.
5. Liebisch, H. W., Peisker, K., Radwan, A. S. and Schütte, H-R. (1972) *Z. Pflanzenphysiol.* **62**, 671.
6. Leete, E. (1967) *J. Am. Chem. Soc.* **89**, 7081.
7. McGaw, B. A. and Woolley, J. G. (1979) *Tetrahedron Letters* **34**, 3135.
8. Leete, E. (1979) *Planta Med.* **36**, 97.
9. Willaman, J. J. and Schubert, B. G. (1961) in *Alkaloid-*

Bearing Plants and their Contained Alkaloids p. 1234. U.S. Department of Agriculture Technical Bulletin.
10. Willaman, J. J. and Li, H-L. (1970) *Lloydia* 3A (Suppl.).
11. Platanova, T. F. and Kuzovkov, A. D. (1963) *Med. Prom. SSSR.* **17**, 19.
12. Späth, E. and Kittel, F. (1943) *Ber.* **76**, 942.
13. Martin, A. S., Rovirosa, J., Gambaro, V. and Castillo, M. (1980) *Phytochemistry* **19**, 2007.
14. Avery, A. G., Satina, S. and Rietsema, J. (1959) in *Blakeslee: The Genus* Datura p. 16. Ronald Press, New York.
15. Tsuyuki, T., Inamoto, N. and Simamura, K. (1957) *Radioisotopes* **6**, 93.
16. Buckles, R. E. and Mock, G. V. (1950) *J. Org. Chem.* **15**, 680.
17. Galinovsky, F. and Zuber, H. (1953) *Monatsh. Chem.* **84**, 798.
18. Hess, K. (1913) *Ber.* **46**, 4104.
19. Barger, G., Martin, W. F. and Mitchell, W. (1937) *J. Chem. Soc.*, 1820.
20. Evans, W. C. and Partridge, M. W. (1952) *J. Pharm. Pharmacol.* **4**, 769.
21. Basey, K. and Woolley, J. G. (1973) *Phytochemistry* **12**, 2197.
22. Leete, E. and Nelson, S. J. (1969) *Phytochemistry* **8**, 413.