$-C_8H_7O_2]^+$ (12), 155 $\{M-[C(7)H_2-C(6)HOCOCH_2Ph]\}^+$ (3), 138 (7), 135 (2), 122 (27), 97 (19), 95 (60), 94 (52), 91 (16), 60 (6), 43 (100). Weak signals at *m/z* 303 and 105 suggested contamination with (**2b**).

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REFERENCES

1. Al-Said, M. S., Evans, W. C. and Grout, R. J. (1986) *J. Chem. Soc. Perkin Trans. I* 957.
2. El-Imam, Y. M. A., Evans, W. C., Grout, R. J. and Ramsey, K. P. A. (1987) *Phytochemistry* **26**, 2385.
3. El-Imam, Y. M. A., Evans, W. C. and Plowman, T. (1985) *Phytochemistry* **24**, 2285.
4. Al-Yahya, M. A. I., Evans, W. C. and Grout, R. J. (1979) *J. Chem. Soc. Perkin Trans. I* 2130.
5. Al-Said, M. S., Evans, W. C. and Grout, R. J. (1986) *Phytochemistry* **25**, 851.
6. Johns, S. R., Lamberton, J. A. and Sioumis, A. A. (1970) *Aust. J. Chem.* **23**, 421.
7. Agar, J. T. H. and Evans, W. C. (1976) *J. Chem. Soc. Perkin Trans. I* 1550.
8. Henry, T. A. (1949) *The Plant Alkaloids* 4th Edn., p. 101. Churchill, London.
9. Hegnauer, R. and Fikenscher, L. H. (1960) *Pharm. Acta Helv.* **35**, 43.
10. Klein, G. and Soos, G. (1929) *Österreich. Botan. Z.* **78**, 157.

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HYDROXYNORCYTISINE, A QUINOLIZIDONE ALKALOID FROM *LABURNUM ANAGYROIDES*

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Key Word Index—*Laburnum anagyroides*; Leguminosae; pods; 3-hydroxy-11-norcytisine; cytisine; quinolizidone alkaloids; dansyl derivatives.

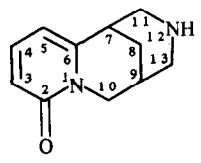
Abstract—A new type of higher plant alkaloid, 3-hydroxy-11-norcytisine, was isolated from the green pods of *Laburnum anagyroides* and characterized spectroscopically as its dansyl derivative.

INTRODUCTION

Laburnum anagyroides Med., formerly *L. vulgare* or *Cytisus Laburnum*, a small tree, native to central and southern Europe, is commonly grown in the U.K. for its long racemes of ornamental golden yellow flowers. All parts of the plant are toxic, especially the seeds [1–4]. Although not always fatal to cattle, sublethal doses can be excreted in the milk and indirectly poison humans. Symptoms of poisoning are irregular, weak heart action, vomiting and unconsciousness, followed by death [5]. The major toxin present is considered to be the quinolizidone alkaloid, cytisine (**1**) (synonyms baptitoxine, sophorine and ulexine) [6–8], first isolated from the species in 1862 [9]. We have now established the presence of a related compound, 5-hydroxy-7,11-diazatricyclo[7,2,1,$0^{2,7}$] dodeca-2,4-dien-6-one or 3-hydroxy-11-norcytisine (**2**), the first recorded example of a quinolizidine alkaloid having a five-membered C ring.

RESULTS AND DISCUSSION

During this work the alkaloids and amines present in *L. anagyroides* extracts were initially recovered by ion-exchange on carboxymethylcellulose and then made easier to detect and isolate by conversion to their fluorescent



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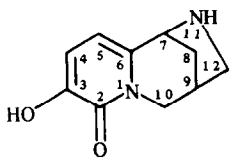

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*Author to whom correspondence should be addressed.

5-dimethylamino-naphthalene-1-sulphonyl- (dansyl or dns) derivatives.

Didns-3-hydroxy-11-norcytisine was first recognised as a yellow-green-fluorescent spot running close to the origin on standard 2D-chromatograms. Its R_fs were 0.05 and 0.34 in solvents A and B, respectively (see Experimental). After isolation, its ^{1}H NMR spectrum showed singlets at δ2.91 and 2.85, corresponding to the $(Me)_2N$ groups of dansyls in different states of combination. The degree of separation of these singlets indicated that one of the dansyls was linked to O, the other to N. The mass spectrum of the isolate showed a clear $[M]^+$ of precise m/z 658.1918, corresponding to $C_{34}H_{34}N_4O_6S_2$ (calculated m/z 658.1919). After subtracting two dns groups and adding 2H, this gave a molecular formula for the free compound of $C_{10}H_{12}N_2O_2$. Its aliphatic network was established from the individual proton couplings shown in Fig. 1, while chemical shifts suggested that two ends of this network were joined by N. Subtracting this part of the structure from the molecular formula left a residue of $C_5H_3NO_2$. In the isolate, one of these H atoms had been displaced by dns, so must have originally formed part of a phenolic OH group, while the chemical shifts of the others at δ5.54 and 6.87 were almost identical to those of two of the aromatic ring protons of dns-cytisine. The implication was that both parent alkaloids had an identical A ring skeleton, but this was substituted at position 3 or 5 in the isolate. NOE experiments on free cytisine indicated that the aromatic doublet missing from the spectrum of the isolate corresponded to the proton at position 3. The conclusion that the isolate was didns-3-hydroxy-11-norcytisine is supported by the data given in Fig. 1, which shows a more comprehensive comparison of its ^{1}H NMR assignments with those of dns-cytisine.

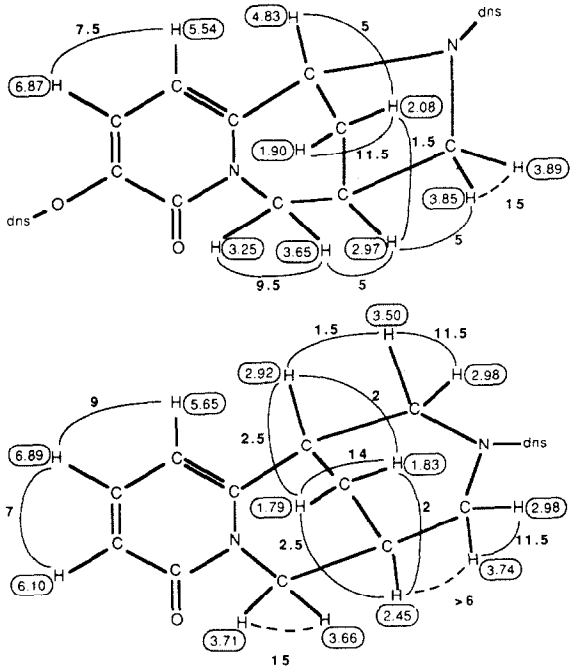

Fig. 1. Assignments of 400 MHz ^{1}H NMR spectra of didns-3-hydroxy-11-norcytisine (upper) and dns-cytisine (lower). Encircled numbers are $y=\delta$ppm. H$\overset{x}{\frown}$H $x=J_{H,H}$ Hz. Couplings indicated - - - were deduced from J values but all the others were positively demonstrated by individual decoupling experiments.

Dansylation has been used for the assay of cephaeline, emetine, ephedrine and morphine [10, 11] but this is the first time it has been applied to the isolation and characterisation of a new alkaloid. The method also revealed 14 slow running spots, having the chromatographic characteristics of dns-alkaloids, in extracts of *L. anagyroides*. The major component of this complex, having R_fs of 0.00 and 0.14 in solvents A and B respectively (see Experimental) was isolated and shown to be dns-cytisine: here recovery was poor as the solvent extraction methods used were adapted for the more polar dns-amines. However, recovery of didns-3-hydroxy-11-norcytisine, the second most prominent component, probably exceeded 50%, illustrating that dansylation can detect NH-containing alkaloids at the ng/g fr. wt level.

EXPERIMENTAL

Plant material. L. anagyroides pods were supplied by Westfield College, London NW3 7ST, U.K. and authenticated by Dr. G. J. Cunnell.

Amine dansylation and TLC. The procedures were as previously described in ref. [12], the dansylated products being chromatographed on silica gel in one of the following solvents before examination under 366 nm UV light: A, C_6H_{12}–EtOAc (2:3); B, C_6H_6–Et_3N (5:1); C, $CHCl_3$–*n*-BuOH–H_2O (5:3:1); D, $CHCl_3$–Et_3N (8:3); E, $CHCl_3$–C_6H_6–Et_3N (3:2:1).

The basic nitrogen fraction of *L. anagyroides* isolated by ion-exchange, was routinely analysed by dansylation followed by two-dimensional chromatography in solvents A and B.

Isolation of 3-hydroxy-11-norcytisine and cytisine as their dns derivatives. Green pods containing seeds (3 kg) were homogenized with 4 l MeOH and the residue re-extracted with 3 l MeOH–H_2O (7:3). The extracts were filtered, combined and taken to dryness *in vacuo* at 50°. The residue, in 1.5 l H_2O, was applied to a 40 × 3 cm diam. column of CM 52 (microgranular carboxymethylcellulose, Whatman) in the Na^+ form. After washing with 4 l H_2O, until the effluent gave no ninhydrin reaction, the amines were eluted with 4 l 0.5 M HOAc. The flow rate was 0.5 ml/min throughout and the temp. 4°. After evapn to dryness as before and re-evapn × 5, each with 100 ml H_2O, to remove residual acid, the bases were redissolved in 25 ml H_2O and divided into 30 equal fractions. Each was reacted with 1.65 ml dns chloride (Sigma, 30mg/ml Me_2CO) and incubated for 16 hr at 20° in darkened, sealed tubes in the presence of sufficient solid $NaHCO_3$ to saturate the mixt. Aq. 15% (w/v) proline (2 ml) was then added to react with residual dns chloride and, after leaving for 1 hr, the derivatized products were extracted by vortex mixing with 5 × 2 ml EtOAc. The combined organic phases were evapd to a vol. of 2–3 ml *in vacuo* and chromatographed on silica gel (Kieselgel 60G, Merck). The fluorescent bands were eluted with Me_2CO, concd *in vacuo* and rechromatographed in the next solvent. Thus, the sample was fractionated successively in solvents A–D and finally purified on Kieselgel 60HR in solvent E prepared from Aristar components. The products obtained were desiccated over P_2O_5 for 2 days. Yields of dns derivatives (free bases) were cytisine, 200 mg (90 mg); 3-hydroxy-11-norcytisine, 2 mg (0.6 mg).

Synthesis of authentic dns-cytisine. Cytisine (Sigma), 10 mg in 0.5 ml H_2O, was reacted with 1 ml dns chloride (5 ml/ml in Me_2CO) followed by 1 ml aq. proline soln. Dns-cytisine was extracted from the reaction mixt with 3 × 2 ml EtOAc and purified on Kieselgel 60HR in solvent D prepared from Aristar components. Other details were as described in the previous paragraph.

^{1}H NMR. Spectra were recorded in $CDCl_3$ at 400 MHz with ref. to $CHCl_3$ at 7.27 ppm. *Free cytisine.* 1.95 (*d*, H, J = 2.5 Hz, C-

8H), 1.96 (*d*, H, $J=2.5$ Hz, C-8H), 2.33 (*m*, H, $J=2$, 7 Hz, C-9H), 2.91 (*m*, H, $J=1.2$, 2.5 Hz, C-7H), 3.01 (*m*, 2H, $J=12$ Hz, C-11H and C-13H), 3.06 (*dd*, H, $J=2.5$, 12 Hz, C-11H), 3.11 (*dd*, H, $J=2$, 12 Hz, C-12H), 3.90 (*qd*, H, $J=1.5$, 7, 15, C-10H), 4.13 (*d*, H, $J=15$ Hz, C-10H), 6.00 (*dd*, H, $J=1.2$, 7 Hz, C-5H), 6.45 (*dd*, H, $J=1.5$, 9 Hz, C-3H), 7.30 (*q*, H, $J=7$, 9 Hz, C-4H). *Dns-cytisine*. 1.79 (*br d*, H, C-8H), 1.83 (*br d*, H, C-8H), 2.45 (*br s*, H, C-9H), 2.79 [s, 6H, $N(Me)_2$ of dns], 2.92 (*br s*, H, C-7H), 2.98 (*d*, 2H, C-11H), 3.50 (*dd*, H, C-11H), 3.66 (*d*, H, C-10H), 3.71 (*d*, H, C-10H), 3.74 (*t*, H, C-13H), 5.65 (*d*, H, C-5H), 6.10 (*d*, H, C-3H), 6.89 (*q*, H, C-4H), 7.06 (*d*, H, $J=8$ Hz), 7.33 (*t*, H, $J=8$, 8.5 Hz), 7.40 (*t*, H, $J=8.5$ Hz), 7.76 (*d*, H, $J=8.5$), 8.09 (*d*, H, $J=8$ Hz), 8.44 (*d*, H, $J=8.5$); protons in 7.06–8.44 region are from the $C_{10}H_6$ of dns; additional J values are shown in Fig. 1. *Didns-3-hydroxy-11-norcytisine*. 1.90 (*d*, H, C-8H), 2.08 (*m*, H, C-8H), 2.85 [*s*, 6H, $N(Me)_2$ of dns], 2.91 [*s*, 6H, $N(Me)_2$ of 2nd dns], 2.97 (*m*, H, C-9H), 3.25 (*d*, H, C-10H), 3.65 (*q*, H, C-10H), 3.85 (*dd*, H, C-12H), 3.89 (*d*, H, C-12H), 4.83 (*d*, H, C-7H), 5.54 (*d*, H, C-5H), 6.87 (*d*, H, C-4H), 7.13 (*d*, H, $J=7.5$ Hz), 7.23 (*d*, H, $J=7.5$ Hz), 7.41 (*t*, H, $J=7.5$ Hz), 7.50 (*t*, H, $J=7.5$ Hz), 7.52 (*t*, H, $J=8$ Hz), 7.65 (*t*, H, $J=8$ Hz), 8.08 (*d*, H, $J=8.5$ Hz), 8.19 (*dd*, H, $J=1$, 7.5 Hz), 8.26 (*dd*, H, $J=17.5$ Hz), 8.52 (*d*, H, $J=7.5$ Hz), 8.55 (*d*, H, $J=7.5$ Hz), 8.63 (*d*, H, $J=8$ Hz); protons in the 7.13–8.63 region are from the $C_{10}H_6$s of 2 dns; additional J values are shown in Fig. 1.

EIMS. *Dns-cytisine*: *m/z*, (rel. int.); 424 $[M+1]^+$ (17), 423 $[M]^+$ (75), 408 $[M-Me]^+$ (18), 191 $[M-dns+2H]^+$ (27), 190 $[M-dns+H]^+$ (8), 189 $[M-dns]^+$ (36), 171 $[C_{12}H_{12}N+H]^+$ (52), 170 $[C_{12}H_{12}N^+$ fm. dns] (37), 169 $[C_{12}H_{11}N]^+$ (29), 155 $[C_{11}H_9N$ fm. dns$]^+$ (14), 154 $[C_{11}H_8N]^+$ (15), 147 [cytisine residue $-C_2H_4N]^+$ (20), 146 [cytisine residue $-C_2H_5N]^+$ (39), 128 $[C_{10}H_6+2H]^+$ (12), 127 $[C_{10}H_6+H]^+$ (14). *Didns-3-hydroxy-11-norcytisine*: *m/z*. (rel. int.); 658 $[M]^+$ (0.3), 426 $[M-dns+2H]^+$ (1.0), 425 $[M-dns+H]^+$ (3.6), 424 $[M-dns]^+$ (1.6), 191 $[M-2dns+H]^+$ (1.4), 171 $[C_{12}H_{12}N+H]^+$ (10.5), 170 $[C_{12}H_{12}N$ fm. dns$]^+$ (9.8), 169 $[C_{12}H_{11}N]^+$ (4.2), 155 $[C_{11}H_9N$ fm. dns$]^+$ (2.2), 154 $[C_{11}H_8N]^+$ (3.1), 128 $[C_{10}H_6+2H]^+$ (1.9), 127 $[C_{10}H_6+H]^+$ (2.5), 126 $[C_{10}H_6$ fm. dns$]^+$ (1.1).

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REFERENCES

1. Wheelhouse, C. G. (1870) *Brit. Med. J. I*, 79.
2. Vance, J. (1877) *Lancet II*, 414.
3. Roberts, A. M. (1877), *Lancet II*, 341.
4. Biggs, M. G. (1883) *Brit. Med. J. I*, 1117.
5. Vickery, M. L. and Vickery, B. (1981) *Secondary Plant Metabolism* pp. 266, 269. Macmillan, London.
6. Dale, H. H. and Laidlaw, P.P. (1912) *J. Pharm. Exp. Ther.* **3**, 205.
7. Ing, H. R. (1931) *J. Chem. Soc.* 2195.
8. Leonard, N. J. (1953) in *The Alkaloids, Chemistry and Physiology* (Manske, R. H. F. and Holmes, H. L., eds) Vol 3, p. 119. Academic Press, London.
9. Husemann, A. and Marmé, W. (1862) *Edin. Med. J.* **7**, 908, 1025.
10. Frei, R. W., Santi, W. and Thomas, M. (1976) *J. Chromatog.* **116**, 365.
11. Tagliaro, F. and Frigerio, A. (1985) *J. Chromatog.* **330**, 323.
12. Hayman, A. R. and Gray, D. O. (1987) *Phytochemistry* **26**, 839.

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(+)-*N*-METHYLTILIAMOSINE, AN ALKALOID FROM *TILIACORA RACEMOSA*

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Key Word Index—*Tiliacora racemosa*; Menispermaceae; leaves; new diphenylbisbenzylisoquinoline alkaloid; *N*-methyltiliamosine.

Abstract—The leaves of *Tiliacora racemosa* yielded *N*-methyltiliamosine, a new diphenylbisbenzylisoquinoline alkaloid whose constitution was established from spectral as well as synthetic methods.

INTRODUCTION

The presence of *N*-methyltiliamosine in the leaves of *Tiliacora racemosa* was indicated earlier [1]. Evidence in support of the identification of this alkaloid are now presented in this communication.

*Authors to whom correspondence should be addressed.

RESULTS AND DISCUSSIONS

The alkaloid, $C_{37}H_{38}N_2O_6$ ($[M]^+$ *m/z* 606), $[\alpha]_D^{25°}$ $+510°$ (*c* 1.5, $CHCl_3$), was isolated in low yield by prep. TLC. The UV [λ_{max}^{EtOH} 240 sh (log ε 4.65), 291 nm (log ε 4.0); $\lambda_{max}^{EtOH-0.1\ N\ NaOH}$ 304 nm (log ε 3.84)], IR [ν_{max}^{KBr} 3375 (hydrogen bonded OH)], 400 MHz ^{1}H NMR (Table 1) and mass [*m/z* 606, 607, 605, 591, 380, 379, 366, 365, 349, 303, 190] spectra were comparable to tiliamosine (**1**) [2]