Phytochemistry, Vol. 25, No. 6, pp. 1511–1513, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

THE C_{19}-DITERPENOID ALKALOIDS OF *DELPHINIUM BICOLOR*

PALANIAPPAN KULANTHAIVEL, MICHAEL BENN* and WALTER MAJAK†

Chemistry Department, The University, Calgary, Alberta, Canada, T2N 1N4; †Research Station, Agriculture Canada, Kamloops, B.C., Canada, V28 8A9

(*Received* 4 *November* 1985)

Key Word Index—*Delphinium bicolor*; Ranunculaceae; diterpenoid alkaloids; methyllycaconitine.

Abstract—Eight C_{19}-diterpenoid alkaloids were isolated from *Delphinium bicolor*, including the neurotoxic methyllycaconitine and a new alkaloid, bicolorine 6-*O*-acetate.

INTRODUCTION

Of the species of *Delphinium* endemic to Canada, *D. bicolor* Nutt. is encountered as an early blossoming plant of forage on Western range-land. Known as low larkspur, it has a reputation for being toxic to cattle [1, 2]. We suspected that the toxicity would be due to diterpenoid alkaloids, as has been established for several other species [3–6], and accordingly undertook an analysis of *D. bicolor* for such compounds. In previous communications [7–10] we described two new alkaloids, A and B, isolated during preliminary stages of this study. We now report our further findings.

RESULTS AND DISCUSSION

Whole plants were collected while in full blossom. Extraction and fractionation of the plant bases by conventional procedures resulted in the isolation of eight alkaloids. Spectroscopic investigations revealed all of these to be C_{19}-diterpenoids, seven of which were known: alkaloids A (**2**) and B (**10**) (bicoloridine and bicolorine, see later), condelphine (**1**), delcosine (**9**), isotalatizidine (**6**), karacoline (**5**) and methyllycaconitine (**3**). The eighth was identified as a new alkaloid on the basis of the following analyses of spectroscopic data: MS (*m/z* 435) suggested a molecular composition $C_{24}H_{37}NO_6$; the IR spectrum contained absorptions for hydroxyl and ester functionalities; the ^{1}H NMR spectrum similarly revealed the presence of a *C*-methyl, acetate, *N*-ethyl and methoxyl; and as well there were absorptions with the positions and multiplicities expected for hydroxylation at C-1 (α) and C-14 (α), and acetoxylation at C-6 (β) of an aconitane skeleton. Such alkaloids are almost invariably β-methoxylated at C-16, so we arrived at **4** as the structure for the compound, with which the ^{13}C NMR data (see Table 1) were in full accord. This corresponded to the 6-*O*-acetate of alkaloid B, an apparently previously unknown compound, and indeed upon saponification it gave **10**.

It now seems appropriate to give alkaloids A and B more specific names, and we have decided to call them bicoloridine and bicolorine respectively. Our new alkaloid is then bicolorine 6-*O*-acetate.

The presence of methyllycaconitine as a major alkaloid (we estimate it to comprise 30–50% of the total bases) would account for the reputed poisonous properties of *D. bicolor*, for it is a very potent nicotinic neuromuscular toxin [4–6].

EXPERIMENTAL

Instrumental methods were as described previously [11]. Prep. TLC was performed using 1 mm thickness plates of silica gel 60 PF 254 (E. Merck), and compounds were recovered by scraping off the appropriate zones (detected with I_2 vapour) and extracting them with MeOH–$CHCl_3$ (*ca* 3:1 v/v).

Plant material. Plants were collected along the Sheep Creek and Sibbald Flats forestry roads SW of Calgary. Voucher specimens are deposited in the Herbarium of the University of Calgary.

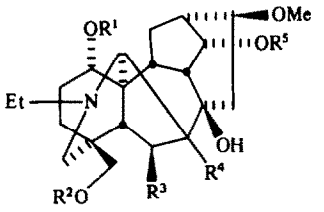
Extraction and isolation of the alkaloids. Freshly collected

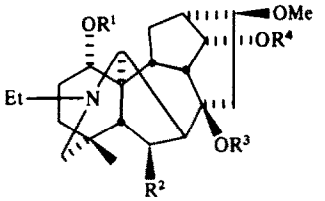
Table 1. ^{13}C NMR data* for bicolorine 6-*O*-acetate (**4**)

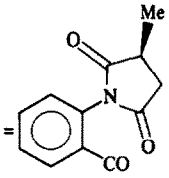
Carbons	Shift (multiplicity)	Carbons	Shift (multiplicity)
1	72.72 (*d*)	13	45.19 (*d*)
2	29.71 (*t*)†	14	76.19 (*d*)
3	31.88 (*t*)	15	43.56 (*t*)
4	33.16 (*s*)	16	81.92 (*d*)
5	44.31 (*d*)	17	65.51 (*d*)
6	72.55 (*d*)	18	27.35 (*q*)
7	48.55 (*d*)	19	61.34 (*t*)
8	74.60 (*s*)	>N–CH_2	48.45 (*t*)
9	52.36 (*d*)	Me	13.08 (*q*)
10	39.34 (*d*)	OMe at C-16	56.30 (*q*)
11	48.25 (*s*)	–C=O	170.81 (*s*)
12	28.94 (*t*)†	Me	21.75 (*q*)

*At 50.2 MHz, in $CDCl_3$, shifts relative to TMS = 0.
†Assignments may be interchanged.

*To whom correspondence should be addressed.







1 $R^1 = R^3 = R^4 = H$, $R^2 = Me$, $R^5 = Ac$
3 $R^1 = R^5 = H$, $R^3 = OMe$, $R^4 = OH$, $R^2 =$
6 $R^1 = R^3 = R^4 = R^5 = H$, $R^2 = Me$
8 $R^1 = R^2 = R^5 = Me$, $R^3 = OMe$, $R^4 = OH$
9 $R^1 = R^5 = H$, $R^2 = Me$, $R^3 = OMe$, $R^4 = OH$

2 $R^1 = R^4 = H$, $R^2 = OAc$, $R^3 = Me$
4 $R^1 = R^3 = R^4 = H$, $R^2 = OAc$
5 $R^1 = R^2 = R^3 = R^4 = H$
7 $R^1 = R^4 = Ac$, $R^2 = OAc$, $R^3 = H$
10 $R^1 = R^3 = R^4 = H$, $R^2 = OH$

whole plants were repeatedly macerated (Waring blender) in EtOH. Air dried and powdered (Wiley Mill) plant was percolated with EtOH. Evaporation of the EtOH extracts (temp. < 50°) gave dark gums from which the bases were isolated in the usual way [12]. Typically 0.2–0.3% wt/marc of alkaloids were thus obtained, and TLC (MeOH–$CHCl_3$, 1:4) showed no significant differences in the composition of the mixtures obtained from the fresh or air-dried plants.

The crude alkaloidal fraction (4.2 g) was chromatographed on a column of neutral Al_2O_3 (225 g, Woelm, Grade III). Fractions were collected as follows: Fr. 1, C_6H_6 (500 ml); 2, C_6H_6–$CHCl_3$ (1:1; 200 ml); 3, C_6H_6–$CHCl_3$ (1:1; 100 ml); 4–16, C_6H_6–$CHCl_3$ (1:1; 13 × 50 ml); 17–25, C_6H_6–$CHCl_3$ (1:4; 9 × 50 ml); 26–41, $CHCl_3$ (16 × 50 ml); 42–46, $CHCl_3$–MeOH (49:1; 5 × 50 ml); 55–56, $CHCl_3$–MeOH (9:1; 2 × 50 ml); 57–60, MeOH (4 × 50 ml).

Fr. 10–12 (0.290 g) showed identical TLC ($CHCl_3$–MeOH, 9:1) and were combined. Purification by prep. TLC with the above solvent system, followed by crystallization from Et_2O–hexane, afforded condelphine (**1**, 140 mg), mp 160–161°, identified by direct comparison (IR, ^{1}H NMR [13]).

TLC ($CHCl_3$–MeOH, 9:1) of Fr. 20–21 (64 mg) showed predominantly a single component, and purification by prep. TLC gave bicolorine (**2**, 45 mg), identified by direct comparison (IR, ^{1}H NMR [10]). A similar TLC analysis showed that Fr. 22–31 (460 mg) contained **3**, and this was confirmed by ^{1}H NMR. Fr. 32–34 (0.347 g) upon purification by prep. TLC ($CHCl_3$–MeOH, 9:1) gave mainly two bands. The faster moving of these yielded a mixture (240 mg) containing methyllycaconitine (**3**) and **4** in the ratio ~ 3:1 (from ^{1}H NMR). The lower band (10 mg) was homogeneous and yielded karakoline (**5**), mp 181–183°; identified by direct comparison (IR, ^{1}H NMR [13, 14]). Purification of Fr. 35 and 36 (0.187 g) by prep. TLC ($CHCl_3$–MeOH, 9:1) afforded from the upper band a mixture of **3** and **4**, in the ratio 3:1 (70 mg); while a lower band furnished a mixture of **4** and isotalatizidine (**6**, 105 mg). The lower band upon refractionation by prep. TLC, with the same solvent system yielded as the less polar component spectroscopically pure **4** (35 mg), mp 165–167° (CH_2Cl_2–hexane); $[\alpha]_D$ + 19° (*c* 0.2; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3420 br, 1736, 1457, 1439, 1395, 1366, 1253, 1225, 1091, 1042; EIMS *m/z* (%): 435 $[M]^+$ (11), 418 (66), 374 (21); ^{1}H NMR ($CDCl_3$): δ5.37 (*br d*, J = 7.5 Hz, H-6), 4.22 (*t*, J = 5 Hz, H-14), 3.75 (*m*, $W_{1/2}$ = 8 Hz, H-1), 1.12 (*t*, J = 7 Hz, >N–Et), 0.99 (H-18), 3.34 (OMe), 2.04 (OAc). The ^{13}C NMR data is listed in Table 1. The more polar component proved to be isotalatizidine (**6**). Similar treatment of Fr. 39–43 (0.265 g) gave a mixture of **3** and **4** (60 mg), and 160 mg of isotalatizidine (**6**), mp 139–140°, identified by direct comparison (mp, IR, ^{1}H and ^{13}C NMR).

A portion (50 mg) of the mixture of **3** and **4** was acetylated with Ac_2O (1 ml) in pyridine (1 ml) for 2 days. Excess reagent was removed *in vacuo* and the residue was purified by prep. TLC ($CHCl_3$–MeOH, 9:1) to yield from the upper band **7** (12 mg), mp 177–178° ($CHCl_3$–hexane). IR ν_{max}^{KBr} cm^{-1}: 3470 br, 1744, 1736, 1720, 1367, 1248, 1230, 1092; EIMS *m/z* (%): 519 $[M]^+$ (1), 476 (28), 460 (81), 400 (27); ^{1}H NMR ($CDCl_3$): δ5.31 (*br d*, J = 7.5 Hz, H-6), 4.88 (*dd*, J = 10.5, 7.0 Hz, H-1), 4.77 (*t*, J = 5 Hz, H-14), 1.05 (*t*, J = 7 Hz, >N–Et), 0.87 (H-18), 3.23 (OMe), 2.04, 2.03 × 2 (OAc). The lower band gave **3** (30 mg).

Another portion (50 mg) of the mixture of **3** and **4**, in MeOH (5 ml) was stirred with K_2CO_3 (100 mg) in H_2O (1 ml) for 3 hr under N_2. The MeOH was removed under red. pres., and the mixture diluted with 10 ml of H_2O and extracted with $CHCl_3$. The gummy product after removal of $CHCl_3$ was purified by prep. TLC ($CHCl_3$–MeOH, 4:1); which furnished from the upper band **10** (9 mg), identical in all respects (mp, IR, ^{1}H NMR) with the natural substance bicolorine (= alkaloid B) [2]. The lower band afforded lycoctonine (**8**) (12 mg), the IR spectrum of which was superimposable upon that of an authentic specimen.

Purification of Fr. 47–49 (250 mg) by prep. TLC ($CHCl_3$–MeOH, 4:1), and crystallization of the products from Me_2CO–hexane gave delcosine (**9**, 240 mg), mp 202–203°, whose identity was confirmed by comparison (IR, ^{1}H NMR) with an authentic specimen. Fr. 55–56 (76 mg) contained one major substance, but had several minor impurities. Purification by prep. TLC ($CHCl_3$–MeOH, 4:1) provide pure **10** (35 mg), mp 184–186°, again identified by comparison of its IR spectrum with that of an authentic sample.

Acknowledgements—We thank Prof. S. W. Pelletier for a reference specimen of isotalatizidine. Financial support of our work was provided by a grant-in-aid of research (to M.H.B.) from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. Looman, J., Majak, W. and Smoliak, S. (1985) *Stock Poisoning Plants of Western Canada*. Contribution 1982-7E, Agriculture Canada, Ottawa.

2. Moss, E. H. (1959) *Flora of Alberta*, p. 233. University of Toronto Press.
3. Cronin, E. H. and Nielsen, D. B. (1977) in *Effects of Poisonous Plants on Livestock* (Keeler, R. F., van Kampen, K. R. and James, L. F., eds) p. 521. Academic Press, New York; see also the following chapter by Olsen, J. D., p. 535.
4. Aiyar, V. N., Benn, M. H., Hanna, T., Jacyno, J., Roth, S. H. and Wilkens, J. L. (1979) *Experientia* **35**, 1367.
5. Nation, P. N., Benn, M. H., Roth, S. H. and Wilkens, J. L. (1982) *Can. Vet. J.* **23**, 264.
6. Benn, M. H. and Jacyno, J. (1983) in *Alkaloids: Chemical and Biological Perspectives* (Pelletier, S. W., ed.) Vol. 1, p. 233. Wiley-Interscience, New York.
7. Jones, A. J. and Benn, M. H. (1972) *Tetrahedron Letters* 4351.
8. Jones, A. J. and Benn, M. H. (1973) *Can. J. Chem.* **51**, 486.
9. Pelletier, S. W., Mody, N. V., Jones, A. J. and Benn, M. H. (1976) *Tetrahedron Letters* 3025.
10. Codding, P. W., Kerr, K. A., Benn, M. H., Jones, A. J., Pelletier, S. W. and Mody, N. V. (1980) *Tetrahedron Letters* **21**, 127.
11. Rüeger, H. and Benn, M. H. (1982) *Can. J. Chem.* **60**, 2918.
12. Benn, M. H. (1966) *Can. J. Chem.* **44**, 1.
13. Sultankhodzhaev, M. N., Yunusov, M. S. and Yunusov, S. Yu. (1973) *Khim. Prir. Soedin.* 199.
14. Pelletier, S. W., Mody, N. V., Joshi, B. S. and Schramm, L. (1984) in *Alkaloids: Chemical and Biological Perspectives* (Pelletier, S. W., ed.) Vol. 2, p. 205. Wiley-Interscience, New York.