1″, *d*), 3.95 (OMe, *s*), 3.92 (H-6″, *dd*), 3.70 (H-6″, *dd*), 3.56 (H-2″, *dd*), 3.51 (H-5″, *m*), 3.48 (H-3″, *dd*), 3.38 (H-4″, *dd*); ^1H–^1H coupling constants (Hz) J (6, 8) = 2.4, J (1″, 2″) = 7.6, J (2″, 3″) = 9.0, J (3″, 4″) = 9.0, J (4″, 5″) = 9.5, J (5″, 6″) = 2.4 and 6.2, J (6‴,6″) = 12.0; ^{13}C chemical shifts: 153.3 (C-2), 126.9 (C-3), 177.6 (C-4), 160.6/160.3 (C-5/C-9), 97.2 (C-6), 165.6 (C-7), 105.2 (C-8), 111.3 (C-10), 124.0 (C-1′), 131.8 (C-2′/6′), 116.2 (C-3′/5′), 158.7 (C-4′), 104.1 (C-1″), 74.8 (C-2″), 77.2 (C-3″), 71.3 (C-4″), 78.9 (C-5″), 62.5 (C-6″), 56.9 (OMe).

Acknowledgement—S.A.K. thanks the Alexander-von-Humboldt foundation for the award of a fellowship and the University of Khartoum for a sabbatical leave.

REFERENCES

1. Ingham, J. L. (1983) in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Grisebach, H. and Kirby, G. W. eds), **43**, p. 1. Springer, Wien.
2. Mitra, J., Das, A. and Joshi, T. (1983) *Phytochemistry* **22**, 1063.
3. *Chem. Abstr.* **56**: 1176g.
4. Markham, K. R., Chari, V. M. and Mabry, T. J. (1982) in *The Flavanoids, Advances in Research*, (Harborne, J. B. and Mabry, T. J., eds), p. 19. Chapman & Hall, London.
5. Gellért, M., Szendrei, K., Dinya, Z. and Répási, J. (1985) *Flavanoids and Bioflavanoids* (Farkas, L., Gábor, M. and Kállay, F., eds). Akademia Kiado, Budapest.

Phytochemistry, Vol. 28, No. 5, pp. 1561–1563, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00

DELBIDINE, AN ALKALOID FROM A HYBRID POPULATION OF *DELPHINIUM OCCIDENTALE* AND *DELPHINIUM BARBEYI*

BALAWANT S. JOSHI, HARIDUTT K. DESAI, EL-SAYEDA A. EL-KASHOURY, S. WILLIAM PELLETIER* and JOHN D. OLSEN†

Institute for Natural Products Research and the Department of Chemistry, School of Chemical Sciences, The University of Georgia, Athens, GA 30602, U.S.A. †Poisonous Plant Research Laboratory, Agricultural Research Western Region, U.S. Department of Agriculture, Logan, UT 84321, U.S.A.

(*Received* 19 *September* 1988)

Key Word Index—*Delphinium occidentale/barbeyi*; Ranunculaceae; C_{20}-diterpenoid alkaloid, delbidine.

Abstract—A new C_{20}-diterpenoid alkaloid delbidine has been isolated from a hybrid population of *Delphinium occidentale* (S. Wats.) S. Wats. and *Delphinium barbeyi* (Huth) Huth and its structure elucidated by spectroscopic methods. The structure was confirmed by its correlation with geyeridine.

INTRODUCTION

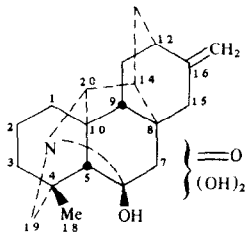
The diterpenoid alkaloids occur mostly in several of the *Aconitum* and *Delphinium* species which belong to the Ranunculaceae. These alkaloids have long been of interest because of their usefulness in medicine and intensely poisonous property. *Delphinium barbeyi* Huth is a herbaceous perennial plant native to the Rocky Mountain region of the United States. It grows above 7000 ft in the mountains of Utah, Wyoming and Colorado [1].

The occurrence of the C_{19}-diterpenoid alkaloids anthranoyllycoctonine [2], delpheline [3], deltaline [3] and lycoctonine [2, 3] from *D. barbeyi* Huth (*D. glaucum* S. Wats) has been recorded by earlier investigators. Recently from a hybrid population of *Delphinium occidentale* (S. Wats.) S. Wats. and *Delphinium barbeyi* (Huth) Huth delcosine [4], deltamine [4] and dictyocarpine [4] have been reported. The isolation of an unusual alkaloid barbeline containing a C(19) = N azomethine group in the C_{19}-diterpenoid alkaloid skeleton has also been reported [5] from this hybrid population. In the present note we wish to report the isolation and structure elucidation of a new C_{20}-diterpenoid alkaloid from this hybrid population.

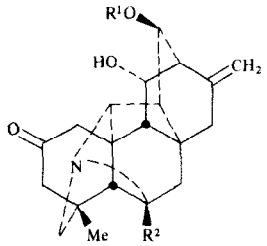
*Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

Ethanol extraction and subsequent isolation of the basic fraction of the *Delphinium* hybrid gave a crude alkaloid which was found by TLC to be a mixture of a number of alkaloids. The mixture was purified by vacuum liquid chromatography [6] on alumina and a polar fraction was collected by elution with acetone–25% methanol. From this fraction, delbidine (**3**) was obtained



1



2 $R^1 = R^2 = H$
3 $R^1 = H, R^2 = OH$
4 $R^1 = Ac, R^2 = OH$

as colourless cubes mp > 360°. The IR spectrum exhibited bands at 3508, 3360 (OH), 1685 (C=O), 1660 (C=CH_2) cm^{-1} and the ^1H NMR spectrum (DMSO-d_6) showed signals due to a tertiary methyl group δ1.36 (3H, *s*), and an exocyclic methylene group at δ4.50, 4.70 (each 1H, *br s*). The ^{13}C NMR spectrum showed 20 lines for 20 carbon atoms of the molecule. The proton noise-decoupled spectrum and DEPT studies indicated six quaternary carbon singlets, seven methine carbon doublets, six methylene carbon triplets and one methyl quartet (Table 1). On the basis of these data, the molecular formula $C_{20}H_{25}NO_4$ (M_r 343) was derived for delbidine. As the new alkaloid contains 20 carbon atoms, biogenetic considerations and the absence of an *N*-methyl group in the molecule suggest that delbidine is a C_{20}-diterpenoid alkaloid having the hetisan-type skeleton [7]. The quaternary carbon at δ97.9 is indicative of a carbinolamine carbon. IR and ^{13}C NMR spectral evidence suggest the presence of two secondary hydroxyl groups (3508, 3360 cm^{-1}; δ73.3, 69.9) and a ketonic function (1685 cm^{-1}; δ212.9). Thus, a partial structure (**1**) having one carbonyl and two hydroxyl groups can be derived. The keto group may be located on the methylene carbons C-1, C-2, C-3, C-7, C-11, C-13 or C-15. The quaternary carbon signals at δ42.3, 45.2, 55.6 and 148.1 should be assigned to C-4, C-8, C-10 and C-16, respectively, as in 2-dehydrohetisine (**2**) [8]. On the basis of the chemical shifts of the quaternary carbons, location of the carbonyl group on the adjacent carbons at C-1, C-3, C-7 and C-15 can be readily discounted, as this would lead to a downfield shift of the quaternary carbons. This evidence reduces location of the keto group to three positions C-2, C-11 or C-13. When the keto group is present at C-11 or C-13, the quaternary carbon at C-4 shows a chemical shift ~36.5–37.5 ppm as in 11-dehydrohetisine, 13-dehydro-2, 11-diacetylhetisine [8], spiradine A[Yu, D.-Q., personal communication] or spirasine X [9]. The chemical shift for C-2 in alkaloids which do not bear an OH group at this position appears ~ 19.3 ppm. Delbidine does not show a singlet ~δ36–38 or a triplet ~δ19 ppm indicating that the keto group is located at C-2. Location of the carbonyl group at C-2 is also supported by the CD measurement which shows a very small contribution to a negative Cotton effect [8]. The partial structure (**1**) can therefore be expanded in which the two hydroxyl groups have to be on C-1, C-3, C-7, C-11, C-13 or C-15. When C-15 bears a hydroxyl group, C-16 appears downfield at ~δ156–157 as in hypognavine [10] and sanyonimine [11]. Location of a hydroxyl group at C-1, C-3 or C-7 adjacent to the quaternary carbons C-10, C-4 and C-8 will bring about a downfield shift of these carbons. On the basis of these data delbidine has been assigned the structure (**3**).

Table 1. ^{13}C NMR assignments for delbidine (**3**), geyeridine (**4**) [12] and 2-dehydrohetisine (**2**) [8]

	^{13}C NMR, ppm (multiplet)		
	3	**4***	**2**
C	CD_3SOCD_3	$CDCl_3$	$CDCl_3$
1	44.2 (*t*)	43.2	45.2
2	212.9 (*s*)	210.0	214.9
3	51.5 (*t*)	51.4	49.9
4	42.3 (*s*)	42.8*	42.2
5	60.9 (*d*)	59.2	60.4
6	97.9 (*s*)	100.2	65.2
7	33.3 (*t*)	32.9	36.1
8	45.2 (*s*)	45.9*	44.4
9	51.3 (*d*)	52.2	55.0
10	55.6 (*s*)	55.7	55.7
11	69.9 (*d*)	69.8	71.5
12	53.7 (*d*)	48.6	52.0
13	73.3 (*d*)	75.3	75.6
14	51.1 (*d*)	49.7	50.8
15	44.0 (*t*)	42.9	33.9
16	148.1 (*s*)	143.2	145.4
17	106.1 (*t*)	109.9	108.2
18	30.2 (*q*)	30.1	28.7
19	62.7 (*t*)	59.9	64.4
20	68.9 (*d*)	68.4	70.3
CO	—	170.6	—
Me	—	21.3	—

*Values rounded to the first decimal. These values have been reversed from the literature values, [12], for the sake of consistency of assignments, C-4 being ~ 3.0 ppm upfield when compared to C-8.

The structure of delbidine was confirmed by correlation with geyeridine (**4**) [12], the structure of which has been established by extensive ^1H NMR and 2D NMR COSY studies. Hydrolysis of geyeridine hydrochloride with methanolic potassium hydroxide gave a crystalline compound that proved to be identical in all respects with delbidine.

EXPERIMENTAL

MS: direct inlet, 70 eV; Finnegan Quadrupole 4023; ^{13}C NMR: 15.03, 22.49 MHz in $CDCl_3$ with TMS as int. standard in the Fourier mode. Plant material was collected, 26 and 27 June 1980, near the summit of the Wasatch Plateau in Fairview Canyon, Utah. The plant in pre-bud stage of growth ranged from 30 to 51 cm in height. A voucher plant specimen from the plant community collected is on file in the Intermountain Herbarium, Department of Biology, Utah State Univmrsity, Logan Utah 84321-5500 (specimen no. UTC 198416). The plant was initially identified in the vegetative stage as *Delphinium barbeyi* (Huth) Huth [5]. However, more careful study of the plant population in the mature stage of growth by Dr M. J. Warnock (Division of Life Sciences, Geology and Geography, Sam Houston State University, Huntsville, TX 77341) has revealed that the plants consist of a hybrid population of *Delphinium occidentale* (S. Wats.) S. Wats. and *Delphinium barbeyi* (Huth) Huth.

Isolation of delbidine (3). The ground plant was extracted by percolation with 95% EtOH and evapd under red. pres. The EtOH extract was processed in the usual manner by acid–base extraction (pH 8.5) for the isolation of alkaloids. The crude base (11.5 g) was dissolved in $CHCl_3$ (30 ml), MeOH (1 ml) and chromatographed on a VLC column (90 g Al_2O_3, EM-1085-4) and eluted (28 fractions, 100 ml each) with hexane, Me_2CO and Me_2CO–MeOH. Fraction 26 (190 mg) when suspended in CH_2Cl_2 gave a ppt. (45 mg) which crystallized from MeOH as colourless cubes mp > 360°, $[\alpha]_D^{25°}$ + 22.3° (MeOH; *c* 0.268); MS, *m/z* 343 (M^+, 10%), 326 (5), 287 (7), 269 (20), 176 (19), 91 (49), 55 (100). IR ν_{max}^{nujol} 3508, 3360, 1685, 1660, 1460, 1370, 1350, 1332, 1315, 1295, 1280, 1265, 1220, 1200, 1180, 1085, 1065, 1040, 1030, 1000, 960, 940, 910, 880, 860, 810 cm^{-1}. ^1H NMR (DMSO-d_6): 1.36 (3H, *s*, C-18 Me), 4.5 (1H, *br s*, C-17 H), 4.7 (1H, *br s*, C-17 H).

Hydrolysis of geyeridine hydrochloride (4) to give (3). Geyeridine HCl (4; 5.1 mg) was dissolved in 3% KOH in MeOH (3 ml) and kept at room temp. for 16 hr. Usual work-up gave a gum (2.9 mg) which crystallized from MeOH to afford 1 as colourless cubes, mp > 360° TLC, CO-TLC and IR spectra showed identity with delbidine.

Acknowledgement—We are grateful to Prof. F. R. Stermitz for sending us a sample of geyeridine·HCl.

REFERENCES

1. Beath, O. A. (1939) *Univ. Wyo. Agr. Exp. Sta. Bull.* **231**, 18.
2. Cook, W. B. and Beath, O. A. (1952) *J. Am. Chem. Soc.* **74**, 1411.
3. Carmack, M., Ferris, J. P., Harvey, J., Magat, P. L., Martin, E. and Mayo, D. W. (1958) *J. Am. Chem. Soc.* **80**, 497.
4. Maddry, J. A. (1985) Ph.D. Thesis, University of Georgia.
5. Joshi, B. S., El-Kashoury, E. A., Desai, H. K., Holt, E. M., Olsen, J. D. and Pelletier, S. W. (1988) *Tetrahedron Letters* **29**, 2397.
6. Pelletier, S. W., Chokshi, H. P. and Desai, H. K. (1986) *J. Nat. Prod.* **49**, 892.
7. Chem. Abs. (chem. subst. index) (1972) **77**, 1852cs.
8. Glinski, J. A., Joshi, B. S., Jiang, Q. P. and Pelletier, S. W. (1988) *Heterocycles* **27**, 185.
9. Sun, F., Liang, X. T. and Yu, D.-Q. (1987) *J. Nat. Prod.* **50**, 923.
10. Sakai, S. I., Yamaguchi, K., Yamamoto, I., Hotoda, K., Okazaki, K., Aimi, N. and Okamoto, T. (1983) *Chem. Pharm. Bull. Jpn.* **31**, 3338.
11. Sakai, S. I., Yamaguchi, K., Takayama, H., Yamamoto, I. and Okamoto, T. (1982) *Chem. Pharm. Bull. Jpn.* **30**, 4576.
12. Grina, J. A., Schroeder, D. R., Wydallis, E. T. and Stermitz, F. R. (1986) *J. Org. Chem.* **51**, 390.

Phytochemistry, Vol. 28, No. 5, pp. 1563–1565, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00
Pergamon Press plc.

CYCLOPEPTIDE ALKALOIDS FROM *ZIZYPHUS RUGOSA* BARK

Y. C. TRIPATHI, S. K. MAURYA, V. P. SINGH and V. B. PANDEY

Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi -221 005, India

(*Received* 29 *June* 1988)

Key Word Index—*Zizyphus rugosa*; Rhamnaceae; alkaloids; nummularine-P; sativanine-H; rugosanine-B.

Abstract—The isolation of cyclopeptide alkaloids, nummularine-P, sativanine-H and rugosanine-B, a new 13-membered cyclopeptide alkaloid from the bark of *Zizyphus rugosa* is reported. The structure of the new alkaloid was elucidated by spectroscopic methods as well as by chemical degradation.

INTRODUCTION

Zizyphus rugosa Lam (Family: Rhamnaceae) is a large shrub distributed throughout India. In the Indian system of medicine the bark of this plant is used in the treatment of diarrhoea [1]. In continuation of our search for peptide alkaloids from the bark of *Z. rugosa* [2, 3], we now report here the isolation and characterization of a new 13-membered cyclopeptide alkaloid, rugosanine-B together with two known peptide alkaloids nummularine-P [4] and sativanine-H [5].

RESULTS AND DISCUSSION

Column chromatography of the basic fraction of *Z. rugosa* bark and repeated preparative TLC of chloroform–methanol (5:1) eluants on silica gel furnished