Phytochemistry, Vol 29, No 1, pp 293–295, 1990
Printed in Great Britain

0031-9422/90 $3 00+0 00
Pergamon Press plc

BARBISINE, A C_{20}-DITERPENOID ALKALOID FROM *DELPHINIUM BARBEYI*

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(*Received in revised form* 8 *June* 1989)

Key Word Index—*Delphinium barbeyi*, Ranunculaceae, diterpenoid alkaloid, barbisine

Abstract—The structure and stereochemistry of barbisine, a new C_{20}-diterpenoid alkaloid from *Delphinium barbeyi*, were deduced by a combination of NMR spectroscopy and a single-crystal X-ray diffraction analysis

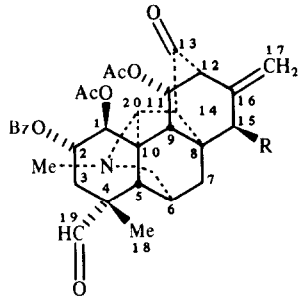
INTRODUCTION

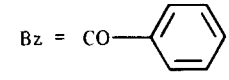
Vakognavine (**1**), and its congener 15-deacetylvakognavine (**2**), from *Aconitum palmatum*, are the only examples known of an *N*-C(19)-*seco* C_{20}-diterpenoid alkaloid [1–3] We report here the isolation from *Delphinium barbeyi* of a new representative (**3**) of this type. Previous studies from our laboratory on the phytochemistry of this taxon have dealt with norditerpenoid alkaloids [4, 5].

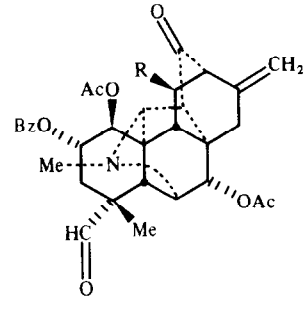
RESULTS AND DISCUSSION

Chromatography of that portion of the alkaloids from *D. barbeyi* which was extracted from an aqueous solution at pH 6 by chloroform yielded barbisine (**3**), mp 251–254° The ^{1}H NMR (see Experimental) and ^{13}C NMR (Table 1) spectral data of **3** showed that it is a C_{20}-diterpenoid alkaloid possessing *N*-methyl and exocyclic methylene groups. The presence of a hydroxyl group (IR band at 3300 cm^{-1}) and three ester functions, as two acetates and a benzoate, was also evident from the examination of the NMR data Spin decoupling established that two of the three ester functions, were located on adjacent carbons of ring A. Thus, by irradiating at δ5.17 (H-1, *d*, J=3 1 Hz) the quartet (J=3 1 Hz) signal at δ5.30 collapsed to a triplet; this signal was split indicating the presence of three vicinal protons. The observed 3.1 Hz coupling constants exclude axial–axial relationships of the vicinal protons, but either axial–equatorial or equatorial–equatorial orientations remain. In the ^{1}H NMR spectrum of barbisine monoacetate (**4**), formed when **3** was treated with acetic anhydride–pyridine, a signal at δ4.78 (*d*, J =4.5 Hz) coupled to another at δ3.43 corresponds to a carbinyl resonance (seen at δ3.78 in **3**) and thereby establishes the presence of a secondary alcohol in **3** Furthermore the downfield shift of the adjacent proton (from δ3 10 in **3**) indicates that these protons are likely to be *cis* to each other

Assuming on biogenetic grounds that H-9 is β, the above data can be best rationalized by locating the secondary hydroxyl in **3** at C-11 and oriented β. This assignment was supported by the small coupling constant (4.5 Hz) between H-9 and H-11 and nearly zero between H-11 and H-12 [6] The remaining two oxygen atoms of the molecular formula were accounted for by an aldehyde function (δ9 23 *s*, ^{1}H NMR and δ196 6 ^{13}C NMR) and a keto function (δ208 8 ^{13}C NMR). The position of the aldehyde group was assigned to C-4 by analogy with known vakognavine derivatives [3] This assignment was supported by the identical chemical shifts for C-3, C-4,







1 R = OAc
2 R = OH

3 R = OH
4 R = OAc

Table 1 ^{13}C NMR data* of compounds **3** and **4** (δ ppm $CDCl_3$)

C†	3	4	C	3	4
1	68 8	68 5	16	135 5	135 0
2	68 4	67 8	17	115 7	115 4
3	29 6	29 5	18	26 1	26 0
4	43 9	43 6	19	196 6	193 1
5	59 3	57 5	20	67 2	67 6
6	61 7	61 2	N-Me	34 9	35 0
7	74 5	73 9	C=O | Me	170 0	169 9
8	46 9	47 0		20 9	20 9
9	56 4	56 4	C=O | Me	170 0	169 0
10	54 1	53 5		20 6	20 5
11	63.2	65 6	C=O | Me	—	168 6
12	61 0	56.5		—	20 5
13	208 8	206 9	Ph-C=O	165 3	165 1
14	50 6	50 8	1	129 6	129 5
15	28 9	28 8	2, 6	129 6	129 5
			3, 5	128 6	128 6
			4	133 5	133 3

*Assignments were made by comparison with those of vakognavine [3]

†Multiplicities were determined by a DEPT experiment

C-5 and C-18 of barbisine compared with those of vakognavine [3]. The negative Cotton effect exhibited by **3** near 300 nm indicated that the keto group is at C-13 [7, 8] (The contribution to the CD curve by the aldehyde group was masked by the addition of HCl) By contrast, alkaloids with a C-11 keto group exhibit a positive Cotton effect in the same region [7, 8] The position of the keto group at C-13 was further confirmed by identical CD curves exhibited by barbisine and vakognavine

The remaining problem was to establish the location of the third ester function. The multiplicity (*s*) of the proton on the carbon bearing the ester required attachment at C-7 or C-15. However, the available data did not permit a choice between the two possibilities The other ambiguity is the distribution of the ester functions over C-1, C-2 and C-7 or C-15, although by analogy with the vakognavines we can assign the benzoate to C-2 In view of these uncertainties an X-ray analysis of barbisine was undertaken. The X-ray analysis leads to structure **3** for barbisine with a final agreement factor of $R = 8.3\%$ and of $R_w = 10\,5\%$ An ORTEP plot of **3** is given in Fig 1.

EXPERIMENTAL

General Mps· corr For chromatographic separations on a Chromatotron [9, 10] silica gel HF-254+366 (EM 7744) was used TLC was carried out on silica gel 60 H (EM 7736)

Plant material Above ground parts of *D barbeyi* (Huth) Huth were collected on 4 and 5 August 1975 The stage of growth varied from plants having no conspicuous flower buds to those having well-formed buds and occasionally flowers, with the bud stage being predominant The collection site was a predominantly east-facing slope *ca* mid-way (2926–3200 m altitude) between Ferron Reservoir and the summit of the skyline divide of the Wasatch Plateau, in the Manti-Lasal National Forest, west of Ferron, Utah Plants were identified by Dr Leila McReynolds Shultz (Curator, Intermountain Herbarium, Department of Biology, Utah State University, Logan, UT 84321-5500) The large collection of plants was air-dried in direct sunlight The entire collection was ground and mixed thoroughly in a commercial feed grinder The total yield of 328 lbs was stored in sealed plastic bags at 5° until analysed or used in other expts

Isolation of barbisine Extn of plant material and fractionation of the alkaloid mixt have been described previously [5] Fraction A (12–14, 0 38 g) [4] was purified on a Chromatotron to furnish 54 mg of a mixt of alkaloids Crystallization of this mixture from Me_2CO–hexane gave **3** (28 mg), mp 248–252 Similar treatment of fraction D (14–16, 0 939 g) [4] gave 102 mg of **3** Repeated crystallization from Me_2CO–hexane furnished pure **3**, mp 251–254° $[\alpha]_D^{24} - 62\,9°$ ($CHCl_3$, *c* 0 273) CD curve (MeOH) $[\theta]_{324} - 198\,675$ (sh), $[\theta]_{311} - 351\,938$ (max), $[\theta]_{304} - 334\,909$ (max), $[\theta]_{276}$ 0, $[\theta]_{248} + 414\,379$ (max) and (MeOH + HCl) $[\theta]_{323} - 41\,154$ (sh), $[\theta]_{311} - 66\,698$ (max), $[\theta]_{302} - 65\,278$ (max), $[\theta]_{271} - 9\,933$ (min), $[\theta]_{250} - 48\,249$ (max), $[\theta]_{244}$ 0 and $[\theta]_{240}$

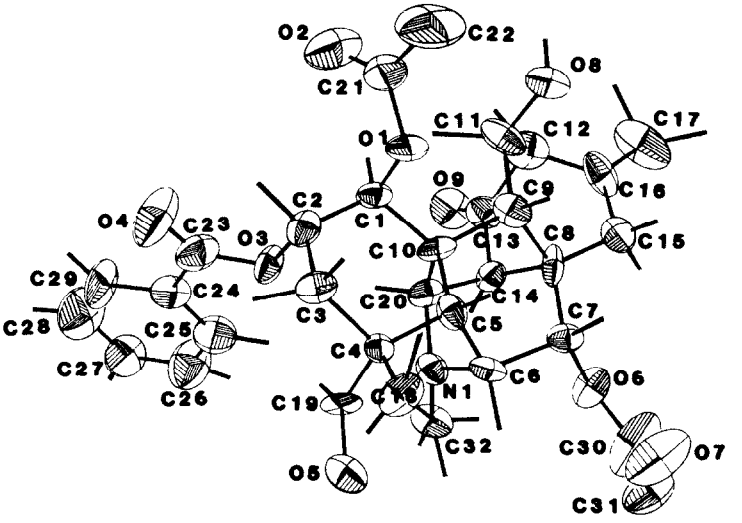


Fig 1 ORTEP plot of barbisine (**3**)

+70 130 (max) IR (Nujol) ν_{max} 3300, 1750, 1720, 1715, 1685, 1650 and 1600 cm^{-1}. EIMS *m/z* 578 $[M+1]^+$ $C_{32}H_{35}NO_9+H$); 549 $[M-28]^+$, 490 $[M-87]^+$ and 105 $[C_7H_5O]^+$. ^1H NMR (250 MHz, $CDCl_3$). δ9.23 (*s*, H-19), 7.85, 7.56 and 7.43 (each *m*, aromatic protons), 5 30 (*q*, $J=3$ 1 Hz, H-2β), 5 17 (*d*, $J=3.1$ Hz, H-1α), 5.05 (*s*, H-7β),5.03 and 4 95 (each *d*, $J=2.1$ Hz, H-17a and 17-b), 3.78 (*d*, $J=4$ 5 Hz, H-11α), 3.62 (*s*, H-20), 3.10 (*d*, $J=4$ 5 Hz, H-9β), 2.45 (*s*, *N*-Me), 2 14 and 2.10 (each *s*, OAc), 1.11 (*s*, Me-18). For ^{13}C NMR data see Table 1

Acetylation of barbisine (**3**). A mixture of **3** (37 mg), 1 ml of pyridine and 1 ml of Ac_2O was kept at room temp. for 2 days. Usual work-up gave 39 mg of **4**, mp 310–315° (decom.) Me_2CO–hexane). $[\alpha]^{23}-57.1°$ ($CHCl_3$; *c* 0.375). IR (Nujol) ν_{max} 1750, 1720, 1715, 1685, 1650 and 1600 cm^{-1}. MS *m/z* 620 ($[M+1]^+$ $C_{34}H_{37}NO_{10}+H$), 591 $[M-28]^+$, 532 $[M-87]^+$ and 105 $[C_7H_5O]^+$ ^1H NMR (90 MHz, $CDCl_3$) δ9 10 (*s*, H-19), 7 85 and 7 70-7 30 (*m*, aromatic protons), 5.38 (*q*, $J=3$ 1 Hz, H-2β), 5.11 (*d*, $J=3.1$ Hz, H-1α), 5 04–4 86 (*m*, H-7β, H-17a and 17b), 4 78 (*d*, $J=4$ 5 Hz, H-11α), 3 70 (*s*, H-20), 3.43 (*d*, $J=4$ 5 Hz, H-9β), 2 48 (*s*, *N*-Me), 2 11, 2 01 and 2.00 (each *s*, OAc), 1 10 (*s*, Me-18). For ^{13}C NMR data see Table 1

X-Ray analysis of barbisine (**3**). Single crystals of **3** were prepared by slow evapn from Me_2CO–hexane. A crystal of barbisine ($C_{32}H_{35}NO_9$) was mounted on a Syntex P3 automated diffractometer Unit cell dimensions were determined by least squares refinement of the best angular positions for 15 independent reflections ($2\theta>15°$) during normal alignment procedures using molybdenum radiation ($\lambda=0$ 71069 Å) and yielded $a=9.338(2)$, $b=19.077(6)$, $c=16.812(5)$ Å, $\alpha=\beta=\gamma=90°$, and $v=2994.8(15)$ $Å^3$ For $Z=4$ the calculated density was 1 281 g/cm^3 The space group was $P2_12_12_1$ Data (2259 independent points after removal of space group forbidden data) were collected at room temperature using a variable scan rate, a θ–2θ scan mode and a scan width of 1.2° below $K\alpha_1$ and 1.2° above $K\alpha_2$ to a maximum 2θ value of 50° Backgrounds were measured at each side of the scan for a combined time equal to the total scan time. The intensities of three standard reflections were remeasured after every 97 reflections As the intensities of these reflections showed less than 5% variation, corrections for decomposition were deemed unnecessary Data were corrected for Lorentz, polarization and background effects Observed reflections [1347 $I>3.0\,\sigma(I)$] were used for solution of carbon and oxygen positions of the structure by direct methods using SHELX86 [11]. Refinement [12] of scale factor, positional and anisotropic thermal parameters for all non hydrogen atoms was carried out to convergence The positions of 29 hydrogen atoms were located from a difference Fourier synthesis and were included (with hydrogen positional and thermal parameters held fixed) in the final cycles of refinement The positions of the protons bonded to acetate methyl carbon atoms, C-22 and C-31, were not apparent. [function minimized, $\Sigma(|F_o|-|F_c|)^2$] leading to a final agreement factor, $R=8$ 3%. [$R=(\Sigma||F_o|-|F_c||/\Sigma|F_o|)\times100$]. Scattering factors were taken from ref [13] In the final stages of refinement a weight of $1/\sigma(F)^2$ was used $R_w=10$ 5.

A complete list of X-ray data has been deposited with the Cambridge Crystallographic Data Centre, U.K

Acknowledgements—We thank Mr Courtney Pape for the MS data and Dr B S. Joshi, Dr H K Desai and Mr Q. Jiang for reading the manuscript and making useful suggestions

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