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NORDITERPENOID ALKALOIDS FROM *DELPHINIUM PEREGRINUM* VAR. *ELONGATUM*

GABRIEL DE LA FUENTE* and LASTENIA RUIZ-MESÍA

Instituto de Productos Naturales y Agrobiologia, CPNO Antonio González, CSIC-Universidad de La Laguna, La Laguna, Tenerife, Spain

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Key Word Index—*Delphinium peregrinum* var. *elongatum*; Ranunculaceae; diterpenoid alkaloids; 14-*O*-acetylperegrine; peregrine; dehydrobicoloridine; bicoloridine; bicoloridine alcohol; peregrine alcohol; nudicaulidine; dihydrogadesine; peregrinine; hetisinone; hetisine; atisinium chloride.

Abstract—Three new norditerpenoid alkaloids, dehydrobicoloridine, bicoloridine alcohol and peregrinine, together with the known alkaloids 14-*O*-acetylperegrine, peregrine alcohol, peregrine, bicoloridine, nudicaulidine, dihydrogadesine, hetisinone, hetisine and atisinium chloride were isolated from *Delphinium pereginum* var. *elongatum*. The structures of the new alkaloids were determined by NMR spectroscopy and partial synthesis.

INTRODUCTION

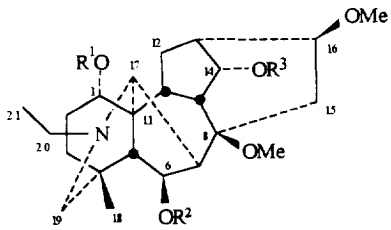
In a previous work on *Delphinium peregrinum* var. *elongatum*, we isolated the alkaloids bicolorine, dihydrogadesine, nudicaulidine, peregrine and 13-acetylhetisinone [1]. Now from the same species we have isolated three new norditerpenoid alkaloids, dehydrobicoloridine (**3**), bicoloridine alcohol (**5**) and peregrinine (**9**), together with the known alkaloids 14-acetylperegrine (**1**), peregrine alcohol (**6**), hetisinone (**10**), hetisine (**11**) and atisinium chloride (**12**).

RESULTS AND DISCUSSION

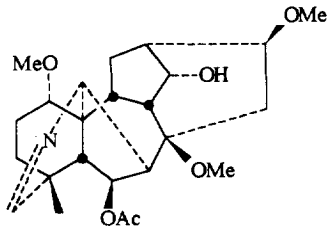
The new bases, whose elementary composition were determined by HR mass spectrometry and ^{13}C NMR, showed characteristic signals of norditerpenoid alkaloids in their NMR spectra [2, 3] and characteristic fragmentation of such compounds in their mass spectra [4]. The NMR spectra of dehydrobicoloridine (**3**), $C_{25}H_{37}NO_6$ gave signals at δ_H 1.05 (3H, *t*, $J = 7.2$ Hz), δ_C 47.3 *t* and 14.1 *q* of an *N*-ethyl group, δ_H 0.92 (3H, *s*) and δ_C 19.5 *q* of an angular methyl group, δ_H 3.11 and 3.38 (3H each, *s*), δ_C 48.3 *q* and 56.4 *q* of two methoxy groups, and δ_H 2.04 (3H, *s*), δ_C 21.6 *q* and 171.1 *s* of an acetate group (IR, 1730 and 1250 cm^{-1}). The ^{13}C NMR spectrum (Table 1) contained only three singlets upfield from 80 ppm at δ_C 38.4 (C-4), 48.3 (C-11) and 78.1 (C-8), indicating that the compound is an aconitine-type norditerpenoid possessing a tertiary methoxy group at C-8 (δ_C 48.3 *q*) [2, 3]. The one-proton signals at δ_H 4.11 (*dt*, $J = 4.8$ and 4.1 Hz), which become a *t* of $J = 4.1$ Hz when D_2O was added, and 5.36 (*dd*, $J = 6.7$ and 1.3 Hz), and the methine carbon resonances at δ_C 75.3 and 74.9, suggested the presence of a secondary hydroxyl group at C-14α and an acetate group at C-6β in the molecule. Moreover, the carbon resonances at δ_C 82.7 *d* and 56.4 *q* allow us to locate the other methoxyl group at C-16β [2, 3]. The loss of a molecule of acrolein from the ions at *m/z* 447 $[M]^+$ and 388 $[M - OAc]^+$ in the mass spectrum [5], the strong absorption bands at 985 and 885 cm^{-1} in the IR [6] and the one-proton signals at δ_H 3.74 (*d*, $J = 4.9$ Hz) and 3.73 *s* indicated the existence of a C_1–O–C_{19} ether in the compound. The ^{13}C NMR spectrum of **3** was similar to that of bicoloridine (**4**) (Table 1) and showed two methine carbon resonances at δ_C 68.7 (C-1) and 90.3 (C-19); this, together with the disappearance of the triplet at ~60 ppm present in **4**, provided additional proof of the C_1–O–C_{19} bridge in **3**. The structure of dehydrobicoloridine (**3**) was confirmed by partial synthesis from bicoloridine (**4**). Treatment of **4** with $KMnO_4$ in aqueous acetone gave *N*-deethyldehydrobicoloridine (**13**) in 65% yield [7, 8]. The NMR spectra, unlike those of bicoloridine, did not show signals for the *N*-ethyl group but had signals at δ_H 3.86 (1H, *s*, H-19), 3.87 (1H, *d*, $J = 3.9$ Hz, H-1β), δ_C 68.7 *d* (C-1) and 86.7 *d* (C-19), as expected for a C_1–O–C_{19} ether. Also, the IR spectrum gave bands at 885 and 990 cm^{-1} and the mass spectrum contained an ion at *m/z* 363 corresponding to the loss of acrolein from the $[M]^+$ at *m/z* 419, characteristic of such a function. Dehydrobicoloridine (**3**) was subsequently obtained in 44% yield via treatment of **13** with ethyl bromide and potassium carbonate in dry acetone [9].

The NMR spectra of bicoloridine alcohol (**5**), $C_{23}H_{37}NO_6$, showed that its structure was very similar

*Author to whom correspondence should be addressed.



1 R^1 = Me; R^2 = R^3 = Ac
2 R^1 = Me; R^2 = Ac; R^3 = H
4 R^1 = H; R^2 = Ac; R^3 = H
5 R^1 = R^2 = R^3 = H
6 R^1 = Me; R^2 = R^3 = H



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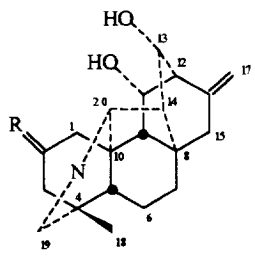

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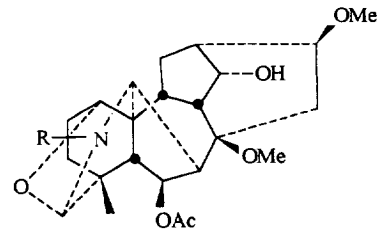
11 R = β H, α OH



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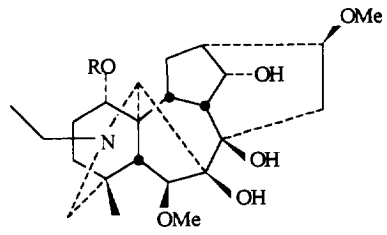
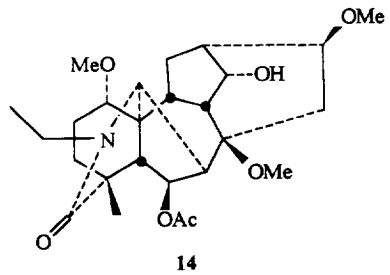
13 R = H



7 R = Me
8 R = H



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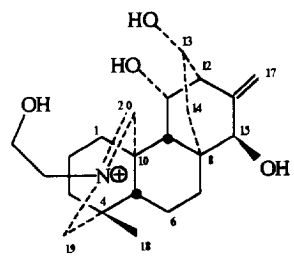

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to that of bicoloridine (**4**), having the same functional groups, except for the absence of the acetate group. The one-proton signal for the C-6 carbinyl proton at δ_H 5.31 (*d*, J = 7.4 Hz) in **4**, showed at 4.37 (*d*, J = 7.3 Hz) in **5**, and clearly indicated that a hydroxyl group is situated at C-6β in alkaloid **5**. Also, the expected β-effects of ~2 and ~3 ppm were observed on the C-5 and C-7 carbon resonances of **5**, respectively. Basic hydrolysis of bicoloridine (**4**) with methanolic KOH gave bicoloridine alcohol (**5**) in 88% yield.

From the NMR data (Tables 1 and 2) peregrinine (**9**), $C_{24}H_{35}NO_6$, lacks an *N*-ethyl group and has a tertiary methyl group, δ_H 1.13 (3H, *s*) and δ_C 22.6 *q*, three methoxy groups, δ_H 3.07, 3.21 and 3.37 (3H each, *s*) and δ_C 48.4, 56.5 and 55.9 (*q* each), and an acetate group, δ_H 2.04 (3H, *s*), δ_C 21.5 *q* and 170.6 *s* (IR 1730 and 1245 cm^{-1}). The ^{13}C NMR spectrum contained only three singlets upfield from 80 ppm at δ_C 45.3 (C-4), 47.9 (C-11) and 77.3 (C-8), suggesting that peregrinine is an aconitine-type norditerpenoid alkaloid with a tertiary methoxyl group at C-8 (δ_C 48.4 *q*) [2, 3]. The other two methoxyl groups were situated at C-1α and C-16β to account for the one-proton signals at δ_H 3.26 (*t*, J = 6.7 Hz, H-1β) and 3.42 (*m*, H-16α), which in turn gave one-bond connectivities with the methine carbon resonances at 82.5 and 82.1 ppm respectively, in the HMQC spectrum (Table 2) [11]. The one-proton signal at δ_H 4.05 (*dt*, J = 5.1 and 5.3 Hz), becoming a *t*, J = 5.3 Hz, when D_2O was added, that correlated with the carbon resonance at δ_C 75.5 *d* (HMQC), was in agreement with the existence of a secondary hydroxyl group at C-14α in the molecule [2, 3]. The norditerpenoid alkaloids gave C-4 and C-19 resonances in the range of δ_C 32–35 ppm and 56–62 ppm, respectively, if they contain C(4)Me, C(3)H_2 and C(5)H [2, 3]. In the ^{13}C NMR spectrum of peregrine (**9**), C-4 appeared at δ_C 45.3 *s* and the C-19 methylene

Table 1. ^{13}C NMR assignments for bicoloridine (**4**), dehydrobicoloridine (**3**), bicoloridine alcohol (**5**), *N*-deethyldehydrobicoloridine (**13**), peregrine (**2**), peregrine alcohol (**6**), peregrinine (**9**) and lactam (**14**)

C	4	3	5	13	2	6	9	14
1	72.6	68.7	72.9	68.7	84.7	85.6	82.5	82.6
2	29.7	22.9	29.6	22.9	26.5	26.5	24.6	26.4
3	31.6	30.0	31.8	30.4	37.1	37.5	31.2	34.7
4	32.8	38.4	32.6	38.9	34.5	34.6	45.3	46.4
5	52.8	53.8	54.8	53.5	56.4	58.9	52.0	55.0
6	72.3	74.9	72.5	74.5	73.4	73.0	75.3	76.8
7	42.3	48.5	45.5	49.6	42.4	45.9	46.5	46.7
8	79.9	78.1	81.1	78.1	79.1	80.9	77.3	77.6
9	44.4	43.6	44.4	43.7	44.6	43.8	43.8	43.8
10	44.4	37.4	43.5	37.5	46.2	46.3	45.8	46.3
11	48.9	48.3	48.9	46.3	48.2	48.3	47.9	46.5
12	30.6	29.7	30.3	29.8	28.6	28.5	28.7	27.2
13	40.0	39.2	39.9	39.5	38.6	37.7	38.4	36.8
14	75.9	75.3	75.8	75.3	75.5	75.2	75.5	75.1
15	37.8	35.1	37.4	35.2	33.0	33.1	32.4	28.8
16	83.8	82.7	83.0	82.7	82.5	82.4	82.1	81.7
17	65.5	62.5	65.1	54.6	64.7	64.3	63.4	63.4
18	27.3	19.5	27.5	19.6	25.9	26.0	22.6	21.4
19	61.6	90.3	62.2	86.7	57.6	58.1	168.5	173.1
20	43.8	47.3	48.6		49.3	49.6		41.8
21	12.9	14.1	13.1		13.6	13.8		12.8
1′					56.0	56.3	55.9	55.5
8′	48.0	48.3	48.5	48.4	48.3	48.6	48.4	48.6
16′	56.4	56.4	56.6	56.5	56.4	56.5	56.5	56.6
CO	170.9	171.1		171.0	170.2		170.6	170.7
CH_3	21.5	21.7		21.6	21.7		21.5	21.7

Chemical shifts in ppm down-field from TMS.
Carbon multiplicities determined by DEPT pulse sequence.

carbon resonance was absent; consequently, the methine carbon resonance at δ_C 168.5 (HMQC δ 7.20 *s*) was attributed to the existence of a C(19) = N- azomethine group in **9** (IR 1655 cm^{-1}). The one-proton doublet at δ_H 5.07 (J = 6.8 Hz) indicated that the acetate group in **9** is at C-6 [2, 3]. Because the one-proton broad singlet at δ_H 1.65, which ascribed to H-5 on account of its coupling with the protons at δ_H 3.99 *br s* (H-17) and 7.20 *s* (H-19) (Table 2), did not show coupling with the H-6 signal at δ_H 5.07 in the ^1H COSY spectrum, the acetate group was placed in the β-position (90° dihedral angle between H-5 and H-6α). The rest of the ^1H and ^{13}C NMR signals (Tables 1 and 2) were in agreement with the proposed structure and assignments were made by comparison with the spectra of perergine (**2**) [10] and ^1H COSY and HMQC data (Table 2).

The structure of peregrinine (**9**) was confirmed by synthesis from peregrine (**2**). Treatment of peregrine with KMO_4 in aqueous acetone gave, as the more polar compound, peregrinine in 21% yield, identical with the natural alkaloid, and the lactam **14** in 29% yield [7, 8]. The NMR spectra of **14** were similar to those of peregrine (Tables 1 and 3) but the ^1H NMR spectrum of **14** did not show signals for the C-19 methylene protons. The ^{13}C NMR spectrum gave a new singlet at δ_C 173.1 with the disappearance of the triplet at δ_C 57.6 for C-19 seen in the spectrum of peregrine (**2**). Like *D. bicolor* [12], *D. speciosum* [13] and *D. caeruleum* [14], *D. peregrinum* var. *elongatum* produces aconitine-type norditerpenoid alkaloids with a β-oxygen function at C-6, together with lycoctonine-type alkaloids. It seems reasonable to suppose that at least in these plants the formation of lycoctonine-type alkaloids occurs by hydroxylation first at C-6β and then at C-7. *D. munzianum* also produces aconitine-type alkaloids with a C-6β oxygen function but lycoctonine-type alkaloids were not isolated [15].

EXPERIMENTAL

General. Mps: uncorr. IR: $CHCl_3$ and KBr. OR: $CHCl_3$, 1 dm cell. EIMS 70 eV. NMR spectra were recorded in $CDCl_3$ on Bruker WP–200 SY and AMX spectrometers, using TMS and solvent as int. standards. DEPT and 2D NMR expts were carried out with standard pulse sequences. Alumina Merck Art. 1077 and silica gel Merck Art. 7734 was used for CC and alumina Merck Art. 5581 and silica gel Schleicher and Schuell 394732 were employed for TLC. Visualization was made using Dragendorff's reagent.

Plant material. Plants were collected at Medina Plage, Rabat, Marocco, during the flowering period, and auth-

Table 2. ^{1}H, ^{1}H COSY and HMQC NMR data for peregrinine (**9**)

Proton		Correlated atom	
		HMQC	COSY
1β	3.26 *t* (6.7)	82.5 *d*	H-2α, H-2β
2α	1.79 *m*	24.6 *t*	H-1β, H-2β, H-3β
2β	1.49 *m*	24.6 *t*	H-1β, H-2α, H-3β
3β	1.33 *m*	31.2 *t*	H-2α, H-2β
5	1.65 *br s*	52.0 *d*	H-17 (W), H-19
6α	5.07 *d* (6.8)	75.3 *d*	H-7
7	2.79 *d* (7.0)	46.5 *d*	H-6α
9	2.83 *t* (5.6)	43.8 *d*	H-14β, H-10
10	2.01 *m*	45.8 *d*	H-9
12β	1.91 *m*	28.7 *t*	H-13
13	2.38 *br t* (6.2)	38.4 *d*	H-12β, H-14β
14β	4.05 *dt* (5.1, 5.3)	75.5 *d*	H-9, H-13
15α	2.13 *dd* (15.7, 4.5)	32.4 *t*	H-15β, H-16α
15β	2.28 *dd* (15.9, 8.3)	32.4 *t*	H-15α, H-16α
16α	3.42 *m*	82.1 *d*	H-15α, H-15β
17	3.99 *br s*	63.4 *d*	H-5 (W)
18	1.13 *s*	22.6 *q*	
19	7.20 *s*	168.5 *d*	H-5
1′	3.21 *s*	55.9 *q*	
8′	3.07 *s*	48.4 *q*	
16′	3.37 *s*	56.5 *q*	
Ac	2.04 *s*	21.5 *q*	

Chemical shits in ppm down-field from TMS.
Coupling constants in parentheses in Hz.

Table 3. ^{1}H COSY and HMQC NMR data for lactam (**14**)

Proton		Correlated atom	
		HMQC	COSY
1β	3.28 *dd* (9.0, 7.1)	82.6 *d*	
5	1.75 *dd* (3.2, 1.5)	55.0 *d*	H-17 (W)
6α	5.11 *dd* (6.9, 1.5)	76.8 *d*	H-7
7	2.73 *d* (6.8)	46.7 *d*	H-6α
9	2.91 *t* (5.1)	43.8 *d*	H-14β
13	2.44 *m*	36.8 *d*	H-14β
14β	3.97 *dt* (8.4, 4.7)	75.1 *d*	H-13, H-9
15α	1.94 *dd* (17.1, 7.6)	28.8 *t*	H-15β, H-16α
16α	3.46 *m*	81.7 *d*	H-15α, H-15β
17	3.63 *d* (3.1)	63.4 *d*	H-5 (W)
18	1.19 *s*	21.4 *q*	
20A	2.83 *sext* (7.2)	41.8 *t*	H-20B, H-21
20B	3.87 *sext* (7.2)	41.8 *t*	H-20A, H-21
21	1.15 *t* (7.2)	12.8 *q*	H-20A, H-20B
1′	3.24 *s*	55.5 *q*	
8′	3.07 *s*	48.6 *q*	
16′	3.38 *s*	56.5 *q*	
Ac	2.06 *s*	21.7 *q*	

Chemical shifts in ppm down-field from TMS.
Coupling constants in parentheses in Hz.

enticated by Profs J. Molero Briones and C. Blanché Verges, Botany Department, Faculty of Pharmacy, University of Barcelona, where a voucher specimen FC-9074-BCF has been deposited.

Extraction and isolation. Air-dried epigeal parts were extracted with EtOH at room temp. during 2 days. The EtOH extract (673 g) was treated with 5% H_2SO_4 and filtered. The acid soln was basified with $NaCO_3$ to pH 9 and extracted with Et_2O to give crude alkaloid material (4.79 g), which has already been studied [1]. Then, the aq. soln was adjusted to pH 12 with 20% NaOH and extracted with $CHCl_3$ to provide an additional crude alkaloid material (9.2 g). CC on alumina of this fr. using a hexane–EtOAc step gradient, EtOAc and a EtOAc–MeOH step gradient, followed by further CC when necessary, allowed the isolation, in order of increasing polarity, of 14-*O*-acetylperegrine (**1**) (15 mg), peregrine (**2**) (1.2 g), dehydrobicoloridine (**3**) (18 mg), bicoloridine (**4**) (60 mg), bicoloridine alcohol (**5**) (49 mg), peregrine alcohol (**6**) (14 mg), nudicaulidine (**7**) (10 mg), dihydrogadesine (**8**) (115 mg), peregrinine (**9**) (52 mg), hetisinone (**10**) (8 mg), hetisine (**11**) (15 mg) and atisinium chloride (**12**) (35 mg). Known alkaloids were identified by comparison with authentic samples (TLC, mp, IR, MS, ^{1}H and ^{13}C NMR).

14-O-*Acetylperegrine* (1). Resin. $[\alpha]_D$ − 49.6° (*c* 0.24) [15].

Peregrine (**2**). Crystalline, mp 120–122° from hexane–EtOAc. $[\alpha]_D$ + 20° (*c* 0.2) [1, 10].

Dehydrobicoloridine (**3**). Amorphous $[\alpha]_D$ + 29.1° (*c* 0.15). $[M]^+$ *m/z* 447.2603 for $C_{25}H_{37}NO_6$ (Calc. 447.2620). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 2950, 1730, 1455, 1375, 1250, 1080, 985, 885, 810. ^{1}H NMR (200 MHz): δ 0.92 (3H, *s*, H-18), 1.05 (3H, *t*, *J* = 7.2 Hz, H-21), 1.49 (1H, *br s*, H-5), 2.04 (3H, *s*, OAc), 2.40 (1H, *t*, *J* = 6.6 Hz, H-9), 3.11 and 3.38 (3H each, *s*, 2 × OMe), 3.73 (1H, *s*, H-19), 3.74 (1H, *d*, *J* = 4.9 Hz, H-1β), 4.11 (1H, *dt*, *J* = 4.8 and 4.1 Hz, becoming *t*, *J* = 4.1 Hz when D_2O added, H-14β), 5.36 (1H, *dd*, *J* = 6.7 and 1.3 Hz, H-6α). EIMS *m/z* (rel. int.), 447 (15) $[M]^+$, 432 (8), 430 (2), 416 (3), 405 (25), 404 (100), 392 (17), 391 (67), 388 (5), 387 (2), 373 (3), 372 (11), 358 (3), 333 (19), 332 (22), 298 (2), 284 (3), 228 (3), 187 (4), 185 (5), 160 (7), 135 (11), 122 (37), 105 (23), 97 (25), 91 (32), 81 (34), 77 (25), 67 (30), 59 (46), 55 (74). For ^{13}C NMR (50 MHz) see Table 1.

Bicoloridine (**4**). Crystalline, mp 203–205° from hexane–EtOAc. $[\alpha]_D$ + 8° (*c* 0.75) [16, 17]. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3498, 2950, 1730, 1460, 1368, 1295, 1255, 1152, 1115, 1190, 1052, 975, 940, 910, 875. ^{1}H NMR see refs [1, 16]. EIMS *m/z* (rel. int.), 449 (20) $[M]^+$, 434 (15), 433 (19), 432 (58), 418 (22), 406 (17), 404 (4), 400 (3), 392 (7), 393 (3), 390 (13), 388 (12), 378 (10), 375 (6), 374 (16), 373 (3), 372 (7), 358 (12), 342 (4), 314 (4), 298 (4), 291 (4), 242 (3), 216 (4), 188 (6), 160 (7), 151 (12), 135 (12), 122 (20), 91 (38), 83 (44), 71 (52), 58 (100). For ^{13}C NMR see ref. [10].

N-*Deethyldehydrobicoloridine* (**13**) *from bicoloridine* (**4**). To a soln of **4** (12 mg) in a mixt. of Me_2CO (7.5 ml) and H_2O (0.5 ml) was added $KMnO_4$ (30 mg) dissolved in Me_2CO (13 ml) and H_2O (7 ml) and the reaction mixt. stirred at room temp. for 18 hr. Excess $KMnO_4$ was destroyed with $NaHSO_3$ and the mixt. diluted with H_2O, basified with NH_4OH and extracted with $CHCl_3$. After solvent removal, the reaction product was chromatog-

raphed on silica with hexane–EtOAc (1:9) to give *N*-deethyldehydrobicoloridine (**13**) (7.5 mg), 65%) as an amorphous compound. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3000, 2925, 1725, 1455, 1365, 1245, 1090, 1035, 990, 960, 920, 885. ^{1}H NMR (200 MHz): δ 0.88 (3H, *s*, H-18), 1.55 (1H, *br*, *s*, H-5), 2.04 (3H, *s*, OAc), 2.38 (1H, *t*, J = 6.5 Hz, H-9), 2.62 (1H, *d*, J = 6.6 Hz, H-7), 3.09 and 3.38 (3H each, *s*, 2 × OMe), 3.86 (1H, *s*, H-19), 3.87 (1H, *d*, J = 3.9 Hz, H-1β), 4.11 (1H, *br s*, becoming *t*, J = 4.2 Hz when D_2O added, H-14β), 5.47(1H, *dd*, J = 6.6 and 1.4 Hz, H-6α). EIMS *m/z* (rel. int.), 419 (19) $[M]^+$, 408 (8), 402 (2), 401 (1), 389 (6), 388 (3), 387 (3), 386 (3), 377 (26), 376 (100), 374 (15), 371 (3), 370 (3), 360 (4), 362 (22), 359 (2), 346 (5), 345 (8), 344 (24), 342 (3), 332 (4), 328 (6), 321 (6), 302 (4), 299 (5), 286 (5), 272 (4), 268 (6), 256 (4), 245 (8), 228 (5), 221 (7), 213 (13), 202 (8), 183 (10), 165 (10), 143 (23), 131 (33), 121 (35), 105 (43), 91 (71), 79 (40), 70 (38), 67 (39), 57 (50), 55 (79). For ^{13}C NMR (50 MHz) see Table 1.

Dehydrobicoloridine (**3**) *from* N-*deethyldehydrobicoloridine* (**13**). To a mixt. of **13** (6 mg), Me_2CO (5 ml) and K_2CO_3 (10 ml) was gradually added EtBr (1.5 ml) during 5 days, while refluxing. After solvent removal, the resulting reaction product was chromatographed over silica with EtOAc to yield dehydrobicoloridine (**3**) (2.8 mg), 44%), identical with the natural alkaloid (TLC, IR, MS and ^{1}H NMR).

Bicoloridine alcohol (**5**). Resin. $[\alpha]_D$ − 3.3° (*c* 0.24). $[M]^+$ *m/z* 407.2639 for $C_{23}H_{37}NO_5$ (Calc. 407.2672). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3495, 2950, 2850, 1460, 1380, 1150, 1090, 1045, 975, 940, 910, 875, 845. ^{1}H NMR (200 MHz): δ1.05 (3H, *s*, H-18), 1.08 (3H, *t*, J = 7.1 Hz, H-21), 1.92 (1H, *d*, J = 11.6 Hz, H-19A), 2.59 (1H, *d*, J = 7.3 Hz, H-7), 2.79 (1H, *d*, J = 2.1 Hz, H-17), 2.90 (1H, *t*, J = 6 Hz, H-9), 3.32 and 3.37 (3H each, *s*, 2 × OMe), 3.72 (1H, *m*, H-1β), 4.11 (1H, *t*, J = 4.9 Hz, H-14β), 4.37 (1H, *d*, J = 7.3 Hz, H-6β), 4.61 (1H, *s*, OH, disappearing on addition of D_2O). EIMS *m/z* (rel. int.), 407 (14) $[M]^+$, 406 (20), 392 (7), 389 (2), 390 (17), 376 (16), 375 (2), 374 (7), 360 (2), 358 (2), 349 (2), 344 (2), 336 (2), 242 (3), 220 (3), 214 (3), 206 (5), 160 (7), 143 (13), 138 (16), 120 (35), 111 (20), 109 (27), 107 (59), 98 (30), 91 (41), 83 (100). For ^{13}C NMR (50 MHz) see Table 1.

Bicoloridine alcohol (**5**) *from bicoloridine* (**4**). Compound **4** (15 mg) was refluxed with 5% KOH–MeOH (5 ml) for 8 hr. The reaction mixt. was poured into H_2O and extracted with $CHCl_3$. The solvent was removed and the reaction product chromatographed over alumina with EtOAc to give bicoloridine alcohol (**5**) (12 mg, 88%) identical with the natural alkaloid (TLC, IR, MS and ^{1}H NMR).

Peregrine alcohol (**6**). Crystalline, mp 140–142° from hexane–EtOAc. $[\alpha]_D$ − 10° ($CHCl_3$; *c* 0.42) and − 7° (*c* 0.21, EtOH). $[M]^+$ *m/z* 421.2824 for $C_{24}H_{39}NO_5$ (Calc. 421.2828). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3495, 2950, 1460, 1375, 1320, 1295, 1185, 1160, 1085, 925, 900, 870, 840. ^{1}H NMR (200 MHz): δ0.96 (3H, *s*, H-18), 1.05 (3H, *t*, J = 7.2 Hz, H-21), 1.43 (1H, *br s*, H-5), 1.55 (1H, *dm*, J = 13 Hz, H-3α), 2.57 (1H, *d*, J = 10.4 Hz, H-19α), 2.61 (1H, *d*, J = 7 Hz, H-7), 3.12 (1H, *s*. H-17). 3.27, 3.31 and 3.37 (3H each, *s*. 3 × OMe), 3.55 (1H, *d*, J = 5.8 Hz, disappearing on addition of D_2O, 14αOH), 4.04 (1H, *d*, J = 5.8 and 4.9 Hz, becoming *t*, J = 4.8 Hz, when D_2O added, H-14β), 4.29 (1H, *d*, J = 7.4 Hz, H-6α), 4.79 (1H, *s*, disappearing when D_2O added). EIMS, *m/z* (rel. int.), 421 (14) $[M]^+$, 406 (15), 392 (12), 391 (70), 390 (100), 388 (19), 374 (13), 372 (14), 360 (7), 359 (8), 358 (28), 346 (9), 340 (7), 330 (10), 328 (9), 326 (9), 288 (6), 208 (8), 148 (8), 122 (7), 91 (7), 71 (12), 58 (27). For ^{13}C NMR (50 MHz) see Table 1 [10]. This compound was identical to the hydrolysis product of peregrine (**2**) [1, 10] (TLC, mp, IR, MS, ^{1}H and ^{13}C NMR) and with an authentic sample isolated from *D. peregrinum* [27].

Nudicaulidine (**7**). Amorphous $[\alpha]_D$ + 41.6° (*c* 0.14) [18]. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3431, 3015, 2944, 2822, 2390, 1522, 1462, 1426, 1218, 1091, 929, 853. ^{1}H NMR (200 MHz): δ 0.97 (3H, *s*, H-18), 1.08 (3H, *t*, J = 7.1 Hz, H-21), 1.44 (1H, *br s*, H-5), 2.64 (1H, *d*, J = 11.8 Hz, H-19α), 3.12 (1H, *t*, J = 4.6 Hz, H-9), 3.18 (1H, *d*, J = 2.3 Hz, H-17), 3.23, 3.35 and 3.40 (3H each, *s*, 3 × OMe), 3.63(1H, *s*, disappearing when D_2O added, OH), 3.83 (1H, *s*, H-6α), 3.97 (1H, *br s*, becaming *t*, J = 4.5 Hz when D_2O added, H-14β). EIMS *m/z* (rel. int.), 437 (5) $[M]^+$, 422 (22), 420 (9), 419 (10), 407 (20), 406 (97), 405 (13), 404 (50), 402 (4), 392 (19), 388 (3), 385 (5), 374 (6), 372 (7), 349 (7), 314 (8), 296 (7), 274 (7), 260 (7), 256 (8), 244 (13), 230 (17), 216 (14), 185 (17), 164 (17), 157 (22),135 (34), 117 (34), 109 (32), 98 (31), 91 (64), 83 (39), 71 (100), 57 (60), 55 (73). ^{13}C NMR (50 MHz): δ 36.5 *d* (C-13), 46.1 *d* (C-10), and 76.5 *s* (C-8); for other signals see ref. [18].

Dihydrogadesine (**8**). Crystalline, mp 134–140° from hexane–EtOAc. $[\alpha]_D$ + 48.9° (*c* 0.23) [19]. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3495, 2925, 1455, 1385, 1320, 1290, 1145, 1087, 1055, 950, 905, 860. ^{1}H NMR see ref. [19]. EIMS *m/z* (rel. int.), 423(17) $[M]^+$, 409(26), 408 (100), 406 (71), 393 (27), 392 (100), 391 (23), 390 (95), 388 (33), 374 (10), 365 (4), 360 (5), 358 (5), 352 (4), 346 (4), 334 (3), 264 (5), 206 (3), 148 (4), 122 (6), 98 (4), 91 (5), 71 (59), 58 (20). For ^{13}C NMR (50 MHz) see ref. [26].

Peregrinine (**9**). Amorphous $[\alpha]_D$ + 66.7° (*c* 0.1). $[M]^+$ *m/z* 433.2464 for $C_{24}H_{35}NO_6$ (Calc. 433.2464). IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3450, 2995, 1730, 1655, 1455, 1360, 1245, 1090, 1045, 975, 910. ^{1}H NMR (400 MHz): δ3.55 (1H, *s*, disappearing when D_2O added, OH), 4.05 (1H, *dt*, J = 5.3 and 5.1 Hz, becoming *t*, J = 5.3 Hz, when D_2O added, H-14β); for other signals see Table 2. EIMS *m/z* (rel. int.), 433 (43) $[M]^+$, 418 (37), 416 (2), 425 (2), 403 (23), 402 (87), 401 (19), 386 (10), 384 (15), 374 (9), 373 (6), 372 (24), 371 (59), 370 (26), 359 (5), 358 (18), 356 (6), 347 (15), 342 (27), 332 (17), 331 (43), 327 (17), 326 (17), 317 (58), 310 (17), 298 (17), 282 (10), 248 (7), 240 (6), 226 (6), 222 (6), 211 (6), 183 (10), 180 (10), 167 (12), 151 (24), 143 (14), 125 (25), 105 (23), 97 (34), 91 (42), 83 (35), 79 (21), 71 (100), 57 (92), 55 (59). For ^{13}C NMR (100 MHz) see Table 1.

Peregrinine (**9**) *from peregrine* (**2**). To peregrine (30 mg) dissolved in a mixt. of Me_2CO (15 ml) and H_2O (1 ml) was added $KMNO_4$ (30 mg) dissolved in Me_2CO (13 ml) and H_2O (7 ml). The reaction mixt. was stirred at room temp. for 7 hr. Then, the excess reagent was destroyed with $NaHSO_3$ and the mixt. diluted with H_2O, basified with 10% KOH and extracted with $CHCl_3$. Solvent

removal afforded the reaction product which was chromatographed over alumina with hexane–EtOAc (2:3) to give peregrinine (**9**) (6 mg, 21%), as the more polar compound, identical with the natural alkaloid (TLC, IR, MS and ^{1}H NMR), and the less polar compound, lactam (**14**) (9 mg, 29%), mp 188–191° from hexane–EtOAc. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3495, 3000, 2850, 1730, 1635, 1620, 1460, 1380, 1360, 1240, 1255, 1240, 1090, 1045, 990, 920, 890. ^{1}H NMR (400 MHz): δ3.87 (1H, *d*, J = 8.5 Hz, disappearing on addition of D_2O, 14αOH), 3.97 (1H, *dt*, J = 8.4 and 4.7 Hz, becoming *t*, J = 4.2 Hz, when D_2O added, H-14β); for other signals see Table 3. EIMS *m/z* (rel. int.), 477 (45) [M]$^+$, 462 (30), 460 (2), 447 (40), 446 (93), 445 (10), 435 (17), 434 (64), 432 (7), 430 (16), 438 (11), 418 (36), 417 (8), 416 (33), 415 (100), 414 (31), 406 (26), 403 (28), 402 (29), 400 (8), 388 (17), 386 (59), 385 (24), 383 (23), 382 (10), 370 (36), 362 (42), 356 (56), 354 (53), 344 (32), 326 (25), 314 (22), 307 (21), 284 (22), 270 (20), 209 (21), 199 (27), 183 (28), 172 (30), 167 (27), 138 (37), 125 (42), 105 (30), 91 (42), 71 (92), For ^{13}C NMR (100 MHz) see Table 1.

Hetisinone (**10**). Crystalline, mp 199–201° from hexane–EtOAc. $[\alpha]_D$ +41.8° (*c* 0.8) [20, 21]. IR ν_{max}^{KBr} cm^{-1}: 3479, 2975, 2937, 2919, 2878, 2852, 2362, 1730, 1658, 1633, 1451, 1436, 1387, 1349, 1337, 1286, 1247, 1193, 1136, 1104, 1072, 1054, 1042, 902, 877. ^{1}H NMR (200 MHz): δ1.15 (3H, *s*, H-18), 1.64 (1H, *dd*, J = 13.6 and 2.5 Hz, H-7β), 1.76 (1H, *dd*, J = 13.6 and 3.2 Hz, H-7α), 2.42 (1H, *br s*, H-12), 2.72 (1H, *d*, J = 12.8 Hz, H-19α), 2.75 (1H, *d*, J = 14.6 Hz, H-1β), 2.95 (1H, *s*, H-20), 3.31 (1H, *br s*, H-6), 3.33 (1H, *d*, J = 14 Hz, H-1α), 4.23 (2H, *d*, J = 10.3 Hz, H-13β and H-11β), 4.70 and 4.88 (1H each, *s*, H-17). EIMS *m/z* (rel. int.), 327 (100) [M]$^+$, 326 (11), 312 (8), 310 (34), 309 (15), 299 (16), 298 (15), 296 (4), 282 (32), 281 (67), 280 (52), 279 (53), 271 (6), 254 (12), 253 (17), 176 (11), 126 (13), 223 (5), 203 (6), 184 (3), 159 (7), 146 (7), 114 (7), 91 (12), 55 (10), For ^{13}C NMR see ref. [22].

Hetisine (**11**). Crystalline, mp 260–262° from EtOAc–MeOH. $[\alpha]_D$ +10.3 (*c* 0.12) [20, 21]. IR ν_{max}^{KBr} cm^{-1}: 3359, 3330, 2972, 2920, 2886, 1655, 1488, 1455, 1420, 1388, 1331, 1306, 1155, 1114, 1072, 1042, 1030, 1012, 979, 954, 904, 892, 863, 792. ^{1}H NMR (200 MHz): δ0.99 (3H, *s*, H-18), 2.43 (1H, *s*, H-12), 2.53 (1H, *d*, J = 11.7 Hz, H-19β), 2.86 (1H, *d*, J = 15.5 Hz, H-1α), 3.29 (1H, *s*, H-6), 3.39 (1H, *d*, J = 12 Hz, H-19α), 3.90 (1H, *br s*, H-20), 4.10–4.24 (3H, *m*, H-2β, H-11β, H-13β), 4.67 and 4.86 (1H each, *s*, H-17). EIMS *m/z* (rel. int.), 329(100) [M]$^+$, 328 (10), 314 (7), 312 (52), 311 (10), 300 (10), 294 (6), 284 (10), 283 (19), 282 (19), 260 (5), 247 (3), 217 (3), 176 (5), 157 (3), 143 (4), 131 (3), 129 (4), 115 (3), 105 (5), 76 (4), 55 (6), 43 (5). For ^{13}C NMR see ref. [22].

Atisinium chloride (**12**). Crystalline, mp 303–303° (dec.) from EtOAc–MeOH [23]. IR ν_{max}^{KBr} cm^{-1}: 3376, 3263, 3067, 2930, 2867, 1678, 1632, 1451, 1418, 1369, 1264, 1222, 1071, 1000, 981, 893, 857, 799. EIMS *m/z* (rel. int.), 343(49) [M]$^+$, 342 (100), 328 (8), 326 (3), 325 (4), 314 (7), 312 (7), 300 (8), 272 (2), 270 (3), 260 (7), 257 (6), 241 (15), 186 (22), 171 (5), 159 (12), 145 (4), 131 (5). 117 (5), 105 (6), 91 (7), 79 (4), 72 (7). For ^{1}H and ^{13}C NMR see refs [24, 25].

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REFERENCES

1. De la Fuente, G., Gavín, J. A. Díaz-Acosta R. and Morales, J. A. (1988) *Heterocycles* **27**, 1.
2. Pelletier, S. W., Mody, N. V., Joshi, B. S. and Schramm, L. C. (1984) in *Alkaloids: Chemical and Biological Perspectives* Vol. 2 (Pelletier, S. W., ed.), p. 205, Wiley Interscience, New York.
3. Pelletier, S. W. and Joshi, B. S. (1991) *Ibidem* Vol. 7 (Pelletier, S. W., ed.), p. 297, Springer, New York.
4. Yunusov, M. S., Rashkes, Y. V., Salimov, B. T., Ametova, E. F. and Fridlyanskii, G. V. (1985) *Chem. Nat. Comp.*, 492.
5. Pelletier, S. W. and Page, S. W. (1973) in *The Alkaloids* Vol. 3 (Saxton, J. E., ed.), p. 235. The Chem. Soc., London.
6. Anet, R., Clayton, D. and Marion, L. (1975) *Can. J. Chem.* **35**, 397.
7. Achmatowicz, O., Tsuda, Y. and Marion, L. (1965) *Can. J. Chem.* **43**, 2336.
8. Wang, F.-P. and Liang, X.-T. (1992) *Chemistry of the Diterpene Alkaloids* in *The Alkaloids* Vol. 42 (Brossi, A., ed.), Chap. 3, Academic Press, New York.
9. Pelletier, S. W. and Badawi, M. M. (1987) *J. Nat. Prod.* **50**, 381.
10. De la Fluente, G., Ruiz-Mesía, L. and Rodriguez, M. L. (1994) *Helv. Chim. Acta* **77**, 1768.
11. Bax, A. and Subramaniam, S. (1986) *J. Magn. Res.* **67**, 565.
12. Kulanthaivel, P., Benn, M. and Majak, W. (1986) *Phytochemistry* **25**, 1511.
13. Beshitaishvili, L. V., Sultankhodzaev, M. N. and Yunusov, M. S. (1984) *Khim. Prir. Soedin.* **20**, 671.
14. Pan, Y. J., Wang, R., Chen, S.-N. and Chen, Y.-Z. (1993) *Planta Med.* **59**, 83.
15. De la Fuente, G., Meriçli, A. H., Ruiz-Mesía, L., Ulubelen, A., Merçli, F. and Ilarslan, R. *Phytochemistry* (in press).
16. Jones, A. J. and Benn, M. H. (1972) *Tetrahedron Letters* 4351.
17. Codding, P. W., Kerr, K. A., Benn, M. H., Jones, A. J., Pelletier, S. W. and Mody, N. V. (1980) *Tetrahedron Letters* **21**, 127.
18. Kulanthailvel, P. and Benn, M. (1985) *Heterocycles* **23**, 2515.
19. González, A. G., De la Fuente, G. and Díaz, R. (1982) *Phytochemistry* **21**, 1781.
20. Glinski, J. A., Joshi, B. S., Jiang, Q. P. and Pelletier, S. W. (1988) *Heterocycles* **27**, 185.
21. Pelletier, S. W., Harraz, F. M., Badawi, M. M., Tantiraksachai, S. Wang F. and Chen, S. (1986) *Heterocycles* **24**, 1853.

22. González, A. G., De la Fuente, G., Reina, M., Díaz, R. and Timón, I. (1986) *Phytochemistry* **25**, 1971.
23. Pelletier, S. W., Aneja, R. and Gopinath, K. W. (1968) *Phytochemistry* **7**, 625.
24. Pelletier, S. W. and Oeltmann, T. N. (1968) *Tetrahedron* **24**, 2019.
25. Pelletier, S. W. and Mody, N. V. (1979) *J. Am. Chem. Soc.* **101**, 492.
26. González, A. G., de la Fuente, G., Riena, M. and Acosta, R. D. (1986) *Heterocycles* **24**, 1513.
27. Ulubelen, A., Meriçli, A. H., Meriçli, F. and Ilarsland, R. (1992) *Phytochemistry* **31**, 1019.