Diterpenoid Alkaloids from *Delphinium davisii*

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Three new hetisane-type diterpenoid alkaloids, davisinol (**6**), 18-benzoyldavisinol (**7**), and Davisin (**9**) have been isolated from *Delphinium davisii* Munz. and their structures established by detailed spectroscopic studies. Accurate ^{1}H- and ^{13}C-NMR assignments have been made for kobusine (**8**), a related hetisane-type alkaloid, and karakoline (**5**), a norditerpenoid alkaloid. The known norditerpenoid alkaloids 14-acetylperegrine (**4**), 6-deacetylperegrine (**3**), and karakoline (**5**) and the diterpenoid alkaloids hetisine and hetisinone were also isolated.

Turkish *Delphinium* species are used externally in the treatment of rheumatic pain and sciatica and also against body lice.[1]

No phytochemical investigations appear to have been carried out on the constituents of *Delphinium davisii* Munz., a plant growing in central Turkey. In continuation of the collaborative studies on the genus *Delphinium* indigenous to Turkey, between the Institute of Natural Products Research and the Faculty of Pharmacy at the University of Istanbul,[2−6] we report in this paper the isolation and structure determination of the diterpenoid alkaloids of *D. davisii* Munz.

Results and Discussion

The aerial parts of *D. davisii* Munz. were extracted as described in the Experimental Section to give four major fractions A−D. Chromatographic separation of fraction A on an Al_2O_3 rotor gave two known norditerpenoid alkaloids that were identified as 14-acetylperegrine (**4**) and 6-deacetylperegrine (**3**). The isolation of peregrine (**2**) from the aerial parts of *D. peregrinum* var. *elongatum* Boiss. and the erroneous structure (**1**) based on ^{1}H- and ^{13}C-NMR spectra were first reported by de la Fuente *et al.* in 1988.[7] Acetylation of Peregrine gave 14-acetylperegrine, and hydrolysis with KOH in MeOH afforded 6-deacetylperegrine. The structure of peregrine (**2**) has been recently revised to **2** by de la Fuente *et al.* on the basis of ^{1}H, COSY, HMQC, HMBC, and ROESY NMR spectra and an X-ray analysis of 6-deacetylperegrine (**3**).[8] The structures of peregrine and 14-acetylperegrine are therefore revised to **2** and **4**, respectively.

The fraction D was separated on an Al_2O_3 rotor of a Chromatotron to give an amorphous homogeneous alkaloid, $[\alpha]_D$ +27.5° designated as davisinol (**6**). Its molecular formula $C_{20}H_{27}NO_2$ was derived from the FABMS and HRMS $[M + H]^+$ *m/z* at 314.2108. The IR spectrum does not show bands for a carbonyl group. Biogenetic considerations and the absence of a methoxyl or *N*-methyl group indicated that davisinol must belong to the hetisane-type, amongst the various skeleta known for diterpenoid alkaloids.[9,10] The ^{13}C-NMR spectrum of davisinol (**6**) shows 20 carbon signals (Table 1). The DEPT spectrum revealed four non-protonated carbon signlets at δ 145.8, 49.6, 43.5, 40.5; seven doublets at δ 75.7, 67.4, 64.8, 59.5, 56.0, 44.0, 41.9, and nine triplets at δ 110.1, 69.2, 58.2, 35.8, 33.6, 29.6, 28.4, 26.5, 18.9. In the ^{1}H-NMR spectrum (Table 1) the characteristic carbon and proton signals for an exocyclic methylene group were clearly observed: δ 4.83 (2H, d, J = 1.8 Hz), 145.8 (s), 110.1 (t). Two other lowfield methylene triplets occur at δ 58.2 and 69.2. The former signal can be assigned to C-19 (δ 2.55, 2.23 AB, J = 12.5 Hz), based upon an observed NOESY correlation between one of the protons on C-19 (δ 2.23) and H-20 (δ 2.40) (Table 1) and the HMBC correlation of H-20 with C-19, C-6 (64.8), C-8 (43.5), and C-13 (29.6) (Table 1). As the normally observed signal for a C-4 methyl is absent in the ^{1}H- and ^{13}C-NMR spectra, the methylene signal at δ 69.2 (δ 3.28, 3.43 AB, J = 10.8 Hz) should be assigned to C-18 bearing a hydroxyl group. This assignment is confirmed by the HMBC spectrum, which shows a three-bond correlation of H-19α at δ 2.55 with C-18, and H-18b at δ 3.28 with C-5 (δ 56.0; δ 1.72). In this partial structure of davisinol with an OH at C-18, the location of the second oxygen function, which can only be a hydroxyl group, remains to be decided. When no hydroxyl group is present at C-1, C-2, or C-3 in ring A, the signal for C-2 appears about δ 19.8.[11] As the most

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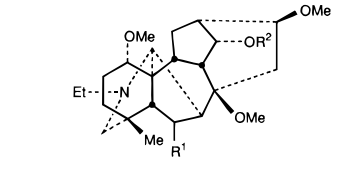
Table 1. NMR Data for Davisinol (**6**)[a]

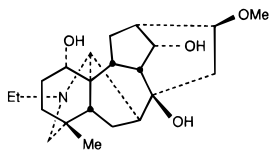
position	δ ^{13}C	δ ^{1}H (mult, J = Hz)	COSY	NOESY	HMBC (H → C)
1	26.5 (t)	α, 1.80 (m)	1β, 2β, 20[c]		3, 10, 5
		β, 1.40 (m)	1α		2, 10, 20
2	18.9 (t)	α, 1.70 (m)			
		β, 1.40 − 1.50 (m)	1α		
3	28.4 (t)	1.48 (m)			
4	43.5 (s)				
5	56.0 (d)	1.72 (s)	20[b]	6, 18α, H19α	6, 8
6	64.8 (d)	3.14 (br s)	7α, 7β, 20[b]	5, 7α, 18a, 18b, 19β	8, 10, 20
7	35.8 (t)	α, 1.65 (d)	6, 15	6, 7β	5, 6, 8
		β, 1.57 (d)	15	7α, 9	
8	40.5 (s)				
9	59.5 (d)	1.38 (s)	11, 12,[b] 14[b]	7β, 11, 14	5, 11, 14, 20
10	49.6 (s)				
11	67.4 (d)	4.01 (d 4.8)	9, 12	9, 12, 13α	8, 10, 16
12	41.9 (d)	2.28 (d 4.8)	9,[b] 11, 13α, 13β	11, 15, 17	
13	29.6 (t)	α, 1.90 (m)	13β	11, 13β, 20	11, 14
		β, 0.91 (m)	13α	13β, 20	
14	44.0 (d)	1.78 (m)	9,[b] 20	9	7
15	33.6 (t)	2.10 (m)	7α,[c] 17[c]	12, 17	8, 9, 16, 17
16	145.8 (s)				
17	110.1 (t)	4.83 (d, 1.8)	15[c]	12	12, 15
18	69.2 (t)	α, 3.43 (AB 10.8)	18b	5, 6, 18b	3, 4, 20
		β, 3.28 (AB 10.8)	18a, 20	6, 18a	3, 5
19	58.2 (t)	α, 2.55 (AB 12.5)	19β	5, 19β	
		β, 2.23 (AB 12.5)	19α	6, 19α, 20	3, 4, 20
20	75.7 (d)	2.40 (s)	14, 18b	13α, 13β, 19α	6, 8, 13, 19

[a] The ^{1}H-^{13}C correlations were based on HETCOR spectrum. [b] Value indicates W-type coupling. [c] Entry indicates long-range coupling.

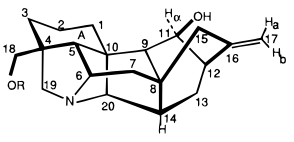
upfield ^{13}C signal in davisinol appears at δ 18.8 (t), it does not bear an OH in ring A. The remaining methylenes on which a hydroxyl may be located are C-7, C-11, C-13, or C-15. The C-6 resonance at δ 64.8 shows correlation to δ 3.14 in the HETCOR spectrum, and this proton shows COSY correlation with H-7α, H-7β (δ 1.65, 1.57), and C-7 does not, therefore, bear an OH group. The C-16 quaternary carbon resonance at δ 145.8 indicated that C-15 does not bear an OH group.[10] This leaves the location of the OH group at C-11 or C-13. Positioning of the hydroxyl group at C-11 and not at C-13 is based on COSY and HMBC results.



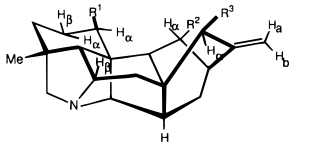
1 R^1 = α-OAc, R^2 = H
2 Peregrine R^1 = β-OAc, R^2 = H
3 6-Deacetylperegrine R^1 = β-OH, R^2 = H
4 14-Acetylperegrine R^1 = β-OAc, R^2 = Ac



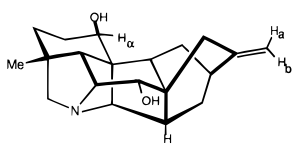
5 Karakoline



6 Davisinol R = H
7 18-Benzoyldavisinol R = CO-Ph



8 Kobusine R^1 = H, R^2, R^3 = β-OH
9 Davisine R^1, R^3 = β-OH, R^2 = H
10 Diacetyldavisine R^1, R^3 = β-OAc, R^2 = H



11 Crassicauline B

It is necessary first to identify either the C-9 or the H-9 signal in the NMR spectrum. The methylene for H-19, which can be recognized with certainty, shows in the HMBC spectrum correlations with C-3 (δ 28.3), C-18, and C-20, all three bonds away (Table 1). In addition, correlation is observed to the quaternary carbon at C-4 (δ 43.5). The H-20 proton at δ 2.40 shows correlation with H-14 (δ 1.78), H-6 (δ 3.14, brs), and H-18b (δ 3.28). It also shows a W-type coupling with H-5 (δ 1.72) and a long-range coupling with H-1α (δ 1.80). The H-14 proton (δ 1.78) showed a W-type coupling with H-9 (δ 1.38) and H-20 (δ 2.40). The H-9 proton is correlated with δ 59.5 (d) in the HETCOR and appears as a sharp singlet. The H-9 proton shows in the COSY spectrum, correlation with H-11 (δ 4.01, d, J = 4.8 Hz) and W-type coupling with H-12 (δ 2.28, d, J = 4.8 Hz) and H-14, as stated above. These correlations are possible only by location of the hydroxyl group at C-11. This conclusion is supported by the HMBC spectrum in which H-9 proton (δ 1.38) is correlated with C-14 (δ 44.0), C-5 (δ 56.0), C-20 (δ 75.7), and C-11 (δ 67.4). These correlations would not be possible if the hydroxyl group were located at C-13. First, H-9 would not be a singlet, if the adjacent group at C-11 were a methylene; second, this proton will not show a COSY correlation with a proton at C-13 bearing a hydroxyl group. The evidence of locating an OH at C-11 results from a few other sets of correlations. The C-17 methylene protons at δ 4.83 show a long-range COSY correlation to H-15 (δ 2.10, m). In the HMBC spectrum, a slice at δ 2.10 (H-15) showed response of signals for C-9 (δ 59.5) or C-17 (δ 110.3), both three bonds removed, and C-8 (δ 40.5) and C-16 (δ 145.8), two bonds away. In the HMBC spectrum, a slice at δ 4.83 (H-17) showed responding carbon signals for C-15 and C-12 (δ 41.9). In the COSY spectrum H-12 is correlated with H-11, H-13α, H-13β, and H-9. These results indicate the

Table 2. NMR Data for Kobusine (**8**)[a]

position	δ ^{13}C	δ ^{1}H (mult, J = Hz)	COSY (LRCOSY)	NOESY	HMBC (1H → C)
1	26.9 (t)	α, 1.76 (m)	*β*	2α	3, 9, 10
		β, 1.45 (m)	α, H2α		3, 5
2	19.5 (t)	α, 1.62 (m)	*β*, 3α	1α	
		β, 1.47 (m)			
3	33.8 (t)	α, 1.40 (dt, 3, 14)	2α, 3*β*		
		β, 1.25 (td, 2.5, 14)	3α		1, 2, 4, 13, 18, 19
4	37.8 (s)				
5	61.0 (d)	1.49 (s)	6, 19*β*[b]	6, 9, 18	18, 19, 20
6	65.1 (d)	3.2 (br s, 1/2w 2.3)	5, 7α, 7*β*, 20[b]	5, 7b, 18, 19*β*	4, 8, 10, 20
7	32.4 (t)	*β*, 2.10 (dd 13.6, 2.3)	6, 7*β*	7*β*, 14	8, 14
		α, 1.63 (dd 13.6, 2.6)	6, 7α	7α, 6	5, 6, 9
8	45.9 (s)				
9	54.9 (d)	1.67 (s)	11, 14,[b] 20[b]	5, 11	11, 14, 20
10	49.1 (s)				
11	67.5 (d)	α, 4.00 (d 4.7)	9, 12	9, 12, 13α	8, 9, 10, 16
12	41.4 (d)	2.43 (m)	11α, 13α, 13*β*	11, 13*β*, 17a	13, 14
13	30.3 (d)	α, 1.77 (dd 9.7, 2.4)	12	14	11
		β, 0.89 (m)	12	11	16, 20
14	41.6 (d)	1.79 (m)	9,[b] 13α, 13*β*, 20	7α, 13*β*, 20	9, 10
15	70.9 (d)	α, 3.85 (s)	7α, 17a, 17b	17b	7, 9, 16, 17
16	150.7(s)				
17	114.3 (t)	α, 5.05 (s)	15α, 17b	12, 17b	12, 15
		β, 5.15 (s)	15α, 7α	15, 17a	12, 15
18	28.8 (q)	0.94 (s)	19α	5, 6, 19*β*	3, 4, 19
19	62.4 (t)	α, 2.32 (AB, 12.4)	18, 19*β*	3α	3, 6, 18, 20
		β, 2.47 (AB, 12.4)	5,[b] 19α	6, 18	3, 4, 18
20	75.0 (d)	2.44 (s)	6,[b] 9,[b] 14	14	1, 6, 8, 19

[a] The ^{1}H-^{13}C correlations were based on HETCOR spectrum. [b] Value indicates W-type coupling.

location of an OH group at C-11. Some of the ^{1}H-NMR assignments have been confirmed by selective irradiation experiments. Irradiation at δ 1.65 (H-7α) collapsed the signal at δ 1.57 (H-7*β*) and irradiation at δ 2.23 (H-19*β*) showed a change of the signals at δ 2.55 (H-19α).

The hydroxyl group at C-11 can be either *ψ*-axial (shown as a dotted line) or *ψ*-equatorial (shown as a thick line) in the twist boat ring formed by C-8, C-9, C-11, C-12, C-13, and C-14. When the OH group is *ψ*-axial, H-11 should show a large coupling (∼ 9−10 Hz) with H-9 (almost 0−2°) and ∼ 4−5 Hz coupling with H-12 (∼ 30°). In davisinol, the coupling with H-9 is almost nil (∼ 90°) and the coupling with H-12 is ∼ 5 Hz (almost 45°) as expected for a *ψ*-equatorial OH group, confirming structure **6** for davisinol.

There are only a few hetisane-type alkaloids that bear a *β*-hydroxyl group at C-11. These are cardionine,[12] isohypognavine,[13] kobusine,[14] pseudokobusine,[15] and talatisine.[16] The ^{13}C-NMR data have been reported for cardionine and isohypognavine but the assignments were not firmly established. Many of the structures were derived from X-ray crystallographic analyses. In order to enable structure derivation by spectral data, we undertook detailed NMR studies of kobusine[14] the absolute stereochemistry of which was established as that of compound **8**.[17] The results of ^{1}H, ^{13}C, COSY HETCOR, and HMBC spectra are summarized in Table 2.

Fraction D gave another homogeneous alkaloid, which was identified as hetisine by comparison of its TLC, IR, ^{1}H-NMR, and ^{13}C-NMR spectral data with those of an authentic sample.[18]

The fraction B was chromatographed on an Al_2O_3 rotor and eluted with hexane $CHCl_3$ (35:65) to give a new homogeneous amorphous alkaloid, $[\alpha]_D$ +42.3°, IR ν_{max} (nujol): 1720, 1465, 1455, 1375 cm^{-1}. Its molecular formula, $C_{27}H_{31}NO_3$, was derived from the FABMS and HRMS, $[M + H]^+$, m/z at 418.2384. The IR (1720 cm^{-1}), ^{1}H NMR [δ (8.02, 2H, d, J = 7.5 Hz, H-2′, 6′), 7.46 (2H, dd, J = 7.6 Hz, H-3′, 5′), 7.58 (1H, dd, J = 7.4 Hz, H-4′)] (Table 3), and ^{13}C-NMR spectral data [δ 129.6 (2C, C-2′, 6′), 128.5 (2C, C-3′, 5′), and 133.1 (C-4′)] indicated that this is a benzoate ester of a hetisane-type alkaloid. The resemblance of the ^{13}C-NMR chemical shifts (Table 3) with those of davisinol (Table 1) suggested that this alkaloid might be 18-benzoyldavisi-

Table 3. NMR Data for 18-Benzoyldavisinol (**7**)[a]

position	δ ^{13}C	δ ^{1}H (mult, J = Hz)	HMBC (H → C)
1	26.4 (t)	α, 1.92 (m)	
		β, 1.51 (m)	
2	18.8 (t)	α, 1.79 (m)	
		β, 1.51 (m)	
3	28.9 (t)	1.62 (m)	2
4	42.3 (s)		
5	56.3 (d)	1.88 (m)	19, 20
6	65.2 (d)	3.27 (br s) 8, 10, 20	
7	35.8 (t)	α, 1.76 (m) 5, 6, 8, 9	
		β, 1.61 (m) 8, 9, 14	
8	40.5 (s)		
9	59.6 (d)	1.45 (s)	5, 7, 10, 11, 14, 20
10	49.5 (s)		
11	67.5 (d)	4.07 (d 4.8)	8, 10, 12, 16
12	41.9 (d)	2.33 (br s 1/2 w 9)	14, 16, 17
13	29.5 (t)	α, 1.95 (m)	20
		β, 1.02 (m)	
14	44.3 (d)	1.90 (m)	9, 13, 20
15	33.6 (t)	α, 2.27 (m)	8, 9, 16, 17
		β, 2.20 (m)	8, 14, 16, 17
16	145.6 (s)		
17	110.7 (s)	4.89 (br s)	12, 15
18	70.8 (t)	α, 4.24 (AB 12.8)	3, 5, 19, (*O*CO)
		β, 4.06 (AB 12.8)	3, 4, 5, (*O*CO)
19	58.4 (t)	α, 2.72 (AB 17.9)	3, 4, 18, 20
		β, 2.44 (AB 17.9)	3, 6, 18, 20
20	75.9 (d)	2.51 (s)	6, 8, 13, 19
O*C*O	166.1 (s)		
C1′	130.1 (s)		
C2′,C6′	129.6 (d)	8.02 (d 7.5)	4′, 3′, 5′
C3′, C5′	128.5 (d)	7.46 (dd 7.6)	2′, 6′
C4′	133.1 (d)	7.58 (dd 7.4)	2′, 3′, 5′, 6′

[a] The ^{1}H-^{13}C correlations were based on HMQC spectrum.

Table 4. NMR Data for Karakoline (**5**)[a]

position	δ ^{13}C	δ ^{1}H (mult, J = Hz)	COSY	HMBC (H → C)
1	72.4 (d)	3.69 (t, 2.5)	2α	2, 3, 11, 17
2	29.6 (t)	α, 1.62 (m)	1, 2β	
		β, 1.55 (m)	2α	
3	31.1 (t)	α, 1.46 (td, 3.0, 13.0)	3β	1, 4, 5, 19
		β, 1.71 (dt, 6.0, 13.0)	3α	1, 2, 4, 18, 19
4	32.8 (s)			
5	46.4 (d)	1.58	17,[b] 19[b]	
6	25.1 (d)	α, 1.55	6β, 7, 17[b]	3, 4, 8, 9, 11, 19
		β, 1.88 (q, 7.0, 15.0)	6α	
7	45.0 (d)	2.05 (m)	6α	7, 17
8	74.2 (s)			
9	46.6 (d)	2.20 (m)	10, 14	8, 13, 14
10	43.9 (d)	1.80 (m)	9, 12	8, 9, 11, 12, 17
11	48.7 (s)			
12	28.5 (t)	α, 2.00 (m)	10, 12β, 13	16
		β, 1.60 (m)	12α	
13	40.0 (d)	2.30 (t, 5.0)	12, 14	9, 10, 12, 14, 15, 16
14	75.7 (d)	4.18 (t, 4.8)	9, 13	8, 16
15	42.2 (t)	α, 2.35 (m)	15β, 16	8, 9, 13, 16
		β, 2.05 (m)	15α, 16	8, 16
16	82.0 (d)	3.35 (dd, 5.0, 9.0)	15α, 15β	16′
17	63.3 (d)	2.74 (s)	5,[b] 6α[b]	6, 9, 19
18	27.5 (q)	0.85 (s)		3, 4, 5, 19
19	60.2 (t)	α, 2.25 (AB, 10.5)		3, 4, 5, 17
		β, 2.05 (AB, 10.5)	5[b]	4, 17
NCH_2	48.3 (t)	NCH_2, 2.40, 2.49 (m)	NCH_2-CH_3	17, 19, NCH_2CH_3
CH_3	13.0 (q)	CH_3, 1.10 (t, 7.5)	NCH_2-CH_3	NCH_2CH_3
OCH_3	56.2 (q)	16′, 3.31 (s)		

[a] The ^{1}H-^{13}C correlations were based on HETCOR spectrum. [b] Values indicate W-type coupling.

nol (**7**). Alkaline hydrolysis of this alkaloid gave davisinol (**6**), confirming the structure assignment. The ^{1}H-NMR spectral data established the location of the benzoate group at C-18. The signal for the H-11 proton in davisinol (**6**) and the benzoate is unchanged and appears at ~ δ 4.00. However, the H-18 protons of **6** at δ 3.28 and 3.43 are shifted downfield to δ 4.08 and 4.24 in the benzoate ester, establishing structure **7** for 18-benzoyldavisinol. Supporting evidence is obtained from the HMBC spectrum (Table 3).

Another alkaloid isolated from the chromatographic separation of B was identified as the norditerpenoid alkaloid karakoline (karacoline, vilmorrianine B) (**5**). This alkaloid was first isolated from *Aconitum karakolicum* Rapcs. and its structure derived from chemical transformations and spectral analysis.[19] Almost identical ^{13}C-NMR chemical shift assignments for karakoline were made by the Chinese and Japanese investigators.[20−22] Because these assignments were not rigorous and were made on the basis of application of the rules of chemical shifts for different substituent groups and comparison of spectra of closely related compounds, we carried out detailed ^{1}H- and ^{13}C-NMR spectral analysis to enable us to make definite assignments for karakoline (**5**). Table 4 summarizes the results of ^{1}H-NMR, ^{13}C-NMR, HMQC, COSY, and HMBC spectral assignments for **5**, a less substituted norditerpene alkaloid. Our results show that the previously assigned ^{13}C-NMR chemical shifts for C-5, C-7, C-10, and C-13 need to be revised.

Fraction C was purified on an Al_2O_3 rotor of a Chromatotron. Gradient elution with hexane, $CHCl_3$, and MeOH gave a homogeneous new alkaloid davisine (**9**), which crystallized from acetone-hexane, mp 130−132 °C, $[\alpha]_D$ + 29.9°. The molecular formula $C_{20}H_{27}NO_2$ was derived from its HRFABMS, $[M + H]^+$ m/z 314.2111. The ^{1}H-NMR spectrum exhibited signals for the presence of an exocyclic methylene (δ 4.94, 4.96, br d) and a tertiary methyl (δ 1.01, 3H, s) and indicated the absence of methoxyl groups. These data indicated that the compound is a diterpenoid and not a norditerpenoid alkaloid. The ^{13}C-NMR spectrum and DEPT experiments indicated the presence of one methyl at δ 28.4; seven methylenes at δ 108.8, 62.8, 33.1, 32.5, 26.8, 27.8, and 27.1; eight methines at δ 75.6, 71.3, 66.1, 65.7, 56.5, 43.4, 41.3, and 33.7; and four quaternary carbons at δ 156.2, 55.0, 45.8, and 37.5. The molecular formula $C_{20}H_{27}NO_2$ indicated eight degrees of unsaturation of which one degree is accounted for by the presence of an exocyclic methylene. The remaining seven degrees of unsaturation indicate the presence of a heptacyclic skeleton as in hetisane-type diterpenoid alkaloids. As there are no other unsaturations in the molecule, both the oxygens of davisine are present as OH groups. This conclusion is also supported by the preparation of diacetyldavisine (**10**). The C-16 carbon signal at δ 156.2 indicated that there is an OH group located at C-15, the absence of which would have caused C-16 to appear around δ 141−148. The remaining hydroxyl should be present in ring A, because of the absence of a methylene in the ^{13}C-NMR ~ δ 18−19.[11] In hetisane-type alkaloids, C-10 normally appears at ~ δ 49−51, and in davisine, the downfield singlet at δ 55.0 is assigned to C-10, this shift being ascribed to the presence of an adjacent OH group. There are eight methine carbons in davisine of which four signals at δ 75.6, 71.3, 66.1, and 65.9 must be due to carbons attached to either nitrogen or oxygen. These are assigned to C-20, C-15, C-1, and C-6, respectively, on the basis of ^{1}H-NMR and HMBC experiments (Table 5).

The configuration of the OH at C-1 (δ 66.1) in **9** is considered as β, by comparison of the chemical shifts with those reported for lassiocarpine (δ 70.4),[23] napelline (δ 70.5),[24] lusidusculine (δ 69.9),[25] and songorine (δ 70.1),[15] which all bear an α-OH at C-1. As a general rule, in norditerpenoid alkaloids, C-1 having an α-OH

Table 5. NMR Data for Davisine (**9**)[a]

position	δ ^{13}C	δ ^{1}H (mult, J = Hz)	COSY	SINEPT (^{1}H → ^{13}C)
1	66.1 (d)	α, 4.20 (br s)	2	3, 5, 10
2	27.1 (t)	1.76 (m)		1, 3, 10
3	27.8 (t)	α, 1.25 (m)	3β	1, 2, 4, 5, 18
		β, 1.78 (m)	3α	1, 2, 4, 19
4	37.5 (s)			
5	56.5 (d)	1.86 (s)	6	6, 8, 9, 10, 19
6	65.7 (d)	3.33 (br s)	5, 7α, 7β, 20[b]	4, 7, 8, 10, 20
7	32.5 (t)	α, 1.65 (dd, 13.0, 3.0)	6, 7β	5, 6, 8, 9
		β, 2.02 (dd, 13.0, 2.5)	6, 7α	5, 6, 8, 9, 14
8	45.8 (s)			
9	41.3 (d)	2.00 (d, 12.0)	11α, 11β, 15[c]	5, 7, 8, 10, 12 14, 20
10	55.0 (s)			
11	26.8 (t)	α, 1.93 (dd, 14.0, 4.0)	9, 12	8, 9, 10, 12, 16
		β, 1.71 (m)	9, 12	10, 12, 13, 16
12	33.7 (d)	2.21 (brd, $W_{1/2}$, 5)	11α, 11β, 13α, 13β	9, 13, 14, 15, 16, 17
13	33.1 (t)	α, 1.09 (dd, 13.0, 2.5)	13β	12, 14, 16, 20
		β, 1.80 (m)	13α	14, 20
14	43.4 (d)	1.85 (m)		7, 8, 10, 13, 20
15	71.3 (d)	3.99 (s)	9,[c] 17	7, 8, 9, 12, 16, 17
16	156.2 (s)			
17	108.8 (t)	α, 4.94 (s)	15α[c]	12, 15, 16
		β, 4.97 (s)		12, 15, 16
18	28.4 (q)	1.01 (s)	19[c]	3, 4, 5, 19
19	62.8 (t)	α, 2.37 (AB 12.8)	19β	3, 6, 18, 20
		β, 2.50 (AB 12.8)	18,[c] 19α	18, 20
20	75.6 (d)	2.43 (s)	6[b]	1, 6, 8, 9, 13, 14, 19

[a] The ^{1}H-^{13}C correlations were based on HETCOR spectrum. [b] Values indicate W-type coupling. [c] Values indicate long-range coupling.

group appears at δ ~72−73, whereas C-1 with a β-OH group appears upfield by ~4 ppm at δ 68−69.[22,26] The only diterpenoid alkaloid with a C-1 β-hydroxyl group found in the literature is crassicauline B (**11**).[27] The ^{13}C-NMR data for **11** are not available. The reason given by the authors for assigning a β-configuration for the C-1-OH is that, in the ^{1}H-NMR spectrum of **11**, H-1 appeared as a multiplet at δ 4.27 and showed a coupling of $W_{1/2}$ = 9 Hz with the geminal proton. The A ring having a C-1$_\alpha$-OH group in norditerpenoid alkaloids has been shown to take a boat conformation because of an intramolecular N − H···O hydrogen bond.[28] In the case of a C-1$_\beta$-OH group, an intramolecular hydrogen bond is no longer possible and the A ring will assume a chair conformation. The H-1 proton in **9** at δ 4.19 and in **10** at δ 5.25 appear as broad peaks having $W_{1/2}$ ~ 5.5 Hz suggesting that the C-1 OH group should have a β configuration.

Another alkaloid isolated from fraction C was identified as hetisinone by comparison of its TLC, ^{1}H-NMR, and ^{13}C-NMR spectra with those of an authentic sample.[12]

Experimental Section

General Experimental Procedures. Melting points are corrected and were determined on a Thomas-Kofler hot stage equipped with a microscope and a polarizer. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter in $CHCl_3$. IR spectra were recorded in Nujol on a Perkin-Elmer model 1420 spectrophotometer. HRMS were determined on a Fisons Auto Space ETOFFPD FAB$^+$ mass spectrometer and Perkin-Elmer SCIEX AP1-1. NMR spectra, including DEPT and 2D experiments, were recorded in $CDCl_3$ on Bruker AC-250 and Bruker AC-300 spectrometers. The pulse sequences employed for the NMR experiments were those of the standard Bruker software. The pulse sequence for the selective INEPT NMR experiments was obtained by modifying the Bruker standard INEPT sequence, and the critical parameters used were as described.[29] Chromatographic separations on a Chromatotron[30] were carried out on rotors coated with 1-mm thick layers of Merck Al_2O_3 60 PF 254, 365 (EM 1104).

Plant Material. The aerial parts of *D. davisii* Munz. were collected in central Turkey (Cankiri-Eskipazar) in August 1994, by Mr. R. Ilarslan and identified by Dr. K. Alpinar. A voucher specimen has been deposited in the Herbarium of the Department of Pharmacy, University of Istanbul, Turkey.

Extraction of Crude Alkaloids. Dried and powdered aerial parts of *D. davisii* (600 g) were exhaustively extracted by percolation at room temperature with 95% EtOH. Evaporation (*in vacuo*) of the combined extracts gave a gummy residue (50 g) that was dissolved in CH_2Cl_2 (500 mL) and extracted twice with 5% H_2SO_4 (200 mL). The acidic extract was washed with CH_2Cl_2 (100 mL × 3) and then basified in the cold, with aqueous NaOH. Extractions with CH_2Cl_2 (pH 10) (250 mL × 10) and (pH 14) (250 mL × 6) and evaporation of the combined extracts *in vacuo* gave a crude mixture of alkaloids (2.84 g). The crude alkaloidal mixture was chromatographed on a basic Al_2O_3 column (5 × 60 cm). The eluting solvent was a gradient of petroleum ether, EtOAc, and EtOH. In all, 79 fractions (100 mL each) were collected. These were combined on the basis of TLC results, and the fractions were pooled to give four fractions—fractions A 1−29 (500 mg), B 30−55 (350 mg), C 56−72 (470 mg), and D 73−79 (300 mg). These were separated on Al_2O_3 rotors of a Chromatotron as described below.

14-Acetylperegrine (4) and 6-Deacetylperegrine (3). Fraction A (500 mg) was chromatographed on a

basic Al_2O_3 rotor. One hundred fractions (20 mL each) were collected by gradient elution with hexane and increasing percentage of $CHCl_3$. Fractions 21−22 (hexane-$CHCl_3$, 80:20) gave 14-acetylperegrine (**4**, 6.5 mg): MS *m/z* $[M + H]^+$ 506; calcd for $C_{28}H_{43}NO_7$, $[M + H]^+$ 506; ^{1}H-NMR δ 0.85 (3H, s, 18-Me), 1.05 (3H, t, J = 7.0 Hz, *N*-CH_2CH_3), 1.45 (1H, br s, H-5), 1.98, 1.99 (each 3H, s, OAc-6, -14), 2.42 (1H, br s, H-20), 2.71 (1H, d, J = 7.4 Hz, H-7), 3.00 (3H, s, OMe-8), 3.27 (3H, s, OMe-1), 3.36 (3H, s, OMe-16), 4.62 (1H, t, J = 5 Hz, H-14), 5.20 (1H, d, J = 7.1 Hz, H-6); ^{13}C-NMR δ 85.0 (d, C-1), 26.9 (t, C-2), 37.0 (t, C-3), 34.1 (s, C-4), 56.4 (d, C-5), 72.9 (d, C-6), 41.7 (d, C-7), 78.4 (s, C-8), 41.0 (d, C-9), 45.8 (d, C-10), 49.1 (s, C-11), 28.4 (t, C-12), 38.9 (d, C-13), 76.1 (d, C-14), 35.7 (t, C-15), 83.4 (d, C-16), 63.9 (d, C-17), 25.9 (q, C-18), 57.3 (t, C-19), 48.6 (t, C-20), 13.5 (q, C-21), 55.9 (q, C-11), 47.8 (q, C-8′), 56.2 (q, C-16′), 171.4 (CO), 21.6, 21.3 (CO*C*H_3). Fractions 33−35 (hexane/$CHCl_3$ 60:40) afforded 6-deacetylperegrine (**3**, 45 mg): MS *m/z* $[M + H]^+$ 422; calcd for $C_{24}H_{39}NO_5$, $[M + H]^+$ 422; ^{1}H-NMR δ 0.94 (3H, s, Me-18), 1.04 (3H, t, J = 7.5 Hz, *N*-CH_2CH_3), 1.42 (1H, brs, H-5), 2.59 (1H, d, J = 7.3 Hz, H-7), 3.25 (3H, s, OMe-8), 3.30 (3H, s, OMe-1), 3.37 (3H, s, OMe-16), 4.02 (1H, m, H-14), 4.28 (1H, d, J = 7.5 Hz, H-6), 4.90 (1H, br s, OH); ^{13}C-NMR δ 85.4 (d, C-1), 26.2 (t, C-2), 37.3 (t, C-3), 34.4 (s, C-4), 58.6 (d, C-5), 75.0 (d, C-6), 46.0 (d, C-7), 80.7 (s, C-8), 43.4 (d, C-9), 45.6 (d, C-10), 48.1 (s, C-11), 28.2 (t, C-12), 37.29 (d, C-13), 72.7 (d, C-14), 31.8 (t, C-15), 82.1 (d, C-16), 64.1 (d, C-17), 25.8 (q, C-18), 57.9 (t, C-19), 49.4 (t, C-20), 13.6 (q, C-21), 56.2 (q, C-1′), 48.3 (q, C-8′), 56.4 (q, C-16′).

18-Benzoyldavisinol (7) and Karakoline (5). Fraction B (350 mg) was chromatographed on a basic Al_2O_3 rotor. Seventy-five fractions (20 mL each) were collected by gradient elution with hexane with an increasing percentage of $CHCl_3$ and with $CHCl_3$ with increasing percentage of MeOH. Fractions 61−71 (hexane-$CHCl_3$, 35:65) gave 18-benzoyldavisinol (**7**, 60 mg): FABMS, $[M + H]^+$ 418; HRMS *m/z* $[M + H]^+$ 418.2384, calcd for $C_{27}H_{32}NO_3$ 418.2382; $[\alpha]^{25}_D$ + 42.34° (*c* 0.206, $CHCl_3$); IR nujol ν_{max} 1720, 1465, 1375, 1336, 1270 cm^{-1}. For ^{1}H- and ^{13}C-NMR data see Table 3. Fractions 76−78 ($CHCl_3$-MeOH, 99:1) gave karakoline (**5**, 70 mg), identical in its TLC, ^{1}H-NMR, and ^{13}C-NMR spectral comparison with an authentic sample.

Hydrolysis of 18-Benzoyldavisinol (7) to Davisinol (6). A solution of 18-benzoyldavisinol (10 mg) in 2% EtOH−KOH (3 mL) was stirred at room temperature for 16 h, cooled to 0 °C, and extracted with $CHCl_3$ (3 × 25 mL). The $CHCl_3$ extract was dried (Na_2SO_4), and evaporated *in vacuo* to give a white foam (8 mg), identical in the TLC, co-TLC, and ^{1}H-NMR spectral comparison with an authentic sample.

Davisine (9) and Hetisinone. Fraction C (470 mg) was chromatographed on a basic Al_2O_3 rotor. Ninety-four fractions (20 mL each) were collected by gradient elution with hexane with an increasing percentage of $CHCl_3$ and with $CHCl_3$ with increasing percentage of MeOH. Fraction 47 ($CHCl_3$-MeOH, 99:2) gave a homogeneous compound that crystallized from Me_2CO−hexane to afford colorless crystals of davisine (**9**, 16 mg): mp 130−132 °C; $[\alpha]^{25}_D$ + 29.9° (*c* 1.56, $CHCl_3$); HRFABMS found *m/z* 314.2111 $[M + H]^+$, calcd for $C_{20}H_{27}NO_2$, 314.2112. For ^{1}H- and ^{13}C-NMR data see Table 5. Fractions 56−58 ($CHCl_3$-MeOH, 99:2) gave hetisinone (18 mg). The TLC, ^{1}H-NMR, and ^{13}C-NMR spectral comparison with an authentic sample established its identity.

1,15-Diacetyldavisine (10). Davisine (12 mg) was dissolved in acetyl chloride (2 mL) and the solution kept at room temperature for 4 days. The reaction mixture was evaporated under vaccum and purified on a Al_2O_3 column and eluted with hexane-$CHCl_3$ (8:2) to afford **10** (10 mg) as an amorphous compound: R_f 0.75 (Al_2O_3, $CHCl_3$-MeOH, 9:1); $[\alpha]^{25}_D$ +6.0° (*c* 0.75, $CHCl_3$); ESIMS *m/z* 398 $[M + H]^+$ 100%; IR (nujol) ν_{max} 1732, 1460, 1370, 1230, 1025, 970, 900 cm^{-1}; ^{1}H-NMR δ 5.25 (1H, br s, $W_{1/2}$ 5.5 Hz, H-1α), 1.90 (1H, s, H-5), 3.30 (1H, brs, H-6), 1.68 (1H, *m*, H-7b), 1.90 (1H, m, H-9), 1.60 (1H, dd, J = 13, 2.5 Hz, H-11b), 2.20 (1H, m, H-12), 1.10 (1H, dt, H-13α), 2.01 (1H, m, H-14), 5.40 (1H, s, H-15α), 4.97 (1H, s, H-17b), 4.93 (1H, s, H-17a), 1.03 (3H, s, H-18), 2.56, 2.37 (2H, AB, J = 12.5 Hz, H-19), 2.54 (1H, s, H-20); ^{13}C-NMR δ 69.8 (d, C-1), 23.9 (t, C-2), 28.3 (t, C-3), 37.6 (s, C-4), 57.4 (d, C-5), 65.5 (d, C-6), 32.4 (d, C-7), 44.2 (s, C-8), 42.7 (d, C-9), 53.4 (s, C-10), 26.4 (t, C-11), 33.5 (d, C-12), 33.2 (*t*, C-13), 43.5 (d, C-14), 72.5 (d, C-15), 151.0 (s, C-16), 111.1 (t, C-17), 28.4 (q, C-18), 62.7 (t, C-19), 75.6 (d, C-20), 171.2, 170.8 (s, *C*O CH_3), 21.3, 21.3 (q, CO*C*H_3).

Davisinol (6) and Hetisine. Fraction D (300 mg) was chromatographed on a basic Al_2O_3 rotor. Ninety fractions (20 mL each) were collected by gradient elution with $CHCl_3$ and an increasing percentage of MeOH. Fractions 7−14 ($CHCl_3$-MeOH, 99:1) afforded davisinol (**5**, 60 mg): HRMS found *m/z* $[M + H]^+$ 314.2108, calcd for $C_{20}H_{28}NO_2$, 314.2131, $[\alpha]^{25}_D$ + 27.5° (*c* 0.189, $CHCl_3$); IR (Nujol) ν max 3345, 1100, 750, and 725 cm^{-1}. For ^{1}H- and ^{13}C-NMR data see Table 1. Fractions 49−56 ($CHCl_3$-MeOH, 85:15) gave hetisine (20 mg) identical in its TLC, ^{1}H-NMR, and ^{13}C-NMR spectral comparison with an authentic sample.

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