Pergamon

Tetrahedron Vol. 50, No. 43, pp. 12283-12292, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00778-0

The Structure of Andersobine, A New Diterpenoid Alkaloid from *Delphinium Andersonii* Gray

Balawant S. Joshi,*[1] Mohindar S. Puar,[2] Yili Bai,[1] A. Mukendi Panu,[1] and S. William Pelletier*[1]

[1]Institute for Natural Products Research and Department of Chemistry, The University of Georgia, Athens, Georgia 30602-2556, U. S. A.

[2]Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033, U. S. A.

Abstract: An investigation of the alkaloidal constituents of *Delphinium andersonii* Gray led to the isolation of andersobine (**4**), a new diterpenoid alkaloid. Its structure has been deduced on the basis of ^1H, ^{13}C, ^1H homonuclear COSY, HETCOR, one dimensional nOe, 2D nOe, and selective INEPT nmr spectral studies.

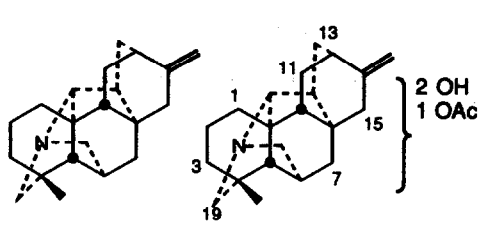
INTRODUCTION

Delphinium andersonii Gray (Ranunculaceae) is a shrub growing in the 'Wildcat Hills' Utah, at an altitude of about 4800 feet. From the aerial parts of the plant, the isolation and structure determination of sixteen norditerpenoid alkaloids: 14-acetylbrowniine, 14-acetyldelcosine, 14-acetylnudicaulidine, andersonidine, andersonine, browniine, 14-deacetylnudicauline, delavaine, delcosine, delectinine, deltaline, dictyocarpine, lycoctonine, methyllycaconitine, nudicauline and takaosamine have been reported.[1-3] The medicinal use of *Aconitums* and *Delphiniums* spans many centuries. Plants that contain norditerpenoid alkaloids are reputed to be used as cardiotonics, sedatives, febrifuges and analgesics.[4] Cattle deaths from grazing of *D. andersonii* have been observed. In the course of our studies on the minor constituents of *D. andersonii*, we isolated by droplet counter current chromatographic separation,[5] a new diterpenoid alkaloid designated as andersobine, and determined its structure as (**4**) by detailed nmr spectral studies.

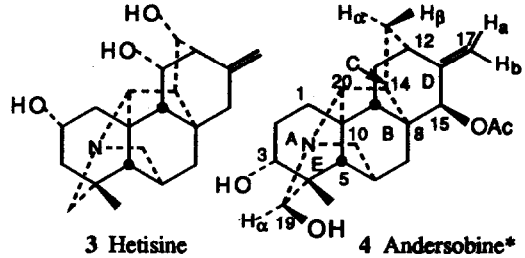
RESULTS AND DISCUSSION

The molecular formula, $C_{22}H_{29}NO_4$, derived for andersobine from the low resolution mass spectrum (EIMS, m/z 371, M^+) and elemental analysis was confirmed by HRMS. Andersobine, mp 310°C, is very sparingly soluble in $CDCl_3$ and the ^1H and ^{13}C nmr spectra were determined in CD_3SOCD_3 and C_5D_5N. As seen in Table 1, the solvent effect on the ^{13}C chemical shifts upon changing from CD_3SOCD_3 to C_5D_5N is minor. The ^1H nmr spectrum in CD_3SOCD_3 indicated the presence of a tertiary methyl at δ 0.95 (3H, *s*) and an acetate methyl at δ 2.02 (3H, *s*). The downfield part of the spectrum showed the presence of an exocyclic methylene group at δ 4.92, 4.83 (each 1H). These inferences were supported by the characteristic carbon resonances in the ^{13}C nmr spectrum at δ 19.1 (*tert.*-$\underline{C}H_3$), δ 170.5 (O$\underline{C}$OCH$_3$), δ 20.8 (OCO$\underline{C}$H$_3$), δ 151.7 (*s*) and δ 109.9 (*t*) (=CH_2) groups. The presence of an acetate accounts for two carbons and andersobine should therefore be an acetate of an alkaloid having the formula $C_{20}H_{27}NO_3$. Biogenetic considerations and the ab-

sence of methoxyl, *N*-methyl or *N*-ethyl groups suggested that andersobine is a diterpenoid and not a norditerpenoid alkaloid. Of the various skeleta known for the diterpenoid alkaloids,[6] andersobine possesses the hetisane skeleton (1) as all the other skeleta have to be *N*-alkyl derivatives. The partial structure of andersobine is thus expressed as (2), possessing one acetoxyl and two hydroxyl groups. This structure is consistent with the ^{13}C nmr spectrum (DMSO-d_6) which shows 22 signals for all the carbon atoms of the molecule. An APT/DEPT spectrum revealed the expected five nonprotonated carbon singlets at δ 170.5, 151.7, 48.5 (two carbons; these appeared as separate signals at δ 50.1, 49.4 in py-d_5), 44.0, nine methine doublets at δ 87.6, 73.0, 71.8, 69.9, 61.7, 60.6, 43.5, 42.9, 33.0, six methylene triplets at δ 109.9, 32.5, 31.8, 28.0, 26.2, 25.6 and two methyl quartets at δ 20.8 and 19.1. A literature search for alkaloids having the hetisane skeleton and possessing three hydroxyl groups ($C_{20}H_{27}NO_3$), indicated six known diterpenoid alkaloids. However, the physical and spectral properties of andersobine are not in accord with the monoacetyl derivatives of these known alkaloids.[7-13]



1 Hetisane skeleton 2 Partial structure of andersobine



3 Hetisine 4 Andersobine*

*For convenience of depiction in the diagram, H-19$_\alpha$ and H-19$_\beta$ indicate ψ-axial and ψ-equatorial protons, respectively, in the chair comformation for the ring E formed by C-4, C-5, C-10, C-20, N, and C-19. There is some confusion in the literature in representation of the configuration of the C-13 proton in the hetisine derivatives (both α and β).[6,14,15] The ring C containing both C-11 and C-13 and formed by the carbon atoms C-8, C-9, C-11, C-12, C-13 and C-14 is selected as the reference ring. H_α and H_β are designated for the ψ-axial and ψ-equatorial protons of this twist boat conformation.

The three oxygen functions of andersobine may be located at C-1, C-2, C-3, C-7, C-11, C-13, C-15 or C-19 positions in the partial structure (2). One of the hydroxyl or acetoxyl group should be located at C-15, adjacent to the exocyclic methylene, as the quaternary carbon signal for C-16 appears at δ 151.7 (δ 152.8, py-d_5), because of the β effect. If a hydroxyl group is absent at C-15, the signal for C-16 is observed at ~ δ 144-147; e.g. hetisine: δ 146.6,[7] Guan-Fu Base Z: δ 144.6,[14] and delatisine: δ 145.7.[16] When a hydroxyl or acetoxyl group is present at C-15, the signal for C-16 appears around δ 151-157; e.g. hypognavine: δ 154.6,[10,17] nominine: δ 156.8,[17] and ryosenamine: δ 155.2.[17,18] By comparison with alkaloids bearing a C-15 hydroxyl group, the resonance at δ 71.8 in andersobine has been assigned to C-15. There are six methines in andersobine appearing downfield of δ 60.0. The methine signal at δ 87.6 (δ 89.3, py-d_5) clearly indicates a carbinolamine carbon resonance indicating the location of a hydroxyl or acetoxyl group at C-19. Such a carbinolamine carbon can be expected to appear around 90.0 ppm, e.g., septentriosine: δ 95.3,[19] triacetylvakhmatine: δ 91.4,[20] and brunonine: δ 94.7.[21] Location of a hydroxyl group at C-20 will make this a quater-

nary carbon, and this position is excluded. Of the remaining five methine carbon signals, two should be attached to the nitrogen atom (C-6 and C-20), two should be oxygenated, and one will be the C-5 methine carbon, as in hetisine.[7]

Table 1. ^1H and ^{13}C Nmr Chemical Shifts and Assignments of Andersobine (4)[a,b]

^{13}C	δ (ppm) $(CD_3)_2SO$	δ (ppm) C_5D_5N	^1H	δ (ppm) $(CD_3)_2SO$	Multiplicity(Hz)	^1H δ (ppm) C_5D_5N	Multiplicity (Hz)
1	25.6 (*t*)	26.7 (*t*)	1β	1.31	*dd*, $J_{1\beta,1\alpha}$=13.0, $J_{1\beta,2\alpha}$=4.0	1.42	*m*
			1α	1.83	*m*, $J_{1\alpha,1\beta}$=13.0	1.82	*m*
2	31.8 (*t*)	29.4 (*t*)	2α	1.42	*m*	1.84	
			2β	1.68	*m*	2.08	*m*
3	73.0 (*d*)	74.5 (*d*)	3	3.30	*m*	3.83	*dd*, $J_{3\beta,2\alpha}$=11.4, $J_{3\beta,2\beta}$=5.5
4	48.5 (*s*)	49.4 (*s*)	4	-	-		
5	61.7 (*d*)	63.0 (*d*)	5	1.38	*s*	1.50	*s*
6	60.6 (*d*)	62.1 (*d*)	6	3.34	br *s*	3.86	*s*
7	28.0 (*t*)	33.0 (*t*)	7β	1.62	*dd*, $J_{7\alpha,7\beta}$=13.0, $J_{7\alpha,6}$=2.5	1.78	*m*
			7α	1.40	*m*		
8	44.0 (*s*)	44.7 (*s*)	8	-	-		
9	43.5 (*d*)	45.1 (*d*)	9	1.68	*m*	1.82	*m*
10	48.5 (*s*)	50.1 (*s*)	10	-	-		
11	26.2 (*t*)	27.0 (*t*)	11β	1.47	*td*, $J_{11\beta,11\alpha}$=13.0, $J_{11\beta,12}$=2.0, $J_{11\beta,9}$=2.0	1.68	*m*
			11α	1.87	*dd*, $J_{11\alpha,11\beta}$=13.0, $J_{11\alpha,12}$=4.0	1.91	m
12	33.0 (*d*)	34.3 (*d*)	12	2.17	*m*	2.14	*m*
13	32.5 (*t*)	33.7 (*t*)	13α	1.15	*td*, $J_{13\alpha,13\beta}$=13.0, $J_{13\alpha,12}$=2.0, $J_{13\alpha,14}$=2.0	1.08	*td*, $J_{13\alpha,13\beta}$=13.0, $J_{13\alpha,12}$=3.0, $J_{13\alpha,14}$=3.0
			13β	1.68	*m*	1.71	*m*
14	42.9 (*d*)	44.1 (*d*)	14	1.80	*d*, $J_{14,13\beta}$=11.6	2.08	*td*, $J_{14,13\beta}$=10.3, $J_{14,13\alpha}$=2.0
15	71.8 (*d*)	73.0 (*d*)	15α	5.29	br *s*, J=< 1.0	5.67	*t*, *J*= < 1.0
16	151.7 (*s*)	152.8 (*s*)	16	-		-	
17	109.9 (*t*)	110.5 (*t*)	17_a	4.92	*t*, *J*=1.6, 1.6	5.18	*t*, *J*=1.6, 1.6
			17_b	4.83	*t*, *J*=1.6, 1.6	5.00	*t*, *J*=1.6, 1.6
18	19.1 (*q*)	20.4 (*q*)	18	0.95	*s*	1.64	*s*
19	87.6 (*d*)	89.3 (*d*)	19	4.07	*s*	4.89	*s*
20	69.9 (*d*)	71.4 (*d*)	20	2.52	br *s*	2.72	*s*
21	170.5 (*s*)	171.2 (*s*)	21	-	-		
22	20.8 (*q*)	21.2 (*q*)	22	2.02	*s*	2.16	*s*
			3-OH	4.40	*d*, *J*=4.6	6.08	*d*, *J*=4.5
			19-OH	5.12	*s*	4.94	*s*

[a] The protons and carbons have been assigned by HETCOR

[b] The multiplicities were determined by APT/DEPT experiments

Table 2. ^1H-^1H Correlations and nOe's of Andersobine(4) in CD_3SOCD_3[a] and C_5D_5N[b]

Observed Proton	Correlations (COSY)[a]	Correlations (COSY)[b]	Correlations nOe's (NOESY)[a]	Correlations nOe's (NOESY)[b]
H-1α	H-1β, H-2α, H-2β	H-1β	H-1β	H-1β
H-1β	H-1α, H-2β	H-1α	H-1α	H-1α, H-2β
H-2α	H-1α	H-3β	H-2β,H-19α H-20	H-20
H-2β	H-1α, H-1β, H-3β	H-3β	H-2α, H-3β	H-1β
H-3β	H-2β, OH-3	H-2α, H-2β	H-2β, H-18	H-5, H-18
OH-3	H-3β	-	H-3β, H-18, H-19	H-18
H-5	H-20 (W)	H-6, H-20 (W)	H-3β, H-18	H-3β, H-7
H-6	H-7α, H-7β, H-20 (W)	H-5, H-7, H-19, H-20 (W)	H-7α, H-18	H-7, H-18
H-7α	H-6, H-7β	H-6	H-6, H-7β, H-14, H-15α	H-5, H-6, H-14
H-7β	H-6, H-7α	-	H-7α	H-7
H-9	H-11β, H-14 (W)	H-11β, H-14 (W)	-	H-11β, H-20
H-11β	H-9, H-11α	H-9, H-11α	H-11α, H-12	H-9
H-11α	H-11β, H-12	H-11β, H-12	H-11β	-
H-12	H-11α, H-13α, H-13β	H-11α, H-13α, H-13β	H-11α, H-11β,-13α, H-13β	H-17β
H-13α	H-12, H-13β, H-14	H-12, H-13β, H-14	H-12, H-13β, H-20	H-13β
H-13β	H-12, H-13α, H-14	H-12, H-13α, H-14	H-12, H-13α	H-13α
H-14	H-9 (W), H-13α, H-13β, H-20	H-9 (W), H-13α, H-13β, H-20	H-7α H-13β, H-15α, H-20	H-7, H-13β, H-15α H-20
H-15α	H-17a, H-17b	H-9, H-17a, H-17b	H-7α, H-14, H-17b, H-17a	H-7, H-14, H-17a, H-22
H-17b	H-15α, H-17b	H-15α, H-17a	H-15α, H-17a	H-12, H-17a
H-17a	H-15α, H-17b	H-15α, H-17b	H-12, H-17b	H-15α, H-17b
H-18	-	-	H-3β, OH-3, H-5, H-6	H-3, H-6, H-19
H-19α	-	H-6, H-18, H-20	H-2α, H-20	H-2α, H-18, H-20
OH-19	H-20	-	-	H-3
H-20	H-5 (W), H-6 (W), H-14	H-5 (W), H-6 (W), H-14	H-2α, H-13α, H-14, H-19	H-2α, H-13α, H-14 H-15α, H-17b, H-19
H-22	-	-	-	H-15α, H-17β

Table 3. Nmr Data of Andersobine (4) from Selective INEPT Experiments

Irradiation of proton assigned to	δ	Enhancement of the carbon signal assigned to* (ppm) Strong	Medium	Weak
(A) *Solvent CD_3SOCD_3*				
CH_3-18	0.95	48.5 (C-4), 73.0 (C-3)	87.6 (C-19)	61.7 (C-5)
H-5	1.38	87.6 (C-19), 69.9 (C-20)		
H-12	2.17	151.7 (C-16), 71.8 (C-15) 43.5 (C-9), 42.9 (C-14)	109.9 (C-17)	
H-3/H-6	3.31	87.6 (C-19), 44.0 (C-8)	48.5 (C-4, C-10)	
H-17b	4.83	151.7 (C-16), 71.8 (C-15)	33.0 (C-12)	
H-17a	4.92	71.8 (C-15), 33.0 (C-12)		
H-15	5.29	170.5 (C-21), 151.7 (C-16)		44.0(C-8), 43.5 (C-9)
(B) *Solvent C_5D_5N*				
H-20	2.72	89.3 (C-19), 62.1 (C-6), 45.1 (C-9)		
H-3	3.83	89.3 (C-19)	49.4 (C-4)	
H-6	3.86	49.4 (C-4), 45.1 (C-9)	71.4 (C-20)	89.3 (C-19)
H-19	4.89	62.1 (C-6)		74.5 (C-3), 71.4 (C-20)
H-15	5.67	171.2 (C-21), 152.8 (C-16)	44.7 (C-8), 45.1 (C-9)	110.5 (C-17)

* Strong 60%, Medium 40-60%, weak <40%

The third oxygen function may be located at C-1, C-2 or C-3 in ring A because of the lack of a triplet for a methylene group around δ 19.8 where the C-2 resonance would appear if A ring is not oxygenated.[16, 22] The quaternary carbon signal of C-10 appears in the normal range at 48.5 ppm, not having the β-effect of an oxygen function located at C-1; e.g. C-10 appears at δ 54.9 in hypognavine,[17] δ 56.8 in vacognavine[23] and δ 53.0 in septentriosine,[19] bearing a C-1 oxygen function. Of the five non-protonated carbons, the signals at δ 170.5, 151.7 and 48.5 have been assigned to C-21, C-16 and C-10, respectively, as discussed. The signal at δ 44.0 should be assigned to C-8 as in hetisine (δ 43.5),[7] and hypognavine (δ 44.3).[17] The remaining quaternary carbon resonance at δ 48.5 should be assigned to C-4. In hetisine,[7] C-4 appears at δ 36.6 and in septentriosine[19] having a hydroxyl group at C-19, the C-4 carbon signal is observed at δ 39.7. This suggested that andersobine might have an oxygen function (OH or OAc) at C-3, contributing to a β effect on C-4, bringing it downfield by ~8 ppm.

On the basis of these data, andersobine was assumed to bear hydroxyl groups at C-3, C-15 and C-19, with one of these being an acetate group. Two separate attempts made to solve the structure of andersobine by X-ray crystallography were unsuccessful in spite of the availability of suitable crystals. In an effort to make other derivatives suitable for X-ray as well as chiroptical studies, the 4-dimethylaminobenzoate ester of andersobine was prepared. X-ray crystallographic analysis of these crystals also failed. Finally, the structure of andersobine as (4) was confirmed by ^{1}H COSY, one dimensional (1D; in C_5D_5N), two dimensional (2D; in C_5D_5N and CD_3SOCD_3) nOe studies and selective INEPT experiments.

Three separate scalar coupled spin systems were delineated by the homonuclear ^{1}H COSY nmr spectra in CD_3SOCD_3: [H-1$_\alpha$-H-1$_\beta$-H-2$_\alpha$-H-2$_\beta$-H-3], [H-6-H-7$_\alpha$-H-7$_\beta$], [H-9-H-11$_\alpha$-H-11$_\beta$-H-12-H-13$_\alpha$-H-13$_\beta$-H-14-H-20] (Tables 1 and 2). The carbon multiplicities and the one-bond ^{1}H-^{13}C coupled protons were assigned using DEPT and heteronuclear COSY (HETCOR) spectra. The C-17 exocyclic methylene protons (δ_H 4.83 and 4.92; δ_C, 109.9) showed significant couplings to a methine proton (δ_H 5.29 br *s*; δ_C, 71.8) in the COSY spectrum attributed to a carbon bearing a hydroxyl or acetoxyl group assigned to H-15. The two exocyclic methylene protons were easily distinguished since one of them (δ 4.92) showed a strong nOe to H-12 only (δ_H 2.17; δ_C, 33.0), whereas its gem-partner (δ 4.83) had dipolar interactions with H-15$_\alpha$. Therefore, the protons at δ 4.92 and δ 4.83 were assigned to H-17a and H-17b, respectively. The methine proton singlet at δ 4.07, correlated with the carbinolamine methine carbon at δ 87.6 in the HETCOR spectrum. This proton showed dipolar interactions with H-2$_\alpha$ (δ_H 1.42; δ_C 31.8), and H-20 (δ_H 2.52 br *s*; δ_C 69.9) in the NOESY spectrum indicating that this resonance at δ 4.07 belongs to H-19 and C-19 is a carbinolamine carbon as predicted. The second oxygenated carbon is thus located at C-19 and the remaining oxygen functionality must be located at C-1, C-2, C-3, C-7, C-11 or C-13. The H-3β proton at δ 3.30 (δ_C73.0) showed coupling to H-2β (δ 1.68 *m*) and to the C-3 hydroxyl proton at δ 4.40 in the COSY spectrum. The nOe's from H-3β to the most up field proton at δ 0.95 (δ_C 19.1) assigned to the C-18 methyl group enabled us to locate the hydroxyl group at C-3, thus excluding placement of the oxygen function at C-1, C-2, C-7, C-11, or C-13. Moreover, H-3β showed an nOe to H-5 (*vide infra*). The H-6 proton (δ_H 3.34 br *s*; δ_C 60.6) also showed an nOe to H-18 (methyl). The 3$_\alpha$ hydroxyl (δ 4.40) showed nOe's to H-3β, H-18 (methyl), and H-19 confirming the location of the hydroxyl groups at C-3 and C-19. These data suggested that C-15 should bear the acetoxyl group. In the selective INEPT experiments[24] (Table 3A and 3B; DMSO-d_6 and py-d_5), irradiation of the H-15 proton (δ 5.29,

DMSO-d_6) caused polarization transfer to the quaternary carbons C-16 (δ_C 151.7) and C-21 (δ_C 170.5), confirming the location of the acetoxyl group at C-15. In addition to these enhancements, a methine (δ_C 43.5) and a quaternary carbon (δ_C 44.0) were also enhanced. The methine (three bonds removed from H-15) and the quaternary carbon (two bonds away) can be assigned to C-9 and C-8, respectively. In another selective INEPT experiment (Table 3B), polarization transfers were observed from H-20 (δ_H 2.72; py-d_5) to the methine carbons at C-19 (δ_C 89.3), C-6 (δ_C 62.1) and C-9 (δ_C 45.1), all three bonds removed from H-20. In the HETCOR spectrum, C-9 (δ_C 45.1; py-d_5) correlated with the signals at δ 1.82, and this proton was assigned to H-9. In the 1D nOe spectrum (in py-d_5; Figure 1) the methine signal at δ 1.50 (δ_C 63.0) showed an nOe to H-3β and the C-18 methyl group and must therefore be H-5. The H-5 proton also showed a long range W-type coupling with H-20 (δ 2.52) in the COSY spectrum. In a selective INEPT experiment (Table 3A), irradiation of H-5 (δ 1.38, δ_C 61.7) showed strong enhancement of signals assigned to C-19 (δ_C 87.6) and C-20 (δ_C 69.9). The remaining methine appearing at δ 1.80 showed a W-type coupling with H-9 in the COSY spectrum and an nOe to H-15α in the NOESY spectrum. This proton signal at δ 1.80 (δ_C 42.9) is assigned to H-14.

We have so far assigned all quaternary carbon signals except the resonance at δ 48.5 (two carbons). This assignment was completed by a selective INEPT experiment. Polarization transfer from the C-18 methyl group (δ 0.95) resulted in a strongly enhanced signal at δ 48.5 assigned to C-4, two bonds removed from H-18. This signal was also enhanced when H-6 was irradiated, indicating that this overlapping signal is due to C-10 (three bond coupling). Selective INEPT studies in py-d_5 showed that the quaternary carbons at δ 49.4 and δ 50.1 should be assigned to C-4 and C-10, respectively (Table 3B).

Six methylene carbons are present in andersobine (**4**) at δ 25.6, 26.2, 28.0, 31.8, 32.5, and 109.9. The lowest field carbon has already been assigned to C-17. The signal for C-11 (δ_C 26.2; δ_H 1.47, 1.87) and C-13 (δ_C, 32.5; δ_H 1.15, 1.68) were identified through the COSY spectrum. The C-12 proton (δ 2.17) (assigned from the nOe with H-17), showed strong coupling to H-11α, H-13α and H-13β in the COSY spectrum. An nOe was also observed in the NOESY spectrum between H-12 and H-11 as well as the H-13 protons. H-13α (δ 1.15) was distinguished by its coupling with H-14 in the COSY spectrum and the expected nOe to H-20. Similarly, only H-11β (δ 1.47) showed coupling to H-9 in the COSY spectrum. An nOe was observed between H-11β and H-12 in the NOESY spectrum. The C-6 proton (δ 3.34) showed correlation to the H-7 protons (δ_H 1.40 *m*; δ_H1.62 *dd* *J*=13.0, 2.5 Hz; δ_C, 28.0) and a long range coupling with H-20 in the COSY spectrum. The H-7α proton (δ 1.40) showed nOe's to H-6, H-7β (δ 1.62), H-14 (δ 1.80) and H-15α. The H-14 proton exhibited a coupling with H-13α (δ 1.15), H-20 and a long range W-type coupling with H-9 (δ 1.68) in the COSY spectrum. The observations of nOe's from H-14 to H-7α, H-13β (δ 1.68 *m*), H-15α and H-20, confirmed the assignments and the spatial relationships of these protons. An nOe from H-13α (δ 1.15) to H-13β (δ 1.68) and H-20 was also observed. The H-2β proton (δ 1.68) was identified from its COSY and nOe to H-3β. H-2α (δ 1.42) showed an nOe to H-19 and H-20 as expected for the A ring in a chair conformation. In C_5D_5N, the H-2α proton appeared at δ 1.84 and showed a correlation to H-3β (δ 3.83). These protons correlated with δ_C (31.8 DMSO-d_6, 29.4 py-d_5) in the HETCOR spectrum. The H-2α proton (δ 1.42) showed COSY correlation with the proton at δ 1.83 assigned to H-1α and the H-2β proton at δ 1.68 correlation to H-1β (δ 1.31). These H-1 protons in turn are correlated to the carbon signal at δ_C 25.6 (DMSO-d_6) and δ_C 26.7 (py-d_5) in the HETCOR spectrum.

Stereochemical assignments of andersobine (4) were confirmed by measurement of vicinal coupling constants as well as the observation of 1D nOe's in C_5D_5N (Figure 1). The equatorial orientation of the C-3 OH group enables the H-3 axial proton to be seen with a large vicinal coupling constant for the axial-axial (11.4 Hz) and a smaller axial-equatorial (5.5 Hz) relation resulting in a double doublet at δ 3.83. This can be explained by H-3 being in a β configuration coupling with its neighboring H-2 axial proton for an A ring in a chair conformation. The H-3 proton also showed a NOESY relation with the H-5 proton, thus establishing their 1,3-diaxial relationship. A strong nOe between H-19$_\alpha$ and H-20 both in 1D and 2D nOe spectra indicated that the OH group at C-19 is in a β-position. H-19$_\alpha$ also showed an nOe in 1D and 2D nOe spectra with H-6 and not with H-5β, as expected. The stereochemistry of the acetoxyl group at C-15 in an equatorial position (boat conformation of the D ring formed by C-8, C-14, C-13, C-12, C-16 and C-15) was established by the NOESY observed between H-15$_\alpha$ and H-14 protons and the absence of an nOe or NOESY relationship between H-15, and H-9.

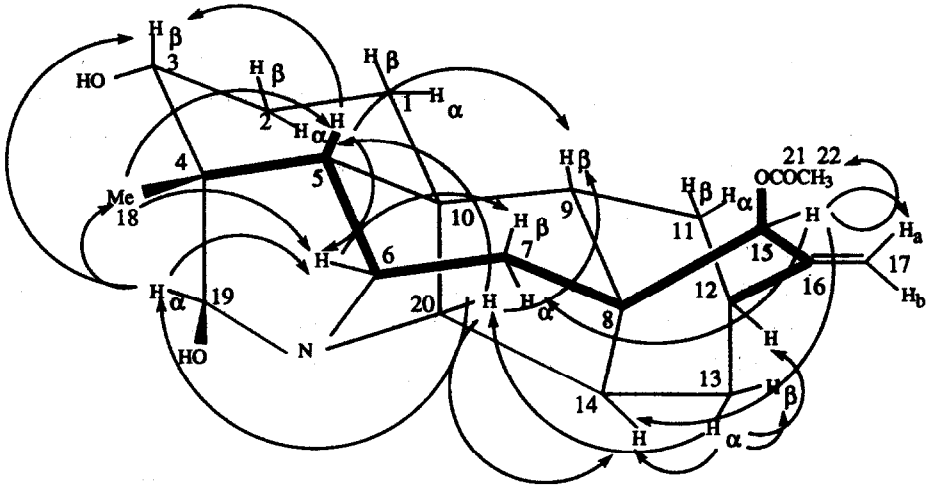


Figure1. 1D nOe's observed for andersobine (4) in C_5D_5N

EXPERIMENTAL

General Procedures

Mp's were taken on a Thomas-Koffler hot stage equipped with a microscope and polarizer. Ir spectra were recorded on a Perkin-Elmer model 1421 spectrophotometer and cd measurements were made on a JASCO spectropolarimeter. ^{1}H and ^{13}C nmr spectra were recorded on Varian XL-300 (300 MHz for ^{1}H and 75.5 MHz for ^{13}C) and Varian XL-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometers. Chemical shift data are given in ppm downfield from TMS. Ei ms were determined by direct inlet on a Finnigan Quadrupole model 4023 spectrometer at an ionizing voltage of 70 eV, and hrms were obtained on a VG-ZAB SE double focusing mass spectrometer at an acceleration voltage of 20 kV. Droplet counter current chromatography was carried out on a Tokyo Rikakikai equipment, model D.C.C.-A. instrument manufactured by Tokyo Rikakikai Co. Ltd, Tokyo, Japan.

Plant material

The aerial parts of *D. andersonii* Gray were collected in 1978 from 'Wildcat Hills' at an altitude of about 4800 ft., 18 miles south west of Snowville, Utah. The plant was identified by Dr. Leila McReynolds Shultz, curator of the Intermountain Herbarium, Utah State University, Logan, Utah. A voucher specimen (UTC accession No. 201385) has been deposited in the Intermountain Herbarium.

Extraction of alkaloids

The dried and ground aerial parts (9.44 kg) were defatted with hexane and then extracted at room temperature with 95% EtOH to give 1.4 kg of extract. A portion of the above extract (670 g) was fractionated by gradient pH extraction to give 1.665 g (pH 10) and 0.16 g (pH 12) of crude alkaloids.

Isolation of andersobine 4

The combined mixture of pH 10 and pH 12 crude alkaloids (1.825 g) was dissolved in about 10 ml of a 50:50 two-phase solvent system prepared by mixing $CHCl_3$, C_6H_6, MeOH, H_2O in the ratio of 5:5:7:2, respectively. The solution was loaded on a droplet counter current chromatography instrument, operated in the ascending mode. Fractions 25-30 ml were collected using an automatic fraction collector set for 90-99 mins: per fraction. The following fractions were collected:

Fraction	Eluent	Weight (mg)
1 - 20	upper phase	400
21 - 24	upper phase	550
25 - 170	upper phase	350
171 - 220	MeOH	600

A crystalline compound was obtained from fractions 21-24. Recrystallization from MeOH afforded colorless plates of andersobine (4, 145 mg), mp 310°C. Ir (nujol) 3470, 1730, 1460, 1375, 1235, 1027, 960, 908, and 870 cm^{-1}. Ms: m/z 371 (M^+;1%), 353 (100), 311 (8), 209 (22), 189 (10), 172 (10), 161 (20), 133 (18), 117 (18), 105 (25), 91 (30), 43 (70). Hrms: (M^+-18, H_2O) m/z 353.2005, Calcd. for $C_{22}H_{27}NO_3$, (M^+ -18, H_2O) 353.1991. Cd (MeOH): $[\theta]_{nm}$ 240 0; 215 +2100; 207 0; 205 -4600. Found: C, 71.04; H, 7.96; N, 3.67; $C_{22}H_{29}NO_4$ requires: C, 71.16; H, 7.82; N, 3.77 %.

Esterification of andersobine

A mixture of andersobine (17 mg), 4-dimethylaminobenzoyl chloride (38 mg), 4-dimethylaminopyridine 14 mg) in dry C_5D_5N (2 ml) was kept at rt for 4 days. The dimethylaminopyridine hydrochloride which had separated was removed by decantation and the mother liquor dissolved in CH_2Cl_2 and chromatographed (VLC) on a small column of basic alumina and collected 8 fractions. Fractions 2-5 (tlc: Al_2O_3; 20% EtOH:Hexane, R_f 0.5) were crystallized from CH_2Cl_2/Et_2O to afford the dimethylaminobenzoate of andersobine (5.0 mg) as colorless crystals, mp 204-207°C. ^1H nmr ($CDCl_3$): δ 7.92 (2H, *d*, *J* = 8.6 Hz, 2',6'-Ar H), 6.64 (2H, *d*, *J* = 8.6 Hz, 3',5'-Ar H), 5.60 (*s*, H-19), 5.44 (1H, br *s*, H-15), 4.99, 4.93 (each 1H, br*s*, H-17), 3.50 (1H, *m*, H-3). ^{13}C nmr ($CDCl_3$): ppm 26.6 (C-1), 29.8 (C-2), 75.7 (C-3), 44.7 (C-4), 63.4 (C-5), 60.9 (C-6), 26.7 (C-7), 44.9 (C-8), 44.5 (C-9), 49.3 (C-10), 24.8 (C-11), 33.7 (C-12), 32.3 (C-13), 42.7 (C-14), 72.7 (C-15), 151.4 (C-16), 110.6 (C-17), 18.4 (C-18), 91.1 (C-19), 171.1 ($\underline{C}OCH_3$), 21.3 ($CO\underline{C}H_3$), 165.4 ($O\underline{C}OCH_3$), 93.6 (C-1'), 131.9 (C-2', C-6'), 110.9 (C-3', C-5'), 148.4 (C-4'), 40.0 [*N*-$(CH_3)_2$].

ACKNOWLEDGMENTS

We thank Dr. A. K. Ganguly for the Cd, Dr. B. Pramanik for the hrms and Dr. J. D. Olsen for the plant collection.

REFERENCES

1. Panu, A. M. Ph. D. Thesis, University of Georgia, Athens, **1986**.
2. Pelletier, S. W.; Panu, A. M.; Kulanthaivel, P.; Olsen, J. D. *Heterocycles*, **1988**, *27*, 2387.
3. Pelletier, S. W.; Kulanthaivel, P.; J. D. Olsen, *Heterocycles*, **1989**, *28*, 107.
4. Benn, M. H.; Jacyno, J. M. The Toxicology and Pharmacology of Diterpenoid Alkaloids in *Alkaloids: Chemical and Biological Perspectives*, Vol. 1, Ed. S. W. Pelletier, Wiley, N. Y. **1983**, pp. 153-210.
5. Pelletier, S. W.; Joshi, B. S.; Desai, H. K. Techniques for Isolation of Alkaloids, in *Advances in Medicinal Plant Research*, Ed. A. J. Vlietinck and R. A. Domisse, Wissenschaftlische Verlagsgesellschaft mbh, Stuttgart, **1985**, pp. 153-157.
6. Pelletier, S. W.; Keith, L. H. *The Alkaloids*, Ed. R. H. F. Manske, Vol. 12, Academic Press, N. Y. **1970**, pp. 136-206; Pelletier, S. W.; Mody, N. V. *The Alkaloids*, Ed. R. H. F. Manske, Vol. 18, Academic Press, N. Y. **1981** pp. 100-216.
7. Glinski, J. A.; Joshi, B. S.; Jiang, Q. P.; Pelletier, S. W. *Heterocycles*, **1988**, *27*, 185.
8. Benn, M. H.; Richardson, J. F.; Majak, W. *Heterocycles*, **1986**, *24*, 1605.
9. Zhang, X.; Snyder, J. K.; Joshi, B. S.; Glinski, J. A.; Pelletier, S. W. *Heterocycles*, **1990**, *31*, 1879.
10. Sakai, S.; Yamaguchi, K.; Yamamoto, I.; Hotoda, K.; Okazaki, T.; Aimi, N.; Haginiwa, J.; Okamoto, T. *Chem. Pharm. Bull.* Japan, **1983**, *31*, 3338.

11. Bando, H.; Wada, K.; Amiya, T.; Kobayashi, K.; Fujimoto, Y.; Sakurai, T. *Heterocycles*, **1987**, *26*, 2623; Takayama, H.; Ito, M.; Sakai, S.; Okamoto, T *ibid.*, **1981**, *15*, 403.
12. Bai, Y. Ph. D. Thesis, University of Calgary, Alberta, **1993**.
13. Karimov, Z.; Zhamierashvili, M. G. *Khim. Prior. Soedin*, **1981**, 335.
14. Reinecke, M. G.; Watson, W. H.; Chen, D. C.; Yan, W. M. *Heterocycles*, **1986**, *24*, 49; Reinecke, M. G.; Minter, D. E.; Chen, D. C.; Yan, W. M. *Tetrahedron*, **1986**, *42*, 6621.
15. Varughese, K. I.; Pelletier, S. W. *J. Nat. Prod.*, **1984**, *47*, 470; Sun, F.; Liang, Y. T.; Yu, D. Q. *J. Nat. Prod.*, **1987**, *50*, 923.
16. Ross, S. A.; Joshi, B. S.; Desai, H. K.; Pelletier, S. W.; Newton, M. G.; Zang, X.; Snyder, J. K. *Tetrahedron*, **1991**, *47*, 9585.
17. Sakai, S.; Yamamoto, I.; Hotoda, K.; Yamaguchi, K.; Aimi, N.; Yamanaka, E.; Haginiwa, J.; Okamoto, T.; *Yakugaku Zasshi*, **1984**, *104*, 222.
18. Sakai, S.; Okazaki, T.; Yamaguchi, K.; Takayama, H.; Aimi, N. *Chem. Pharm. Bull.* Japan, **1987**, *35*, 2615.
19. Joshi, B. S.; Desai, H. K.; Pelletier, S. W.; Holt, E. M.; Aasen, A. J. *J. Nat. Prod.*, **1988**, *51*, 265.
20. Jiang, Q. P.; Pelletier, S. W. *J. Nat. Prod.*, **1991**, *54*, 525.
21. Deng, W.; Sung, W. L. *Heterocycles*, **1986**, *24*, 869.
22. Joshi, B. S.; Chen, D H.; Zhang, X.; Snyder, J. K.; Pelletier, S. W. *Heterocycles*, **1991**, *32*, 1973.
23. Jiang, Q. P.; Pelletier, S. W. *Tetrahedron Letters*, **1988**, *59*, 1875.
24. Bax, A. *J. Magn. Reson.*, **1984**, *57*, 314.

(*Received in USA* 10 *July* 1994; *accepted* 7 *September* 1994)