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The Structure of Ajabicine, a Novel Diterpenoid Alkaloid from *Delphinium Ajacis*

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Abstract: The structure of ajabicine, a novel diterpenoid alkaloid from *Delphinium ajacis*, has been established as **1**. This is the first diterpenoid alkaloid bearing a C-14 exocyclic methylene group.

From the seeds of *Delphinium ajacis* L. [syn. *Consolida ambigua* (L.) P.W. Ball & Heyw] (Ranunculaceae) several lycoctonine-type diterpenoid alkaloids have been isolated. Investigations of *D. ajacis* had led to the isolation of a number of norditerpenoid alkaloids, thirteen from the stem, six from the leaves and eight from the roots.[1] We report here the structure of a novel diterpenoid alkaloid ajabicine (**1**), isolated from the leaves cultivated in Assiyut, Egypt. The amorphous compound (tlc, Al_2O_3, R_f 0.5, Et_2O:5% MeOH) was obtained in 0.0012% yield by acid-base extraction procedure (pH 8) and chromatography on Al_2O_3 by VLC[2] and the Chromatotron.[3] Ajabicine, $C_{22}H_{33}NO_2$ (M^+ m/z 343), showed in the ^{13}C nmr spectrum 22 resonances comprised of one sp^2 singlet (δ 156.6) which is assigned to the carbon containing an exocyclic methylene, three sp^3 singlets (δ 33.3, 49.8, 80.0), seven sp^3 doublets, (δ 38.8, 46.7, 47.1, 48.5, 51,8, 62,8, 73.3), nine methylenes (δ 26.3, 30.8, 32.7, 33.3, 33.5, 35.8, 48.6, 60.2, 103.2), and two methyl carbons (δ 13.3, 27.5). The ^1H nmr spectrum featured signals at δ 4.89, 5.00 (each 1H, *s*, $=CH_2$), 3.96 [1H, *t*, *CH*(OH)], 1.03 (3H, *t*, CH_2CH_3), 0.80 (3H, *s*, *tert*-CH_3). The molecular formula and chemotaxonomic considerations together with the presence of an *N*-ethyl group indicated that ajabicine must be a diterpenoid and not a norditerpenoid alkaloid. This conclusion was supported by the absence of signals for methoxyl groups (^1H and ^{13}C nmr), which are normally present in norditerpenoid alkaloids.[1a,d,4]

All diterpenoid alkaloids containing 20 carbon atoms belong to two broad types: atisine-type (**a**) (modeled on an *ent*-atisane nucleus), and veatchine-type (modeled on an *ent*-kaurane nucleus).[4] All the alkaloids contain an exocyclic methylene group at C-16 and possess three other quaternary carbons at C-4, C-8 and C-10. These ring systems cannot accommodate the quaternary carbon signal of ajabicine at δ 80.0 bearing a hydroxyl group. To account for this carbon signal, the C-8–C-9 or the C-8–C-14 bond must be cleaved, to form a rearranged skeleton.

The results of COSY, TOCSY, HETCOR, and COLOC experiments (in $CDCl_3$) and an inspection of literature values previously reported for norditerpenoid[1a,d] and diterpenoid alkaloids[5] clearly established the presence of partial structures (A), (B) and (C) (Figure 1) in ajabicine. The ^1H and ^{13}C nmr spectra showed better resolution in C_5D_5N than in $CDCl_3$ and were used for assignments of the individual atoms (Table 1). Aside from the partial structures A, B, and C, a total of six degrees of unsaturation remained to be accounted and these must consist of six rings.

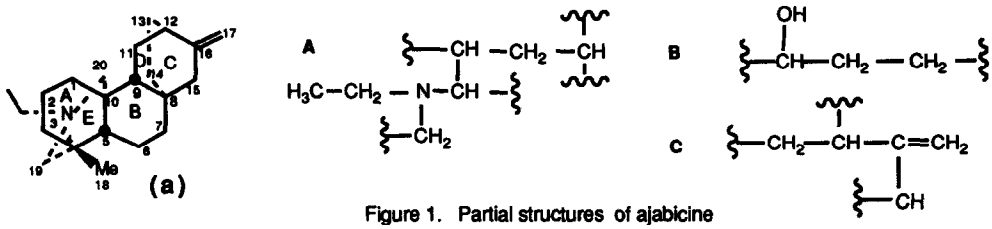


Figure 1. Partial structures of ajabicine

Connectivities of the remaining three quaternary centers, one methine, two methylenes and one methyl group were established by COSY, selective INEPT[6] and FLOCK[7] experiments and these data can be satisfactorily explained by structure 1 for ajabicine. The overall connectivity assignments from the nOe studies (1D and NOESY) are shown in Figure 2. Many of the assignments of the carbon atoms have been confirmed by selective INEPT experiments (Table 2).

Table 1. ^{1}H and ^{13}C NMR Chemical shift assignments of ajabicine 1[a,b]

Position	Carbon δ_C		Correlated H δ_H J(Hz)	Position	Carbon δ_C		Correlated H δ_H J(Hz)
1	73.3 d	H-1	3.96 d,d $J_{1,2a}$=2.9, $J_{1,2b}$=4.2	13	38.8 d	H-13	2.51 m
2	30.8 t	H-2_a	1.87 m	14	156.6 s	–	
		H-2_b	1.74 m	15	33.3 t	H-15_a	2.16 m
3	32.7 t	H-3_a	1.78 m			1H-15_b	1.60 m
		H-3_b	1.44 m	16	33.5 t	H-16_a	1.96 m
4	33.3 s	–				H-16_b	1.84 m
5	47.1 d	H-5	1.55 d $J_{5,6a}$=7.7; $J_{5,6b}\leq1$	17	103.2 t	H-17_a	5.00 d $J_{17a,17b}$=1.9
6	26.3 t	H-6_a	2.22 d,d $J_{6a,6b}$=14.5, $J_{6a,5}$ = 7.7			H-17_b	4.89 d $J_{17b,17a}$ = 1.9
		H-6_b	1.66 d,d $J_{6b,6a}$=14.5; $J_{6b,7a}$=8.0	18	27.5 q	H_3-18	0.80 s
7	46.7d	H-7	2.33 d $J_{7,6a}\leq1$; $J_{7,6b}$=8.0	19	60.2 t	H-19_a	2.20 d AB J_{gem}=11.0
8	80.8 s	–				H-19_b	1.98 d AB J_{gem}=11.0
9	51.8 d	H-9	2.63 d $J_{9,10}$=7.3	20	62.8 d	H-20	3.38 s
10	48.5 d	H-10	2.00 m $J_{10,12b}$=7.5; $J_{10,9}$=7.3	21	48.6 t	H-21_a	2.46 d,q J=12.2, 7.0
11	49.8 s	–				H-21_b	2.32 d,q J=12.2, 7.0
12	35.8 t	H-$12_{a,b}$	2.17 m	22	13.3 q	H_3-22	1.03 t J = 7.0

[a]Spectra were taken on Varian XL-400 (^{1}H, 400 MHz; ^{13}C, 100.5 MHz) in C_5D_5N

[b]Assignments are based on DEPT, HETCOR, COSY, selective INEPT and FLOCK experiments

The H-20 proton at δ 3.38 had long range heteronuclear coupling with C-6, C-7 and C-19 and the H-19_a methylene proton at δ 2.20 correlated with C-4, C-5 and C-20 in the FLOCK experiment. Similarly, the two-bond correlation of H-16_a to C-15 and a three-bond correlation of H-12 to C-16 were also obsersved (Table 3). Stereochemical assignments were confirmed by the vicinal coupling constants and the observed nOes (Figure 2). The OH group at C-1 is in an α-orientation since C-1 appears at 73.3 ppm. Typically, C-1 bearing a β-OH group resonates between 68–69 ppm.[1a,d,8] It has been well established that in norditerpenoid alkaloids having an α-OH group at C-1, the ring A adopts in a boat conformation, enabling hydrogen bonding to the nitrogen.[9] The signal for H-1, which lacked the distinct (dd) appearance expected for an axial hydrogen of a cyclohexane ring adjacent the C-2 methylene group, was found to be a (dd) with coupling constants of 2.9 and 4.2 Hz, expected for a boat conformation as in the case of tatsidine.[10]

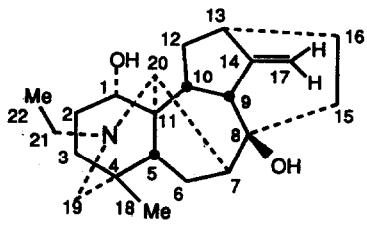
To date more than 250 norditerpenoid alkaloids (C_{19}) have been shown to possess the hexacyclic ring skeleton comprised of one seven membered, three six membered and two five membered

rings as in **1**. On the other hand, the entire group of over 150 naturally occurring diterpenoid alkaloids (C_{20}) are found to be pentacyclic or hexacyclic, containing a bicyclo[2,2,2]octane (atisane-type) or bicyclo[3,2,1]octane (veatchine-type) ring system.[5]

Table 2. Nmr Data from Selective INEPT Experiments on **1** in C_5D_5N

Irradiation of proton assigned to	Enhancement of the carbon signal assigned to *		
	Strong	**Medium**	**Weak**
18-CH_3	C-3, C-4, C-5, C-19		
H-3_b	C-1, C-18	C-4	C-2, C-19
H-5	C-7, C-19, C-20	C-4, C-10, C-11, C-18	
H-6_b	C-4, C-8	C-11	C-7
H-7	C-8	C-11, C-20	C-5, C-9
H-13	C-10	C-14	C-16, C-17
H-9	C-8	C-17, C-12	C-13, C-14, C-16
H-20	C-5, C-6, C-19		C-8, C-10, C-11
H-1	C-3, C-10	C-11	C-20

* Strong 61-100%, Medium 40-60%, Weak < 40%



1 Ajabicine

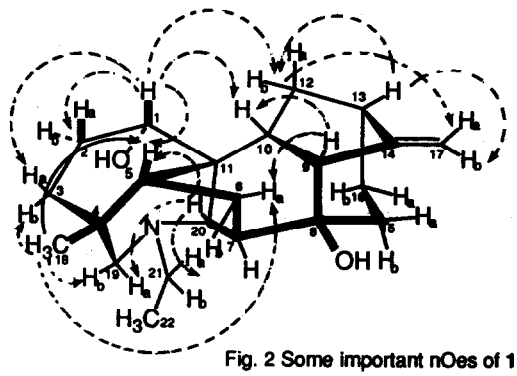
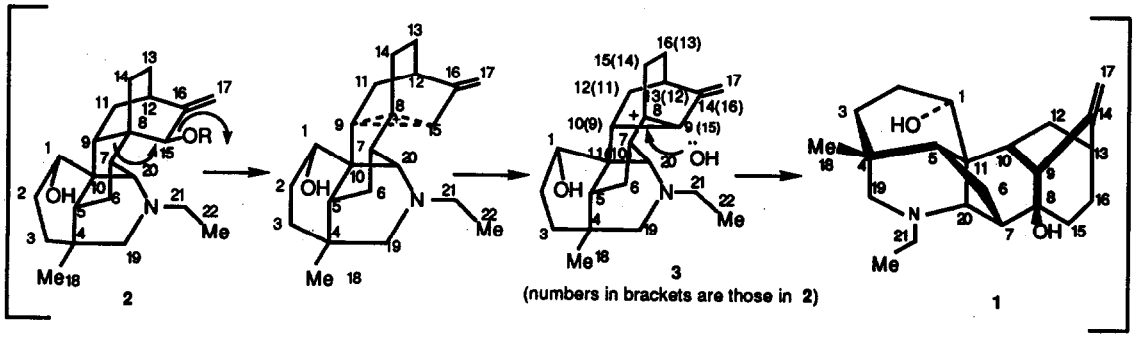


Fig. 2 Some important nOes of **1**

The biogenesis of ajabicine **1** may be assumed to proceed through a rearrangement of the fused bicyclo[2,2,2]octane **2** to give the bicyclo[3,2,1]octane rearrangement product **1** via the homo allylic carbocation **3** which on hydration gives **1**. There are many precedents in the literature for the rearrangement of a bicyclo[2,2,2]octane system to bicyclo[3,2,1]octane.[11]



2 **3** (numbers in brackets are those in **2**) **1**

Table 3. ^{1}H and ^{13}C Long Range Correlations FLOCK (in C_5D_5N) and COLOC (in $CDCl_3$) of 1

	Two or three bond correlated to	
Observed H	FLOCK	COLOC
H-2		C-1, C-11
H-3	C-2	C-1, C-2, C-18
$H\text{-}6_a$		C-11
H-10		C-11
$H\text{-}12_{a,b}$	C-16	
$H\text{-}16_a$	C-15	C-14
$H\text{-}17_a$	C-9, C-13	
$H\text{-}17_b$	C-9	C-9, C-14
$H_3\text{-}18$	C-3, C-4, C-5, C-19	C-4, C-5
$H\text{-}19_a$	C-4, C-5, C-20	C-20
H-20	C-6, C-7, C-19	C-6, C-11
$H\text{-}21_{a,b}$	C-22	

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