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DELATISINE, A NOVEL DITERPENOID ALKALOID FROM *DELPHINIUM ELATUM* L.

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Abstract: Chemical investigation of the seeds of *Delphinium elatum* L. resulted in the isolation of a new diterpenoid alkaloid delatisine whose structure (**1**) was established by ^{1}H COSY, long range COSY, HETCOR, 2D nOe, fixed evolution HETCOR and selective INEPT nmr studies. The structure of the alkaloid was confirmed by an X-ray crystal structure determination.

Introduction

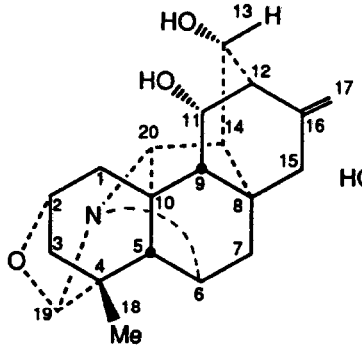
Delphinium elatum L. (Ranunculaceae) has proven to be a rich source of norditerpenoid and diterpenoid alkaloids. Delpheline,[1] deltaline, [2,3] deltamine,[4] elatine[2,3] and methyllycaconitine[1,5] were isolated from the whole plant. However, the following norditerpenoid (i–xiv) and the diterpenoid (xv) alkaloids were found to be present in the seeds: (i) 14-acetyl nudicauline[6]; (ii) delectinine[7]; (iii) delelatine[8]; (iv) delpheline[6]; (v) deltaline[6]; (vi) eladine[6]; (viii) elanine[7]; (viii) elasine[6]; (ix) elatine[6]; (x) isodelpheline[6]; (xi) lycoctonine[6]; (xii) methyllycaconitine[6]; (xiii) nudicauline[6]; (xiv) pacinine[7] and (xv) ajaconine.[7]

We now wish to report the isolation and structure elucidation of a novel furanohetisine-type (C-2/C-19 ether) diterpenoid alkaloid delatisine (**1**), from the seeds of *D. elatum*.

Results and Discussion

Delatisine, mp 274.5–276.5°C was isolated from the seeds of *Delphinium elatum* L. by vacuum liquid chromatography on alumina[9] followed by recrystallization. The preliminary ^{13}C nmr spectrum revealed twenty carbons, none of which could be assigned to a methoxyl or an *N*-methyl group, which were also absent in the ^{1}H nmr spectrum, thus suggesting that delatisine is a diterpenoid alkaloid. A peak at m/z 328 in the EIMS spectrum was potentially due to the loss of 17 mass units (loss of OH group) from the molecular ion (assuming a molecular weight of 345), or due to an $[M+1]^+$ peak. The chemical ionization mass spectrum (CI, CH_4) showed an $[M+H]^+$ peak at m/z 328 indicating the molecular weight of 327 for delatisine (**1**). This result was confirmed by a FAB

mass spectrum in thioglycerol: $[M+H+thioglycerol]^+$ at m/z 436, $[M+H]^+$ at m/z 328, and also FAB mass spectrum in glycerol/NaCl/DMSO indicating $[M+H]^+$ at m/z 328. The molecular formula of delatisine was confirmed as $C_{20}H_{25}NO_3$ (MW: 327) by HRMS (EI 40 eV): m/z 327.1829.



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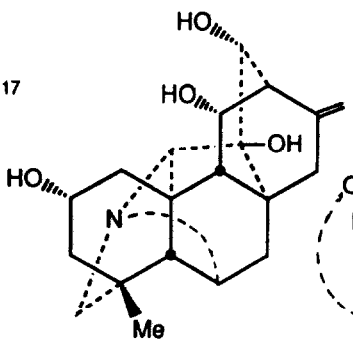

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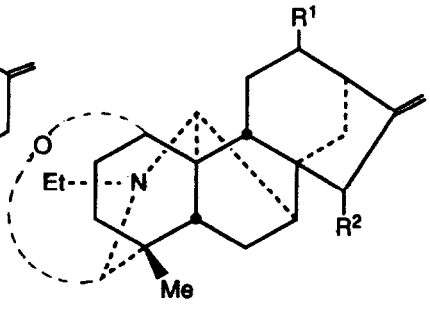


3 **a** R^1 = ···ıı OH; R^2 = ···ıı OAc

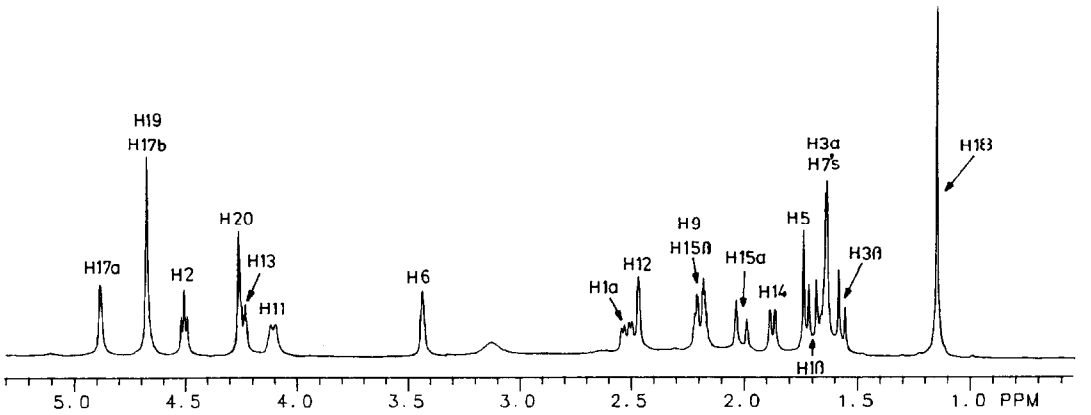
b R^1 = =O; R^2 = ◄ OH

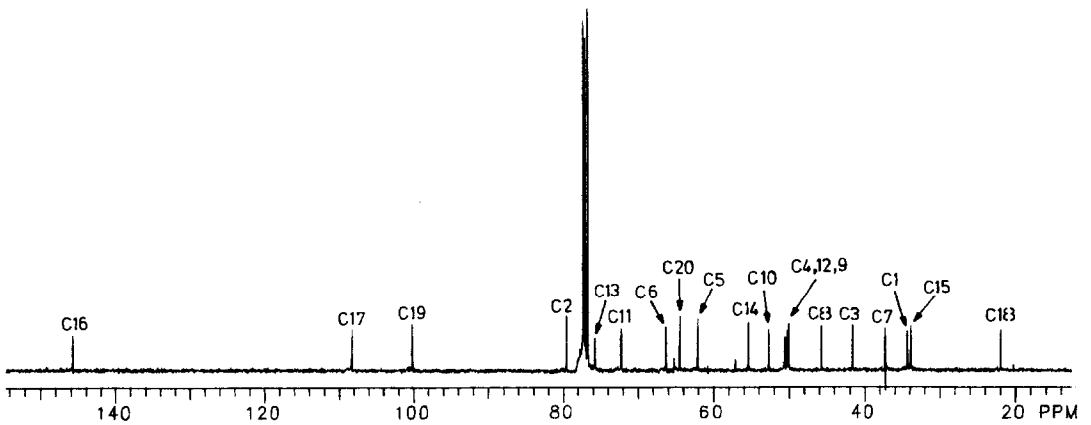
c R^1 = ···ıı OH; R^2 = ◄ OH

d R^1 = ···ıı OAc; R^2 = ◄ OH

A DEPT experiment revealed four nonprotonated carbons at δ 145.7, 52.7, 50.5 and 45.7, ten methines at δ 100.2, 79.6, 75.7, 72.2, 66.3, 64.4, 62.0, 55.4, 50.2 and 50.0, five methylenes at δ 108.2, 41.6, 37.3, 34.3 and 33.9, and one methyl group at δ 21.9. The characteristic carbon and proton resonances of a hetisane-type exocyclic methylene group were easily located at δ 145.7 (*s*) and 108.2 (*t*) for C-16 and C-17, and δ 4.87 and 4.66 for the exocyclic methylene protons. There are six methine doublets downfield of δ 62.0. The methine signal at δ 100.2 clearly indicated a carbinolamine carbon resonance. Of the remaining five signals, two should be attached to the nitrogen atom, and three should be oxygenated, since both the ^1H and ^{13}C nmr spectra indicated that neither an *N*-methyl nor an *N*-ethyl group was present (Figure 1).

This molecular formula raised the question of attaching the three oxygen functionalities to the hetisane skeleton to give four oxygenated methine carbons. This can only be achieved when there is one ether functionality and two hydroxyl groups in the molecule. The location of a hydroxyl group at C-15 was excluded because the carbon chemical shifts of the C-16/C-17 exocyclic double bond (δ 145.7 and 108.2), are typical for a hetisane-type diterpene alkaloid without a hydroxyl group at C-15. Hetisane-type alkaloids with a hydroxyl group at C-15 have the C-16 chemical shift between δ 154-152.[10] At least one oxygen function should be located in the A-ring due to the lack of a triplet carbon signal around δ 19.8 where the C-2 resonance should appear if no oxygen functionality exists from C-1 to C-3.





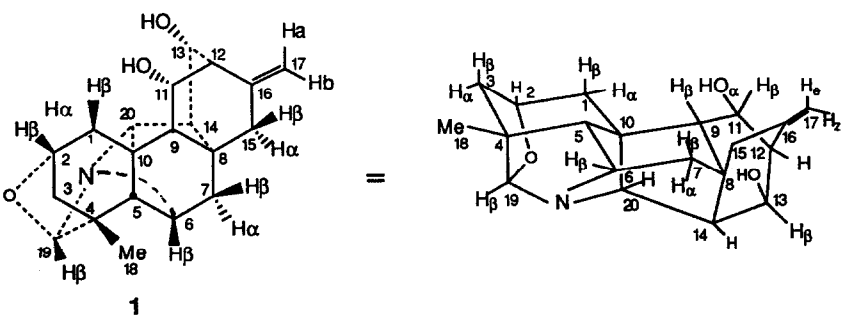


Figure 1. ^{1}H and ^{13}C nmr spectra of delatisine in $CDCl_3$. Labelled peaks indicate the complete proton and carbon assignments of **1** as listed in Table 1.

Table 1. ^{1}H and ^{13}C Chemical shifts assignments of delatisine 1 (in $CDCl_3$)

Carbon	δ(ppm)	Proton	δ(ppm)		J (Hz)
1	34.3 (*t*)	1β	1.70	d,	$J_{1\beta,1\alpha}$=13.1, $J_{1\beta,2}$<1.0
		1α	2.52	dd,	$J_{1\alpha,1\beta}$=13.1, $J_{1\alpha,2}$=5.4
2	79.6 (*d*)	2	4.50	br *t*,	$J_{2,1\alpha}$=5.4 $J_{2,3\alpha}$=5.7
3	41.6 (*t*)	3β	1.57	*d*,	$J_{3\alpha,3\beta}$=11.2, $J_{3\beta,2}$<1.0
		3α *	1.64	*dd*,	$J_{3\alpha,3\beta}$=11.2, $J_{3\alpha,2}$=5.7
4	50.5 (*s*)				
5	62.0 (*d*)	5	1.74	*s*,	$W_{1/2}$=2.7 Hz
6	66.3 (*d*)	6	3.44	br *s*	$W_{1/2}$=7.6 Hz
7	37.3 (*t*)	7α,β	1.63	*m*	
8	45.7 (*s*)				
9	55.4 (*d*)	9	2.18	*dd*,	$J_{9,14}$=2.1, $J_{9,11}$=8.6
10	52.7 (*s*)				
11	75.7 (*d*)	11*	4.11	br *d*,	$J_{11,9}$=8.6, $J_{11,12}$=2.3, $J_{11,13}$=2.1
12	50.2 (*d*)	12	2.46	br *s*,	$J_{12,13}$<1.0, $W_{1/2}$=6.2 Hz
13	72.2 (*d*)	13	4.25	*m*,	$J_{13,14}$=9.1, $J_{13,11}$=2.1
14	50.0 (*d*)	14*	1.87	*dd*,	$J_{14,13}$=9.1, $J_{14,9}$=2.1
15	33.9 (*t*)	15b*	2.16	AB,	J_{gem}=18.0
		15a	2.01	AB,	J_{gem}=18.0
16	145.7 (*s*)				
17	108.2 (*t*)	17a	4.88	br *s*,	$W_{1/2}$=7.2 Hz
		17b	4.67	br *s*,	overlapped with H-19
18	21.9 (*q*)	18	1.15	*s*	
19	100.2 (*d*)	19	4.67	*s*	
20	64.4 (*d*)	20	4.26	*s*,	$W_{1/2}$=4.5 Hz

* The chemical shift was read from cross section of COSY spectrum.

The exocyclic methylene protons (δ 4.88 and 4.67) showed significant couplings to a nonequivalent methylene pair of protons (AB-system) at δ 2.16 and 2.01 in the COSY spectrum (Table 2). These couplings were enhanced in a long range COSY experiment (LRCOSY, delay = 0.2 s, Figure 2a), and the latter methylene group was assigned to H-15. This assignment confirmed that C-15 does not bear a hydroxyl group or ether functionality.

There was a distinctive methine proton at δ 3.44 (br *s*, $W_{1/2}$ = 7.6 Hz) in the ^{1}H nmr spectrum. The resonance of this signal seemed too far downfield for an aliphatic methine proton without a heteroatom attached, yet too far upfield for a carbinol methine proton. The HETCOR experiment indicated that this proton correlated with a methine carbon signal at δ 66.3, thus it most likely was a nitrogen-bonded carbon. The scalar coupling from this proton (δ 3.44) to some signals in an overlapped region around 1.6 ppm, in the COSY spectrum, was of little initial help in assigning this spin system. A NOESY experiment (Table 2) showed prominent nOe's from the signal at δ 3.44 to the methyl singlet at δ 1.15, and most importantly to a sharp methine singlet at δ 1.74 (^{13}C: δ 62.0) characteristic of H-5 in hetisane-type alkaloids, enabling the assignment of the broad singlet (δ 3.44) to H-6. This assignment was confirmed by the vicinal coupling between H-5 and H-6, though it was weak, observed in the cross-section of the LRCOSY spectrum (delay = 0.2 s, Figure 2b). The nitrogenated C-6 therefore confirmed the hetisane skeleton. The proton resonances around δ 1.6 to which H-6 showed coupling in the COSY spectrum must therefore belong to H-7, and C-7 is therefore not oxygenated.

A methine singlet at δ 4.67, overlapped with the more upfield exocyclic methylene proton, correlated to the carbinolamine methine carbon at δ 100.2 in the HETCOR experiment. This proton

also showed dipolar interactions with H-18 (methyl group), H-20 and H-6 in the 2D-nOe spectrum, interactions which were not possible with the exocyclic methylene protons at C-17, indicating that the resonance at δ 4.67 belonged to H-19, and C-19 is a carbinolamine carbon as predicted. Long range (^{4}J) W-coupling between this signal assigned to H-19 and the H-18 methyl protons was also detected in the LRCOSY spectrum (Figure 2, delay = 0.2 s), confirming this assignment. Thus, one oxygenated carbon was located at C-19, and the remaining three must be at C-1, C-2, C-3, C-11 or C-13 since all oxygenated carbons are doublets.

Table 2. ^{1}H-^{1}H Correlations and nOe's of delatisine 1 (in $CDCl_3$, at 20°C)

Protons	nOe's (NOESY)	Correlations (COSY)
H-1β	H-1α, H-2	H-1α
H-1α	H-2, H-1β, H-20	H-1β, H-2
H-2	H-1α, H-1β, H-3α, H-3β	H-1α, H-3α
H-3β	H-3α, H-2	H-3α
H-3α	H-3β, H-2, H-18, H-19	H-2, H-3β
H-5	H-6, H-9, H-18	
H-6	H-19, H-5, H-7, H-18	H-7, H-20 (W-coupling)
H-7	H-6, H-15α, H-15β, H-14	H-6
H-9	H-11, H-5	H-11, H-14 (W-coupling)
H-11	H-12, H-9	H-9
H-12	H-17a, H-13, H-11	H-13
H-13	H-12, H-14, C-13 OH	H-12, H-14
H-14	H-13, H-7, H-15α*	H-13, H-9 (W-coupling)
H-15β	H-15α, H-17a, H-17b	H-15α ,H-17a, H-17b
H-15α	H-15β, H-17a, H-17b, H-14*	H-15β, H-17a, H-17b
H-17a	H-17b, H-12	H-17b, H-15α, H-15β
H-17b	H-17a, H-15α, H-15β	H-17a, H-15α, H-15β
H-18	H-19, H-5, H-6, H-3α	
H-19	H-20, H-6, H-3α, H-18	
H-20	H-19, H-1α	

* The nOe's that only showed up in NOESY at 30°C.

A broad triplet at δ 4.50 (*dd*, J = 5.4, 5.7 Hz) showed scalar couplings in the COSY spectrum to the proton at δ 2.52 (*dd*, J = 13.1, 5.4 Hz) and an overlapped signal δ 1.64. (The coupling constants of the triplet at δ 4.50 were measured by a decoupling experiment: when the signal at δ 2.52 was irradiated, the resonance at δ 4.50 appeared as a doublet, J = 5.7 Hz. Coupling with the signal at δ 2.52 was measured from this latter signal which was a well-resolved *dd*.). These latter two protons (δ 2.52 and 1.64) were thought to belong to separate methylene pairs with geminal partners located at 1.70 and 1.64 ppm, respectively, according to the COSY spectral analysis and the size of the couplings. These gem-relationships could not be established by the normal HETCOR experiment because none of the proton-carbon correlations of methylene groups appeared, even in the cross sections due to the small quantity of the sample (less than 3 mg) and inherent insensitivity of the HETCOR experiment. Thus, the fixed evolution HETCOR experiment (T = 0.021 s) with an optimal refocusing time for methylene groups (1/4J = 0.0019 s) was applied in order to enhance these weak heteronuclear correlations missing in the normal HETCOR spectrum.[11]

The heteronuclear correlations of the methylene groups were all observed in the fixed evolution HETCOR spectrum, and the methylene pair assignments anticipated from the COSY spectrum were confirmed. Thus, a methylene carbon signal at δ 34.3 correlated with two protons

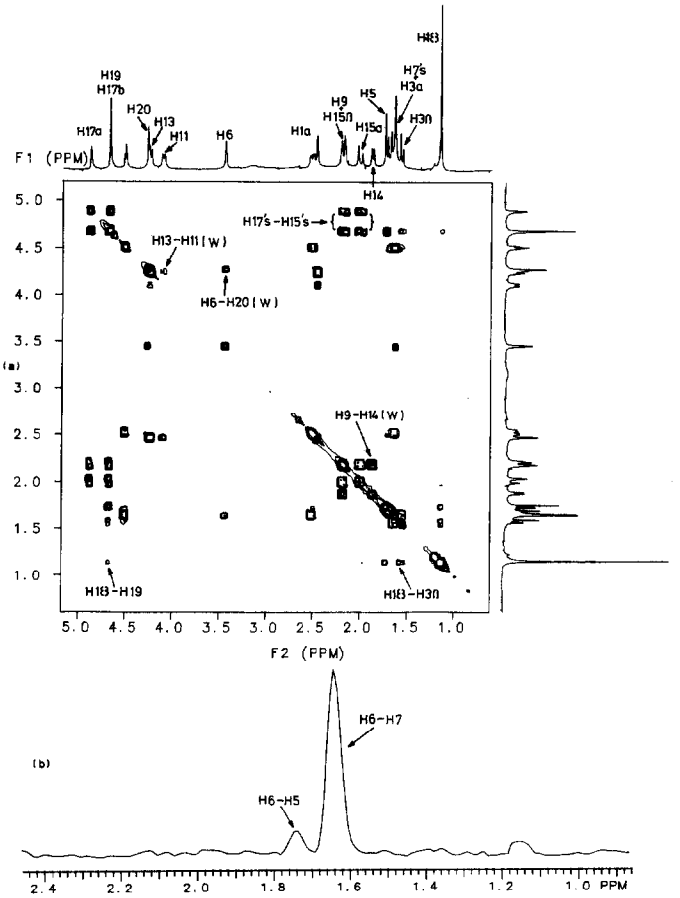


Figure 2 (a) Long range COSY spectrum (delay = 0.2 s) of 1 in $CDCl_3$. The W-couplings are indicated by a 'W' in the spectrum. Other labelled peaks are the characteristic long range couplings between H-17's and H-15's and the long range coupling between H-18 and H-3β used for assigning H-3's. (b) The cross section at H-6 frequency. The weak correlation between H-5 and H-6 is indicated by an arrow.

at δ 2.52 and 1.70, thereby establishing these signals as nonequivalent methylene gem-partners. The correlations between another methylene pair at δ 1.64 and 1.57 and the carbon signal at δ 41.6 appeared as one broad peak in both the contour plot and the cross-section of the fixed evolution HETCOR. These nonequivalent protons (only 28 Hz apart, AB-system) were not well resolved due to the relatively poor resolution of the fixed evolution HETCOR in the proton dimension. (Resolution in this fixed evolution HETCOR experiment was 26.7 Hz per point in the first dimension). However, both protons (δ 1.64 and 1.57) correlated to the same carbon, and therefore they must be the nonequivalent methylene geminal partners. Thus the signals at δ 1.64 and 1.57 were also confirmed to be methylene gem-partners, and the proton at δ 4.50 was confirmed to couple with two flanking methylene pairs which showed no further coupling to other protons. This coupling pattern in which an oxygenated methine proton with vicinal scalar couplings to two methylene

groups, $-CH_2CH(O-)CH_2-$, indicated that only C-2 is oxygenated, and C-1, C-3 are aliphatic methylene groups.

The fixed evolution HETCOR spectrum also enabled assignment of C-15. The proton signals at δ 2.16 and 2.01 assigned to the H-15 methylene protons due to the allylic coupling with the C-17 vinyl protons as discussed earlier, clearly correlated with a carbon triplet at δ 33.9. Thus, the latter was the resonance of C-15.

The triplet at δ 4.50 was then assigned to H-2 since no other location is possible for a $-CH_2-CH(OH)-CH_2-$ system. The coupling constants of H-2 with its neighboring protons (5.4 and 5.7 Hz) suggested that H-2 was β-oriented with only axial-equatorial and equatorial-equatorial vicinal relationships with its neighboring protons. However, when C-2 bears an α-orientated hydroxyl group, H-2 usually appears as a broad singlet (e.g., in tangutisine **2**, H-2 is a broad singlet, $W_{1/2}$ = 10.4 Hz)[12] because the numerous vicinal coupling constants for the axial-equatorial and equatorial-equatorial relations are small and very similar (about 2-4 Hz), resulting in a very broadened signal that often is not split. The splitting of H-2 in delatisine, therefore, must be caused by a dihedral angle resulting in couplings between H-2 and its neighboring protons larger than expected for a A-ring chair conformation. From molecular model analysis, C-2 is in perfect position to form an ether linkage with C-19, which would force $H\text{-}2_\beta$ dihedrally close to $H\text{-}1_\alpha$ and $H\text{-}3_\alpha$ (about 40°), while the dihedral angles with $H\text{-}1_\beta$ and $H\text{-}3_\beta$ would be about 80°. The coupling pattern of H-2, therefore, can be explained by larger coupling between $H\text{-}2_\beta$ and the α-protons at C-1 and C-3 due to formation of ether bridge. A selective INEPT experiment was performed by irradiating H-2 in order to prove the ether linkage between C-19 and C-2. As expected, polarization from H-2 was transferred through three bonds to the carbon signal at 100.2 ppm, which is the C-19 resonance.

With the C-2/C-19 ether linkage, and the C-1 and C-3 methylenes established, only one possibility to locate the two hydroxyl groups remains: C-11 and C-13. The carbinol methine proton at δ 4.11 (br *d*) was coupled to a signal at δ 2.18 (overlapped with the more downfield methylene proton of C-15). This latter proton has a strong dipolar interaction with H-5 (sharp singlet at δ 1.74), as well as the overlapped region with the H-7 protons, and therefore was assigned to H-9, and the broad doublet at δ 4.11 was assigned as the resonance of H-11. (A dipolar interaction between H-15 and H-5 is impossible due to distance constraints. The nOe with the H-7 protons, however, could not be unambiguously assigned to H-9 since the H-15 protons also have potential nOe's with H-7's.). The large vicinal coupling constant between H-9 and H-11 (8.6 Hz) suggested that H-11 is β-orientated, 10° dihedral angle, since a 180° dihedral angle between protons at C-9 and C-11 is not possible in the hetisane skeleton. Another carbinol signal at δ 4.25 (overlapped with the H-20 singlet) showed strong scalar coupling to a doublet at δ 1.87 in the COSY spectrum, which must be the resonances of H-13 and H-14, respectively. Strong W-coupling between H-9 (δ 2.18) and H-14 (δ 1.87) observed in COSY and LRCOSY (Figure 2) experiments confirmed these assignments. The 13–OH is also α-orientated due to the large vicinal coupling constant (9.1 Hz) between H-13 and H-14, 0° dihedral angle (a 180° dihedral angle is not possible between protons at C-13 and C-14 in the hetisane skeleton). Four bond W-coupling between H-11 and H-13 shown

in the LRCOSY spectrum (Figure 2) confirmed the stereochemical assignments for the hydroxyl groups at C-11 and C-13. The structure of this alkaloid was thereby established as **1**.

The methylene gem-protons were distinguished as follows. The methylene pair at δ 2.52 and 1.70 which coupled to H-2 should be the resonances of the H-1's since the δ 2.52 signal showed a significant dipolar interaction with H-20 in the 2D-nOe spectrum. Thus, the proton at δ 2.52 was assigned to H-1α, and its gem-partner at δ 1.70 should be H-1β. The other methylene group which also coupled to H-2 at δ 1.64 and 1.57 in the COSY spectrum must belong to the H-3's. The long range scalar coupling between H-18 and the upfield H-3 observed in the LRCOSY spectrum (Figure 2) confirmed this assignment. An nOe was observed in the 2D-nOe spectrum between H-19 and the signal at δ 1.64 which includes the overlapped resonances of the H-7's and one H-3. The observed nOe, however, must be with the resonance of H-3α since this proton is close to H-19 while the protons on C-7 are too far away to have dipolar interaction with H-19. Thus, the C-3 proton signal at δ 1.64 was assigned to H-3α, and the resonance at δ 1.57 must be H-3β. The appearence of H-3β as a doublet (J_{gem} = 11.2 Hz) confirmed this assignment since the dihedral angle between H-2 and H-3β (and H-2 and H-1β) was about 80° due to the cyclic ether ring constraints, thereby the $J_{2,3\beta}$ was very small. The two exocyclic methylene protons were easily distinguished since one of them (δ 4.88) showed a strong nOe with H-12 only, while its gem-partner (δ 4.67) had dipolar interactions with both H-15α and H-15β. Therefore, they were assigned to H-17a and H-17b, respectively.

The α-methylene proton at C-15 only showed observable dipolar interactions with the H-7 protons (an equivalent methylene pair) in a 2D-nOe experiment at 20°C. The missing nOe's from H-9 or H-14 to either proton of the methylene pair at C-15 would have been key evidence to distinguish these two nonequivalent H-15 protons. In order to reduce the correlation time and thereby enhance the double quantum relaxation pathway and increase the observed nOe's (as described for tangutisine[12]), another NOESY experiment was performed at 30°C. Unfortunately, the chemical shift of H-9 (δ 2.18) was nearly identical to the more downfield H-15 methylene proton (δ 2.16). If this C-15 proton happened to be at the β-position, the only nOe that might be enhanced at 30°C in this spin system would be from H-14 to the more upfield one (δ 2.01). On the contour plot of the second 2D-nOe spectrum, there was still no observable nOe between H-14 and either H-15 proton. However, the cross-section at H-14 did show a cross signal at δ 2.01, which must therefore be H-15α. The signals at δ 2.16 and 2.01 consequently were assigned to H-15β and H-15α respectively.

A methylene carbon at δ 37.3 happened to be very close to the artifact of the instrument (a spike at δ 37.1). It was very difficult to locate the cross peak between this carbon and its attached protons in the HETCOR spectrum. However, the cross-section at this carbon signal in the fixed evolution HETCOR spectrum clearly indicated that it correlated to protons at δ 1.63 (*m*), although the artifact was still present. These protons strongly coupled to H-6 shown in COSY spectrum as previously discussed, and therefore must be the resonances of two equivalent methylene protons at C-7, and the carbon signal at 37.3 ppm belongs to C-7.

All protonated carbons were readily assigned by a HETCOR experiment after the proton assignments were complete. The quaternary carbons were assigned as follows. In the selective

INEPT experiments, irradiating the H-18 methyl singlet caused polarization transfers to C-19, C-5, C-3 and a quaternary carbon at δ 50.5, while irradiating H-2 enhanced two quaternary carbon signals at δ 50.5 and 52.7, and a tertiary carbon signal at δ 100.2. Thus, the signals at δ 50.5 and 52.7 must be the resonances of C-4 and C-10, respectively; the δ 100.2 signal was already assigned to the carbinolamine C-19. When H-6 was irradiated, polarization transfer to a third quaternary carbon, which must be C-8, at δ 45.7 was observed along with transfers to the C-6 and C-20 carbon signals. The complete proton and carbon assignments are listed in Table 1; all nOe's are listed in Table 2.

A comparison of the C-10 and C-7 chemical shifts in delatisine **1** and tangutisine **2** revealed that formation of the ether bridge between C-2 and C-19 caused a significant downfield shift for both C-10 and C-7, from δ 46.9 and 30.3 in tangutisine to δ 52.7 and 37.3 in delatisine, respectively. The C-10 chemical shift of δ 52.7 in **1**, as a result appeared in the typical range (52.0–55.0 ppm) of C-10 in the hetisine type diterpenoid alkaloids with a hydroxyl group at C-9 or C-1, though neither C-1 nor C-9 was oxygenated in **1**. Therefore, these unpredictable ring strain effects on the carbon chemical shifts might lead to a wrong structure for an unknown in the structure elucidation based on the ^{13}C chemical shift rationale.

The structure of delatisine **1** is unusual in that there is no other diterpenoid alkaloid which contains an oxygen bridge between C-2 and C-19 to form a furan ring. A number of alkaloids of the type **3** (a–d) possessing an oxide ring between C-1 and C-19 have been isolated; e.g. (a) dehydrolucidusculine[14,15] (*N*-deethyldehydrolucidusculine,[14,15] 12-acetyldehydrolucidusculine[14]), (b) songoramine[16,17,18] (15-acetylsongoramine[16,17,18]), (c) dehydronapelline[19] (12-*epi*-dehydronapelline[16]) and (d) subdesculine.[20]

Since the structural evidence for delatisine was entirely based on nmr spectral data, a single crystal X-ray diffraction experiment was performed. The structure **1** indicated by the considerations of the ^{1}H and ^{13}C nmr discussed above was confirmed by an X-ray analysis.

Figure 3 shows the ORTEP drawing of delatisine (**1**). The bond distances and angles in delatisine are all within the normal range for that particular bond type. The C(sp3)-C(sp3) lengths average 1.540 Å and range from 1.508 to 1.584 Å. The two C(sp3)-C(sp2) lengths average 1.503 Å; the only C(sp2)-C(sp2) has a typical double bond length of 1.327 Å. The three N-C(sp2) bonds average 1.511 Å; the four single C-O bonds in alcohol and ether functionalities average 1.440 Å. There is only one intermolecular contact distance between non-hydrogen atoms less than 3.3 Å and that is a hydrogen bonding contact between O_{13} and N along the 2_1 axis with a value of 2.733(4) Å

Delatisine contains 6 six-membered rings and 4 five-membered rings. Table 3 lists the ring definitions as well as the Pople-Cremer[21] ring puckering coordinates. Rings A and C are mostly chair-like conformations with a moderate degree of puckering. Ring B, in contrast, is best described as a highly puckered boat conformation. Rings D, E and F are all part of the bicyclo[2.2.2]octane system are highly puckered, nearly pure boat conformations. Rings G and J are best described as twist conformations and ring I is nearest to an envelope conformation; ring H is a nearly equal mix of twist and envelope conformations.

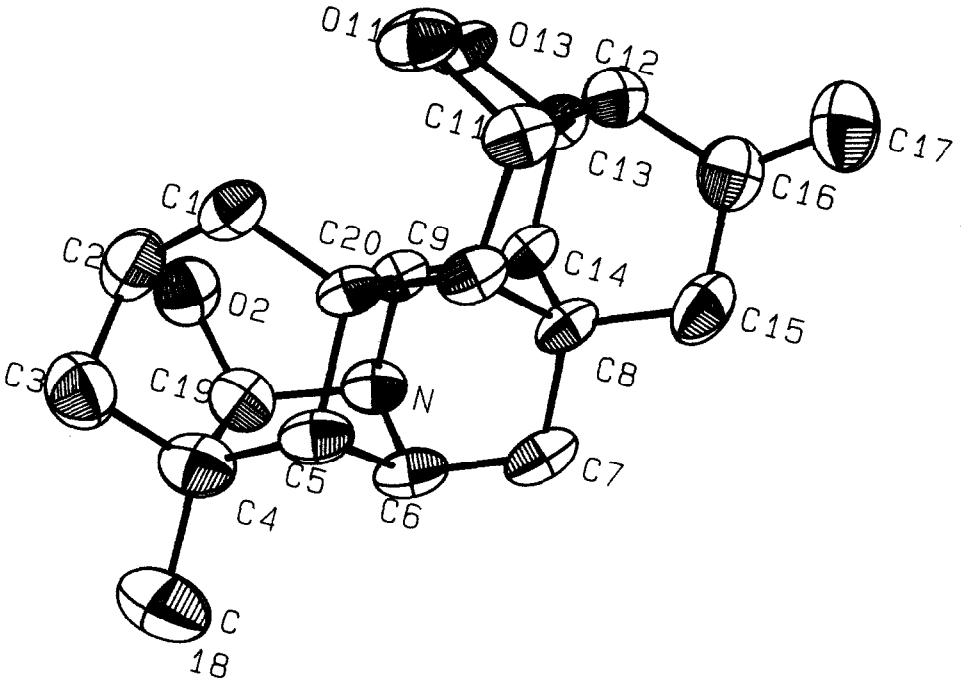


Figure 3. ORTEP drawing of delatisine

Table 3. Ring definitions and the Pople-Cremer ring puckering coordinates for delatisine 1

Ring	Members of ring	q_2	ϕ_2	q_3
Ring A:	$C_1,C_2,C_3,C_4,C_5,C_{10}$	0.233	281	-0.587
Ring B:	$C_4,C_5,C_{10},C_{20},N,C_{19}$	0.971	73	0.035
Ring C:	$C_5,C_6,C_7,C_8,C_9,C_{10}$	0.227	308	-0.692
Ring D:	$C_8,C_9,C_{11},C_{12},C_{13},C_{14}$	0.917	6	0.090
Ring E:	$C_8,C_9,C_{11},C_{12},C_{16},C_{15}$	0.800	351	0.054
Ring F:	$C_8,C_{14},C_{13},C_{12},C_{16},C_{15}$	0.784	0	-0.026
Ring G:	C_4,C_5,C_6,N,C_{19}	0.581	52	
Ring H:	C_5,C_6,N,C_{20},C_{10}	0.604	43	
Ring I:	$C_8,C_9,C_{10},C_{20},C_{14}$	0.547	359	
Ring J:	C_2,C_3,C_4,C_{19},O_2	0.443	21	

Experimental

General Procedures

Mp: are corrected and were determined on a Thomas-Kofler hot stage equipped with a microscope and polarizer. Ir spectra were recorded on a Perkin-Elmer model 1420 spectrophotometer. The nmr spectra were recorded on a Varian XL-400 spectrometer (93.94 kG, 400 MHz for ^1H, 100 MHz for ^{13}C). The nmr sample of delatisine was 3 mg dissolved in 150 μL $CDCl_3$ using a special cylindrical cavity nmr tube (cavity volume of 125 μL) from Wilmad. Residual $CHCl_3$, and $^{13}CDCl_3$ were used as internal references (^1H: δ 7.24, ^{13}C: δ 77.0) for ^1H and ^{13}C nmr, respectively. The pulse sequences employed in the one and two dimensional nmr experiments were the standard Varian software, version 6.1c, except the fixed evolution HETCOR pulse sequence which

was added to the Varian nmr pulse sequence library based on Reynold's program.[11] An evolution time fixed at 0.021 s was employed for the fixed evolution HETCOR, with a refocusing delay of 0.0019 s (1/4J, J = 130 Hz). The HRMS spectrum was recorded on a Finnigan MAT 90 instrument; (EI, 40 eV).

Nmr multipulse sequences

One dimensional ^1H nmr spectra were recorded with a spectral window of 4000 Hz and an acquisition time of 2.0 s, giving a digital resolution of 0.5 Hz per point. The spectral window and the acquisition time for one dimensional ^{13}C nmr spectra were 20000 Hz and 0.5 s, respectively, giving a digital resolution of 2.0 Hz per point. The ^{13}C multiplicities were assigned a DEPT experiment. The DEPT experiment was performed with a narrowed spectral window of 11976 Hz and an acquisition time of 0.5 s, optimized for one bond heteronuclear coupling of 140.0 Hz, in order to get better phased sub-spectra. Long range (two and three bond) heteronuclear ^1H, ^{13}C couplings were detected with one dimensional selective INEPT experiments. The selective INEPT spectra were recorded with the excitation and refocusing delays optimized for different long range heteronuclear coupling constants according to the formulae $\tau = 1/2J$ and $\Delta = 1/3J$, respectively.[13] The parameters for the two dimensional experiments are listed in Table 4.

Abbreviations for the acquisition parameters for the 2D nmr spectra listed in Table 4 are:

SW1 - Spectral window in the second dimension.
SW2 - Spectral window in the first dimension.
AT - Acquisition time (unit: second).
NP - Number of data points in the second dimension.
NT - Number of transients per increment on the first dimension.
NI - Number of increments in the first dimension.
D_3 - Refocusing delay prior to detection (unit: second).
MI - Mixing time in NOESY experiment (unit: second).
TAU - Excitation delay (unit: second).

Table 4. Parameters for recording 2D nmr spectra of **1** (in $CDCl_3$)

Parameters	COSY	LRCOSY	NOESY[a]	NOESY[b]	HETCOR	HETONE[c]
SW1 (Hz)	1773.0	1831.5	2219.8	1880.8	11185.7	3657.6
SW2 (Hz)	1773.0	1831.5	2219.8	1880.8	1776.8	4000.0
AT (s)	0.144	0.140	0.115	0.272	0.023	0.070
NP	512	512	512	1024	512	512
NT	48	256	48	48	144	320
NI	256	128	256	256	256	144
D_3 (s)		0.200				0.00192
MIX (s)			0.800	0.600		
T (s)						0.018

[a] The spectrum was run at 20 °C.
[b] The spectrum was run at 30°C.
[c] Fixed evolution HETCOR.

Isolation of delatisine 1

The seeds of *Delphinium elatum* L. (440 g., from Harris Moran Seeds, lot 1487) were ground and suspended in a mixture of 95% EtOH (640 ml), H_2O (160 ml) and hexane (200 ml). The suspension was heated under reflux for 2 hr and then vigorously stirred at room temperature for 24 hr. The solid residue was removed by filtration and suspended in a mixture of 95% EtOH (640 ml), H_2O (160 ml) and hexane (200 ml). The suspension was stirred at room temperature for 48 hr and the solid residue removed by filtration, and reextracted in a Soxhlet apparatus with 85% EtOH for 96 hr. The EtOH extract was evaporated under reduced pressure to give a residue (28.5 g) which was partitioned between CH_2Cl_2 (2 x 1000 ml) and 1.5 N. H_2SO_4 (1000 ml). The aqueous layer was removed, carefully basified with saturated, aqueous Na_2CO_3 (pH 10) and extracted with Et_2O (4 x 600 ml). The Et_2O extract was washed with H_2O (600 ml), dried over anhydrous Na_2SO_4 and evaporated to afford a white foam (6.55 g), which was chromatographed (vlc) on alumina. The fraction eluted with Et_2O–EtOH (99:1) was crystallized from acetone to afford delati-

sine **1** (82 mg), mp 274.5–276.5°C; HRMS: m/z 327.1829 (M^+, calc. for $C_{20}H_{25}NO_3$, 327.1834); $[\alpha]^{26}D$ +8.6 °(c, 0.21, $CHCl_3$); ir(nujol): 3460cm^{-1} (OH).

X-ray analysis of 1

Single crystal of delatisine **1** prepared by slow crystallization from acetone formed colorless needles, space group $P2_1$, with a = 10.661(3)Å, b = 7.499(4)Å, c = 10.731(2)Å, α = 90.0°, β = 105.82(2)°, γ = 90.0°, V = 825.4(8)Å^3 and $d_{calc.}$ 1.317 g/cm^{-3} for Z = 2. The size of the crystal used for data collection was approximately 0.1 x 0.3 x 0.4 mm. A crystal of **1** was fixed in a random orientation on a glass fiber and mounted on an Enraf-Nonius CAD-4 diffractometer equipped with a graphite crystal monochromator. λ (Cu Kα) = 1.5418Å, μ (Cu Kα) = 6.6 cm^{-1}, F(000) = 352. Cell dimensions were determined by least squares refinement of the angular positions of 25 independent reflections in the 15–25° θ range during the normal alignment procedure. A total of 1941 reflections were collected over a θ range of 2–75° using ω-2θ technique with a variable scan width and scan range. Systematic absences indicated space group $P2_1$. Because of the low value of the absorption coefficient, the data were not corrected for absorption. After Lorentz-polarization correction, averaging redundant data, and eliminating systematic absences, a total of 1563 reflections ($F_0 > 3\sigma$) were considered observed and unique and were used in the structural analysis.

The structural analysis was performed on a VAX 750 using the MolEN structure analysis program system.[22] The structure was solved using SIR88[23] with 8 symbols and using the seminvariant and MESS options. All non-hydrogen atoms were located in several difference Fourier maps and then refined by full-matrix least-squares, first isotropically, then anisotropically. Some hydrogen positions could be located from difference Fourier maps and some hydrogen positions were calculated. All hydrogens atoms, with isotropic thermal parameters fixed, were refined along with positional and thermal parameters of non-hydrogen atoms via full matrix least squares to yield the final reported structure. The final unweighted R value was 0.047. Final positional parameters for all atoms are listed in Table 5. The bond lengths and angles are given in Table 6 and Table 7.

Table 5. Positional parameters and their estimated standard deviations

Atom	x	y	z	B(A2)
O2	0.2395(2)	0.543	0.5486(2)	4.57(5)
O11	0.6330(2)	0.6034(3)	0.9410(2)	4.32(4)
O13	0.6587(2)	0.6244(3)	0.6934(2)	3.51(4)
N	0.3563(2)	0.2583(4)	0.5470(2)	3.46(5)
C1	0.3694(3)	0.5663(4)	0.7785(3)	3.72(6)
C2	0.2365(3)	0.5931(5)	0.6799(3)	4.56(7)
C3	0.1362(3)	0.4677(6)	0.7065(3)	5.05(8)
C4	0.1814(3)	0.2940(6)	0.6578(3)	4.40(7)
C5	0.3147(3)	0.2306(4)	0.7413(2)	3.48(5)
C6	0.3579(3)	0.1137(4)	0.6453(3)	3.77(6)
C7	0.4906(3)	0.0298(4)	0.7044(3)	4.10(6)
C8	0.5921(3)	0.1706(4)	0.7634(3)	3.21(5)
C9	0.5470(3)	0.2985(4)	0.8566(2)	3.10(5)
C10	0.4224(2)	0.3814(4)	0.7611(2)	2.80(4)
C11	0.6643(3)	0.4218(4)	0.9220(2)	3.50(5)
C12	0.7688(3)	0.4109(4)	0.8479(3)	3.52(5)
C13	0.7039(2)	0.4444(4)	0.7039(2)	3.13(5)
C14	0.5948(3)	0.3021(4)	0.6543(2)	2.92(5)
C15	0.7248(3)	0.0846(5)	0.8229(3)	4.76(7)
C16	0.8266(3)	0.2267(5)	0.8670(3)	4.35(7)
C17	0.9527(4)	0.1934(7)	0.9159(4)	6.2(1)
C18	0.0765(3)	0.1503(8)	0.6325(4)	6.4(1)
C19	0.2256(3)	0.3524(5)	0.5378(3)	4.15(7)
C20	0.4569(2)	0.3791(4)	0.6270(2)	2.83(5)
H1A	0.362(2)	0.582(5)	0.857(3)	4.7*
H1B	0.434(3)	0.649(5)	0.764(3)	4.7*
H2	0.185(3)	0.723(6)	0.677(3)	5.9*
H3A	0.038(3)	0.506(6)	0.655(4)	6.5*
H3B	0.140(3)	0.444(7)	0.804(3)	6.5*
H5	0.330(2)	0.182(5)	0.823(3)	4.3*

Table 5. Positional parameters and their estimated standard deviations - continued

Atom	x	y	z	B(A2)
H6	0.306(3)	0.011(6)	0.606(3)	5.0*
H7A	0.525(3)	-0.047(6)	0.641(3)	5.1*
H7B	0.486(3)	-0.044(6)	0.780(3)	5.1*
H9	0.528(2)	0.227(5)	0.924(3)	3.8*
H11	0.707(3)	0.370(5)	1.003(3)	3.9*
H12	0.831(3)	0.495(6)	0.884(3)	4.5*
H13	0.762(2)	0.435(5)	0.658(3)	3.9*
H14	0.617(2)	0.248(5)	0.581(3)	3.6*
H15A	0.754(3)	0.012(7)	0.751(3)	5.9*
H15B	0.740(3)	0.018(7)	0.882(3)	5.9*
H17A	0.988(4)	0.069(7)	0.928(4)	7.8*
H17B	1.019(3)	0.304(7)	0.948(4)	7.8*
H18A	0.087(3)	0.031(7)	0.578(4)	7.3*
H18B	0.060(3)	0.095(6)	0.702(4)	7.3*
H18C	-0.017(3)	0.185(8)	0.581(4)	7.3*
H19	0.152(3)	0.340(5)	0.443(3)	4.9*
H20	0.454(2)	0.510(5)	0.583(3)	3.4*

Starred atoms were refined isotropically.
Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: (4/3) * [a2*B(1,1) + b2*B(2,2) + c2*B(3,3) + ab(cos gamma)*B(1,2) + ac(cos beta)*B(1,3) + bc(cos alpha)*B(2,3)].

Table 6. Bond distances for non-hydrogen atoms (Å)

Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance
O2	C2	1.469(5)	C6	C7	1.519(6)
O2	C19	1.436(5)	C7	C8	1.521(5)
O11	C11	1.430(5)	C8	C9	1.553(4)
O13	C13	1.427(4)	C8	C14	1.537(4)
N	C6	1.509(4)	C8	C15	1.527(5)
N	C19	1.540(5)	C9	C10	1.568(4)
N	C20	1.485(4)	C9	C11	1.560(5)
C1	C2	1.533(6)	C10	C20	1.579(4)
C1	C10	1.529(4)	C11	C12	1.537(5)
C2	C3	1.508(6)	C12	C13	1.532(4)
C3	C4	1.530(7)	C12	C16	1.504(5)
C4	C5	1.534(6)	C13	C14	1.561(4)
C4	C18	1.523(6)	C14	C20	1.532(4)
C4	C19	1.550(5)	C15	C16	1.502(6)
C5	C6	1.517(5)	C16	C17	1.327(6)
C5	C10	1.584(4)			

Numbers in parentheses are estimated standard deviations in the least significant digits.

Table 7. Bond angles for non-hydrogen atoms (°)

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
C2	O2	C19	107.7(3)	C7	C8	C9	112.7(3)
C6	N	C19	102.3(3)	C7	C8	C14	106.7(3)
C6	N	C20	99.5(2)	C7	C8	C15	110.7(3)
C19	N	C20	104.8(3)	C9	C8	C14	99.6(2)
C2	C1	C10	109.7(3)	C9	C8	C15	113.9(3)
O2	C2	C1	111.7(3)	C14	C8	C15	112.5(3)
O2	C2	C3	102.9(3)	C8	C9	C10	100.4(2)
C1	C2	C3	110.9(3)	C8	C9	C11	107.7(3)
C2	C3	C4	99.3(3)	C10	C9	C11	119.5(3)
C3	C4	C5	113.1(3)	C1	C10	C5	112.7(3)
C3	C4	C18	112.3(4)	C1	C10	C9	123.7(3)
C3	C4	C19	103.8(4)	C1	C10	C20	107.9(2)
C5	C4	C18	113.8(4)	C5	C10	C9	105.0(2)
C5	C4	C19	97.5(3)	C5	C10	C20	102.2(2)
C18	C4	C19	115.2(3)	C9	C10	C20	103.0(2)
C4	C5	C6	100.8(3)	O11	C11	C9	116.0(3)
C4	C5	C10	112.3(3)	O11	C11	C12	110.8(3)

Table 7. Bond angles for non-hydrogen atoms (°) - continued

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
C6	C5	C10	99.2(2)	C9	C11	C12	109.9(3)
N	C6	C5	96.4(3)	C11	C12	C13	108.8(3)
N	C6	C7	114.9(3)	C11	C12	C16	107.8(3)
C5	C6	C7	112.1(3)	C13	C12	C16	110.1(3)
C6	C7	C8	111.2(3)	O13	C13	C12	106.5(3)
O13	C13	C14	114.6(2)	C15	C16	C17	123.9(5)
C12	C13	C14	108.8(3)	O2	C19	N	112.2(3)
C8	C14	C13	110.2(2)	O2	C19	C4	105.2(3)
C8	C14	C20	99.9(2)	N	C19	C4	106.9(3)
C13	C14	C20	113.2(2)	N	C20	C10	102.8(2)
C8	C15	C16	109.8(3)	N	C20	C14	112.7(2)
C12	C16	C15	112.0(3)	C10	C20	C14	106.2(2)
C12	C16	C17	124.1(5)				

Numbers in parentheses are estimated standard deviations in the least significant digits.

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