

STUDIES ON THE CONSTITUENTS OF ACONITUM SPECIES. VII.¹
ON THE COMPONENTS OF ACONITUM JAPONICUM THUNB.

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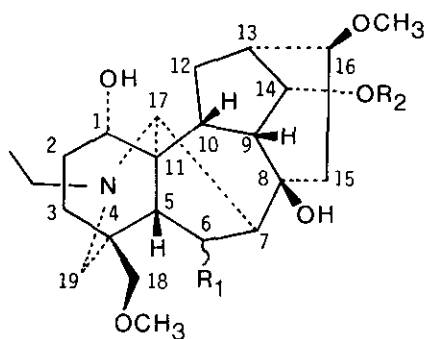
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Abstract — Two new diterpenoid alkaloids, subcumine (1) and subcusine (2), were isolated from the roots of Aconitum japonicum Thunb. The structures of these alkaloids were determined on the basis of spectral data, chemical evidence, and X-ray analysis. We also have isolated two known alkaloids, ezochasmaconitine (3) and anisoezochasmaconitine (4).

Recently, we reported an isolation of eighteen alkaloids^{2,3} from Aconitum japonicum Thunb. In this paper we report isolation and structure determination of two new C₁₉-diterpenoid alkaloids, named subcumine (1) and subcusine (2) together with known alkaloids, ezochasmaconitine (3)⁴ and anisoezochasmaconitine (4)⁴. We also report the revised assignments of ¹³C-chemical shifts of C(9), C(10), and C(13) in the related C₁₉-diterpenoid alkaloids.

Subcumine (1), mp 200-202°C, C₂₆H₄₁NO₇, was deduced a C₁₉-diterpenoid alkaloid from the molecular formula and the ¹H-nmr spectrum which showed five methyl groups assignable to an N-CH₂CH₃, an acetyl, and three methoxyls, and two oxygenated methines at δ 4.02 (d, J= 7.9 Hz) and 4.76 (t, J= 4.6 Hz, C₁₄β-H) (See Experimental). This spectrum was similar to that of 14-acetylneoline⁴ (7, bulatine C⁵, delstaphisagnine⁶) with the exception of the signal at δ 4.02 ppm. The coupling constant of the signal was similar to the value of C₆α-H in the nmr spectra of bicoloridine⁷ (5, alkaloid A⁸) and bicolorine 6-O-acetate⁹ (6) and the

structure was assigned to 1. ^{13}C -Nmr and ir spectra of 1 also supported the structure. Finally, the structure of 1 was determined by X-ray crystal analysis. Perspective view of 1 was illustrated in Fig.1.

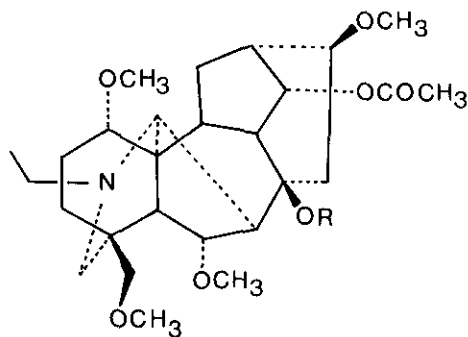


1; $\text{R}_1 = \beta\text{-OCH}_3$, $\text{R}_2 = \text{COCH}_3$

2; $\text{R}_1 = \beta\text{-OCH}_3$, $\text{R}_2 = \text{H}$

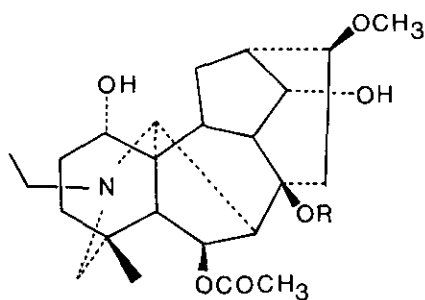
7; $\text{R}_1 = \alpha\text{-OCH}_3$, $\text{R}_2 = \text{COCH}_3$

8; $\text{R}_1 = \alpha\text{-OCH}_3$, $\text{R}_2 = \text{H}$



3; $\text{R} = \text{CO-C}_6\text{H}_5$

4; $\text{R} = \text{CO-C}_6\text{H}_4\text{-OCH}_3(\text{p})$



5; $\text{R} = \text{CH}_3$

6; $\text{R} = \text{H}$

Chart 1

^1H -Nmr spectrum of subcusine (2), mp $194\text{--}196^\circ\text{C}$, $\text{C}_{24}\text{H}_{39}\text{NO}_6$, showed four methyl groups assignable to an $\text{N-CH}_2\text{CH}_3$, three methoxyls, and two oxygenated methines at δ 4.00 (d, $J = 7.8$ Hz, $\text{C}_6\alpha\text{-H}$) and 4.06 (t, $J = 4.5$ Hz, $\text{C}_{14}\beta\text{-H}$). The nmr spectrum of 2 suggested that the acetyl group was free from the structure of 1. Hydrolysis of 1 gave the compound 2, and the structure of subcusine (2) was determined to be deacetylsubcumine.

Table I showed ^{13}C -chemical shifts of 1, 2, 7, and neoline (8). The assignments for C(10) and C(13) were suggested to be revised in most of the C_{19} -diterpenoid alkaloids by Pelletier, *et al.*¹⁰ We agree to their proposal and wish to propose the further revised assignments for C(9) and C(10) of 14-acetylneoline (7) in view of following consideration; in comparison with spectra of 1, 2, 7, and 8, introduction of the acyl group at C(14) shifts the C(9) and C(13) resonances upfield as the result of β effect and shifts C(10) resonance downfield (1.3 ppm) as the result of γ effect in compound 7 and 8. Since C_6 - β oriented methoxyl group is sterically close to C(9) and C(10), the resonances of C(9) and C(10) in compound 1 and 2 are likely affected by sterical effect in addition to the acylation effect.

Table I. ^{13}C -chemical shifts and assignments for subcumine (1), subcusine (2), 14-acetylneoline (delstaphisagnine) (7)⁶, and neoline (8)¹¹

carbon	<u>1</u>	<u>2</u>	<u>7</u>	<u>8</u>	carbon	<u>1</u>	<u>2</u>	<u>7</u>	<u>8</u>
1	72.5	72.4	72.0	72.1	14	76.6	76.0	77.1	75.9
2	27.2	27.3	29.5	29.5 ^a	15	40.3	40.6	42.6	42.7
3	30.0 ^a	30.0 ^a	30.0	29.9 ^a	16	81.5	81.5	81.9	82.3
4	36.9	37.0	38.0	38.2	17	65.0	65.2	63.3	63.3
5	44.0	44.2	44.5	44.3 ^b	18	77.7	77.7	80.1	80.3
6	82.9	82.4	83.3	83.3	19	57.9	57.6	57.9	57.2
7	51.1	50.8	52.6	52.3	6'	57.2	57.1	57.9	57.8
8	75.1	75.1	74.6	73.4	16'	56.2	56.3	56.1	56.3
9	43.5	46.1	43.3 ^c	48.3	18'	59.1	59.1	59.1	59.1
10	45.1	45.4	46.2 ^c	44.9 ^{b,c}	N-CH ₂	48.5	48.5	48.2	48.2
11	48.7	48.3	49.7	49.6	CH ₃	12.9	12.9	13.0	13.0
12	29.2 ^a	29.3 ^a	29.3	29.8 ^a	O=C	171.5	----	170.4	----
13	37.9	39.8	36.6 ^c	40.7 ^c	CH ₃	21.6	----	21.3	----

a and b: The assignments may be interchanged in any vertical column.

c: The assignments were revised according to the previous data on C_{19} -diterpenoid alkaloids¹⁰ and the reason described in the text.

X-ray crystal structure determination of 1

Single crystals were obtained by recrystallization from acetone-hexane. A colorless crystal, 0.20 x 0.19 x 0.65 mm, was used for the X-ray study. The crystal belongs to an orthorhombic system, space group $\text{P2}_1\text{2}_1\text{2}_1$, with a unit cell dimensions $a = 15.639(3)$, $b = 17.825(3)$, $c = 8.895(3)\text{\AA}$, $U = 2480(1)\text{\AA}^3$, $Z = 4$, $D_x = 1.285$

$\text{Mg} \cdot \text{m}^{-3}$, $T = 293 \text{ K}$, $\mu = 0.086 \text{ mm}^{-1}$, $F(000) = 1040$, $M_r = 479.60$. X-ray diffraction data were collected on a Rigaku automated four-circle diffractometer with graphite-monochromatized $\text{MoK}\alpha(\lambda = 0.71073)$ radiation. Within the range of $2\theta \leq 55^\circ$, 2185 reflections with $F_o > 3\sigma(F_o)$ were obtained. Unique reflections (2077) were used for the structure determination and refinement. The intensities were corrected for Lorenz-polarization effects and for deterioration, but no absorption correction was applied. The structure was solved by the direct phasing method using MULTAN 78¹² and was refined by the block-diagonal least-squares method. Unit weight was given to all reflections, and anisotropic temperature factors were used for all non-hydrogen atoms. The final R and R_w values were 5.31 and 5.03%, respectively. The final difference Fourier map showed no peak higher than $0.45 \text{ e}\text{\AA}^{-3}$. Atomic scattering factors and anomalous scattering corrections were taken from International Tables for X-ray Crystallography¹³. Crystallographic calculations performed on a FACOM N-780 computer of the Institute UNICS-III program system¹⁴. The drawing of the molecule is shown in Fig. I with $\text{C}_6\text{-}\alpha\text{H}$ in it. The list of the bond lengths and angles is normal and given in Table III and IV. Tortion angles within the five, six, and seven-membered rings are given in Table V.

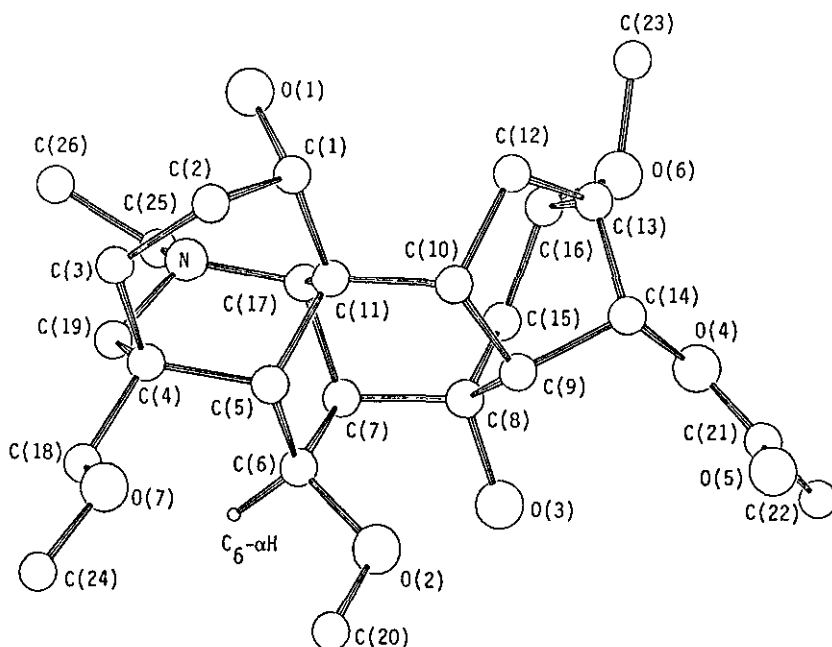


Fig. I. Perspective View of 1

Table II. Atomic parameters.

Positional parameters are multiplied by 10^4 with estimated standard deviations in parentheses. The numbering scheme of atoms is shown in Fig. 1. The equivalent isotropic temperature factor is defined by $B_{eq} = 4/3 \cdot \sum_i \sum_j \beta_{ij} (\alpha_i \cdot \alpha_j)$.

	x	y	z	$B_{eq} (Å^2)$
O(1)	2236(2)	1194(2)	2209(4)	3.5
O(2)	3956(2)	1369(2)	-3604(4)	3.9
O(3)	5483(2)	1256(2)	-2165(4)	3.7
O(4)	5786(2)	-285(2)	-846(4)	4.0
O(5)	5635(3)	-983(4)	-2873(7)	9.2
O(6)	5926(2)	390(2)	2498(4)	4.3
O(7)	1349(3)	2042(2)	-3723(5)	4.8
N	3094(3)	2343(2)	822(5)	3.0
C(1)	2204(3)	855(3)	740(5)	2.7
C(2)	1363(3)	1034(3)	-44(6)	3.2
C(3)	1316(3)	1850(3)	-518(6)	3.5
C(4)	2129(3)	2120(3)	-1383(6)	3.1
C(5)	2748(3)	1458(3)	-1757(5)	2.5
C(6)	3602(3)	1779(3)	-2353(5)	2.8
C(7)	4221(3)	1773(2)	-972(5)	2.6
C(8)	4868(3)	1124(3)	-988(5)	2.6
C(9)	4403(3)	373(3)	-1276(5)	2.7
C(10)	3510(3)	327(3)	-482(5)	2.4
C(11)	2996(3)	1059(2)	-255(5)	2.3
C(12)	3729(3)	-69(3)	1056(6)	2.9
C(13)	4699(3)	-156(3)	1117(6)	3.1
C(14)	4872(3)	-287(3)	-559(6)	3.2
C(15)	5405(3)	1110(3)	464(6)	3.0
C(16)	5147(3)	555(3)	1701(6)	3.1
C(17)	3602(3)	1669(2)	370(5)	2.4
C(18)	1842(3)	2523(3)	-2822(6)	3.4
C(19)	2623(3)	2696(3)	-437(6)	3.3
C(20)	3513(4)	1511(4)	-4968(6)	5.8
C(21)	6080(4)	-634(4)	-1982(8)	5.2
C(22)	7016(4)	-495(4)	-2242(8)	5.6
C(23)	5765(4)	120(4)	3987(7)	5.6
C(24)	964(5)	2410(4)	-4954(9)	6.4
C(25)	3623(4)	2905(3)	1618(6)	3.8
C(26)	3100(4)	3427(3)	2576(8)	5.6

Table III. Bond distances (Å) with e.s.d.'s in parentheses.

O(1)-C(1)	1.441(6)	O(2)-C(6)	1.442(6)	O(2)-C(20)	1.421(7)
O(3)-C(8)	1.440(6)	O(4)-C(14)	1.451(6)	O(4)-C(21)	1.274(8)
O(5)-C(21)	1.224(9)	O(6)-C(16)	1.440(6)	O(6)-C(23)	1.431(7)
O(7)-C(18)	1.405(7)	O(7)-C(24)	1.410(9)	N-C(17)	1.494(6)
N-C(19)	1.482(7)	N-C(25)	1.480(7)	C(1)-C(2)	1.522(7)
C(1)-C(11)	1.565(6)	C(2)-C(3)	1.515(8)	C(3)-C(4)	1.563(7)
C(4)-C(5)	1.561(7)	C(4)-C(18)	1.535(7)	C(4)-C(19)	1.536(7)
C(5)-C(6)	1.547(7)	C(5)-C(11)	1.563(6)	C(6)-C(7)	1.564(7)
C(7)-C(8)	1.537(6)	C(7)-C(17)	1.548(6)	C(8)-C(9)	1.546(7)
C(8)-C(15)	1.541(7)	C(9)-C(10)	1.567(6)	C(9)-C(14)	1.527(7)
C(10)-C(11)	1.545(6)	C(10)-C(12)	1.576(7)	C(11)-C(17)	1.546(6)
C(12)-C(13)	1.527(7)	C(13)-C(14)	1.532(8)	C(13)-C(16)	1.539(7)
C(15)-C(16)	1.533(7)	C(21)-C(22)	1.502(8)	C(25)-C(26)	1.504(9)

Table IV. Bond angles(°) with e.s.d.'s in parentheses.

C(6)-O(2)-C(20)	112.4(4)	C(14)-O(4)-C(21)	119.6(4)
C(16)-O(6)-C(23)	112.0(4)	C(18)-C(7)-C(24)	113.2(5)
C(17)-N-C(19)	113.8(4)	C(17)-N-C(25)	112.1(4)
C(19)-N-C(25)	110.6(4)	O(1)-C(1)-C(2)	111.0(4)
O(1)-C(1)-C(11)	112.8(4)	C(2)-C(1)-C(11)	112.1(4)
C(1)-C(2)-C(3)	111.8(4)	C(2)-C(3)-C(4)	113.2(4)
C(3)-C(4)-C(5)	112.1(4)	C(3)-C(4)-C(18)	108.5(4)
C(3)-C(4)-C(19)	110.2(4)	C(5)-C(4)-C(18)	110.9(4)
C(5)-C(4)-C(19)	108.1(4)	C(18)-C(4)-C(19)	106.9(4)

Table IV continued.

C(4) -C(5) -C(6)	109.2(4)	C(4) -C(5) -C(11)	108.4(4)
C(6) -C(5) -C(11)	104.3(4)	O(2) -C(6) -C(5)	114.1(4)
O(2) -C(6) -C(7)	111.4(4)	C(5) -C(6) -C(7)	105.3(4)
C(6) -C(7) -C(8)	113.9(4)	C(6) -C(7) -C(17)	102.7(4)
C(8) -C(7) -C(17)	109.2(4)	O(3) -C(8) -C(7)	108.9(4)
O(3) -C(8) -C(9)	109.6(4)	O(3) -C(8) -C(15)	104.4(3)
C(7) -C(8) -C(9)	110.1(4)	C(7) -C(8) -C(15)	111.3(4)
C(9) -C(8) -C(15)	112.4(4)	C(8) -C(9) -C(10)	113.0(4)
C(8) -C(9) -C(14)	111.9(4)	C(10) -C(9) -C(14)	101.6(4)
C(9) -C(10) -C(11)	118.5(4)	C(9) -C(10) -C(12)	102.8(3)
C(11) -C(10) -C(12)	112.2(4)	C(1) -C(11) -C(5)	113.1(4)
C(1) -C(11) -C(10)	106.8(4)	C(1) -C(11) -C(17)	116.4(4)
C(5) -C(11) -C(10)	113.7(4)	C(5) -C(11) -C(17)	98.0(3)
C(10) -C(11) -C(17)	108.8(3)	C(10) -C(12) -C(13)	107.0(4)
C(12) -C(13) -C(14)	99.0(4)	C(12) -C(13) -C(16)	112.3(4)
C(14) -C(13) -C(16)	111.9(4)	O(4) -C(14) -C(9)	113.4(4)
O(4) -C(14) -C(13)	110.2(4)	C(9) -C(14) -C(13)	101.8(4)
C(8) -C(15) -C(16)	118.0(4)	O(6) -C(16) -C(13)	112.5(4)
O(6) -C(16) -C(15)	105.3(4)	C(13) -C(16) -C(15)	114.1(4)
N -C(17) -C(7)	116.3(4)	N -C(17) -C(11)	109.6(4)
C(7) -C(17) -C(11)	101.0(3)	O(7) -C(18) -C(4)	110.6(4)
N -C(19) -C(4)	112.3(4)	O(4) -C(21) -O(5)	123.8(6)
O(4) -C(21) -C(22)	113.1(6)	O(5) -C(21) -C(22)	122.6(6)
N -C(25) -C(26)	112.7(5)		

Table V. Torsion angles(°) with e.s.d.'s in parentheses.

C(1) -C(2) -C(3) -C(4)	49.1(6)
C(2) -C(3) -C(4) -C(5)	8.1(6)
C(3) -C(4) -C(5) -C(11)	-57.1(5)
C(4) -C(5) -C(11) -C(1)	50.1(5)
C(2) -C(1) -C(11) -C(5)	4.8(5)
C(11) -C(1) -C(2) -C(3)	-56.0(5)
C(5) -C(6) -C(7) -C(8)	102.2(4)
C(6) -C(7) -C(8) -C(9)	-49.7(5)
C(7) -C(8) -C(9) -C(10)	-37.5(5)
C(8) -C(9) -C(10) -C(11)	30.7(5)
C(9) -C(10) -C(11) -C(5)	61.9(5)
C(6) -C(5) -C(11) -C(10)	-71.6(4)
C(11) -C(5) -C(6) -C(7)	-17.2(4)
C(9) -C(10) -C(12) -C(13)	5.0(5)
C(10) -C(12) -C(13) -C(14)	-34.0(5)
C(12) -C(13) -C(14) -C(9)	51.3(4)
C(10) -C(9) -C(14) -C(13)	-49.0(4)
C(14) -C(9) -C(10) -C(12)	26.4(4)
C(9) -C(10) -C(12) -C(13)	5.0(5)
C(10) -C(12) -C(13) -C(16)	84.3(5)
C(12) -C(13) -C(16) -C(15)	-91.0(5)
C(8) -C(15) -C(16) -C(13)	28.4(6)
C(9) -C(8) -C(15) -C(16)	-24.6(6)
C(15) -C(8) -C(9) -C(10)	87.2(5)
C(8) -C(9) -C(10) -C(12)	-93.6(4)
C(15) -C(8) -C(9) -C(14)	-26.6(5)
C(8) -C(9) -C(14) -C(13)	71.7(5)
C(16) -C(13) -C(14) -C(9)	-67.3(5)
C(14) -C(13) -C(16) -C(15)	19.3(6)
C(8) -C(15) -C(16) -C(13)	28.4(6)
C(9) -C(8) -C(15) -C(16)	-24.6(6)
C(4) -C(5) -C(11) -C(17)	-73.1(4)
C(5) -C(11) -C(17) -N	70.3(4)
C(19) -N -C(17) -C(11)	-61.4(5)
C(17) -N -C(19) -C(4)	47.7(5)

Table V continued.

C(5) -C(4) -C(19)-N	-48.7(5)
C(19)-C(4) -C(5) -C(11)	64.6(5)
C(7) -C(8) -C(9) -C(10)	-37.5(5)
C(8) -C(9) -C(10)-C(11)	30.7(5)
C(9) -C(10)-C(11)-C(17)	-46.2(5)
C(10)-C(11)-C(17)-C(7)	65.5(4)
C(8) -C(7) -C(17)-C(11)	-78.1(4)
C(17)-C(7) -C(8) -C(9)	64.4(5)
C(5) -C(6) -C(7) -C(17)	-15.7(4)
C(6) -C(7) -C(17)-C(11)	43.2(4)
C(5) -C(11)-C(17)-C(7)	-53.0(4)
C(6) -C(5) -C(11)-C(17)	43.1(4)
C(11)-C(5) -C(6) -C(7)	-17.2(4)

EXPERIMENTAL

All melting points are uncorrected. Ir spectra in CHCl_3 solution were taken with a JASCO IRA-2 spectrometer. Nmr spectra were measured in CDCl_3 solution with a JOEL FX-100 and GX-270 spectrometers using TMS as an internal standard. Ms were measured with a Shimadzu LKB-9000B spectrometer, and HR-ms were measured with JMS-D300 mass spectrometer. Optical rotations were measured with Jasco DIP-4 digital polarimeter.

Isolation procedure---We have already reported the isolation and extraction procedure from the roots of the title plant to obtain the crude fraction.

Isolation of ten alkaloids isolated from the Fr-A were reported previously.² The remaining crude bases in the Fr-A were combined and chromatographed on silica gel with a mixture of hexane and ether saturated with 28% aqueous NH_3 ; the content of hexane was decreased gradually. Purification by repeated column chromatography gave subcumine (1, 22.4 mg), subcusine (2, 11.0 mg), 3 (9.0 mg, mp 163-164°C), and 4 (4.7 mg, mp 141-143°C).

Subcumine (1)---mp 200-202°C(from acetone-hexane), $[\alpha]_D^{+18.3^\circ}$ (c= 0.48, CHCl_3).

HR-ms: Calcd for $\text{C}_{26}\text{H}_{41}\text{NO}_7$ 479.2883, Found 479.2884. Ms (m/z): 479 (M^+), 464 (base peak), 462, 446. Ir ν (max, cm^{-1}): 3450, 1720, 1250. $^1\text{H-Nmr}$ (δ , 270 MHz): 1.11 (3H, t, J= 7.3 Hz), 2.06 (3H, s), 3.32 (6H, s), 3.34 (3H, s), 4.02 (1H, d, J= 7.9 Hz), 4.76 (1H, t, J= 4.6 Hz).

Subcusine (2)--- mp 194-196°C(from acetone-hexane), $[\alpha]_D^{+33.3^\circ}$ (c= 0.3, CHCl_3).

HR-ms: Calcd for $\text{C}_{24}\text{H}_{39}\text{NO}_6$ 437.2777, Found 437.2751. Ms (m/z): 437 (M^+), 422 (base peak), 420, 404. Ir ν (max, cm^{-1}): 3450. $^1\text{H-Nmr}$ (δ): 1.07 (3H, t, J= 7.0 Hz), 3.30 (3H, s), 3.33 (6H, s), 4.00 (1H, d, J= 7.8 Hz), 4.06 (1H, t, J= 4.5 Hz).

Hydrolysis of 1---Subcumine (2 mg) was dissolved in 1 ml of 5% methanolic KOH

solution and allowed to stand at room temperature for 4 h. Removal of solvent under reduced pressure gave a residue, which was mixed with a small amount of H₂O and extracted with CHCl₃ (10 ml x 3). The extract was evaporated to yellowish residue which showed a single spot on tlc. The product was identical with subcusine (2) by comparison of ¹H-nmr spectrum and the tlc behavior.

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