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LASSIOCARPINE, A NOVEL C_{20}-DITERPENE ALKALOID ISOLATED FROM *ACONITUM KOJIMAE* OHWI

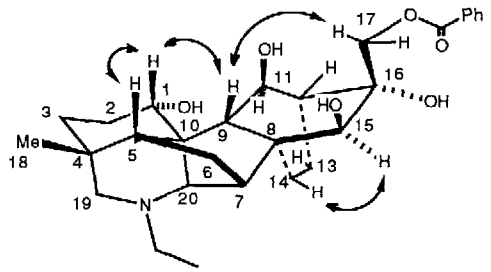
*Hiromitsu TAKAYAMA, Jing-Jing SUN, Norio AIMI, and Shin-ichiro SAKAI**
Faculty of Pharmaceutical Sciences, Chiba University, 1-33, Yayoi-cho, Chiba 260, Japan
Sheng-Teh LU and Ih-Sheng CHEN
Kaohsiung Medical College, No. 100, Shih-Chuan 1st Road, Kaohsiung, Taiwan Republic of China

Summary The structure of **1** proposed for lassiocarpine, a novel type of diterpene alkaloid isolated from *Aconitum kojimae* Ohwi var. *lassiocarpium*, was inferred from the spectroscopic analysis, especially from the HMQC, HMBC, and NOESY experiments.

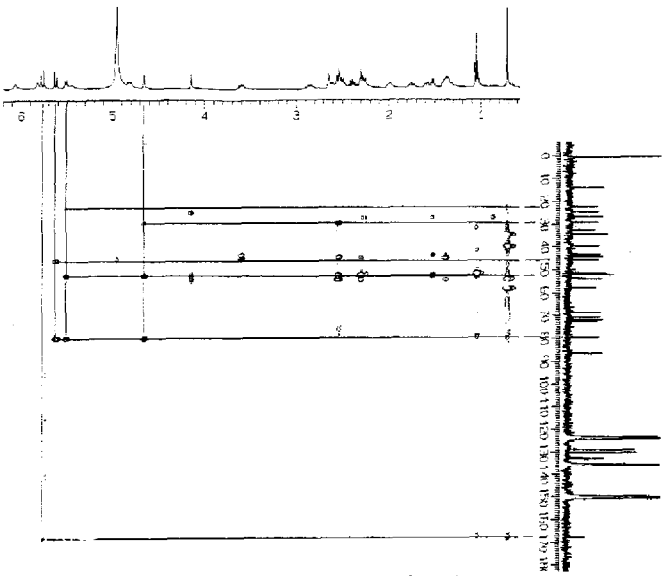
In connection with a study on the diterpene alkaloids from Aconitum species, we investigated the alkaloidal components of *Aconitum kojimae* Ohwi var. *lassiocarpium* Tamura, a plant native to Mt. Lu-lin, Chia-yi country in Taiwan. The crude alkaloid obtained from the root of this plant was purified by aluminum and silica gel column chromatography followed by medium pressure liquid chromatography to yield a new base named lassiocarpine, mp. 141-143°C (acetone) , $[\alpha]_D^{22}$ -17.4° (c=0.49, MeOH). We describe here the structure elucidation of this new minor alkaloid by applying 2D-NMR spectral techniques, viz, proton-detected one-bond (HMQC)[1] and long-range (HMBC)[2] ^1H-^{13}C NMR experiment and NOESY[3] spectrum.

The high resolution mass spectrum of **1** presents the molecular ion m/z 497.2784 , corresponding to the formula $C_{29}H_{39}NO_6$. The IR spectrum indicated the presence of hydroxy groups (3420 cm^{-1}) and an ester function (1710 cm^{-1}). The ^{13}C NMR spectrum of **1** suggested at first that this compound had one benzoyl ester group and five oxygenated carbons (δ 70.4, 71.6, 72.3, 79.1, and 86.2). We have deduced from the ^1H [δ 0.72 (3H, s, H_3-18), 1.05 (3H, t, J=7.3Hz, N-CH_2CH_3), 4.15 (1H, s, H-20), and 3.61 (1H, dd, J=7.3 and 13.6Hz, H-6β)] and ^{13}C NMR spectra that lassiocarpine was a C_{20}-diterpene alkaloid having the atisine-denudatine skeleton.[4] But most characteristic of this new compound is the absence of the exomethylene group at C_{16}-C_{17} position that is usually present in the C_{20}-diterpene alkaloids.

After assigning all the protonated carbons by the HMQC experiments (Table), the placement and the stereochemistry of the functional groups were deduced by the two or three bond heteronuclear connectivities from the HMBC experiments and NOESY spectrum as described in the following examples. A doublet signal at δ5.53 (H-11) connected to δ51.9 (C_9), δ79.1(C_{16}) and δ22.0(C_{13}) that indicated the presence of a secondary hydroxy group on C_{11}. And the β-orientation of this hydroxy group was cleanly assigned by the coupling constant (d, J=8.9Hz) with H-9 and by the appearance of a cross peak between H-11α and H-13 in the NOESY spectrum. Connectivities of the signal at δ4.66 (1H, d, +D_2O→s, H-15) with δ79.1 (C_{16}), δ51.9 (C_9), and δ28.7 (C_{14}) were observed. Furthermore, intense interaction between this signal (δ4.66) and H-14 in the NOESY spectrum demonstrated that lassiocarpine had C_{15}-β-OH just as common C_{20}-diterpene alkaloids. The cross peaks[5] from the δ4.83 (H-1) to δ51.9 (C_{10}) and δ68.3 (C_{20}) and the coupling constants of this proton (δ4.83, dd, J=10.9 and 5.7Hz) showed the presence of an α-hydroxy group on C_1. One of the signal [δ5.63 (J=11.4Hz)] of AB type resonance gave the cross peaks with δ45.5 (C_{12}) and δ79.1 (C_{16}), and the other signal [δ5.77 (J=11.4Hz)] of this AB type resonance had the connectivity with the ester-carbonic resonance at δ167.4. This reveals that primary alcohol at C_{17} exists as a benzoyl ester. The remaining tertiary carbon with alcohol function was unambiguously assigned through the observation of the connectivities of the resonance at δ79.1 (C_{16}) to H-15 (δ4.66), H-17 (δ5.63), and H-11 (δ5.53). The NOE cross peak between H-17 and H-9 established the stereochemistry at C_{16} position[6] as illustrated in the Fig. Another key two- and three-bond heteronuclear connectivities also substantiated the structure of **1** as follows:



NOESY Experiment of Lassiocarpine (1)



Heteronuclear Multiple-Bond Correlation Spectrum (high-field region) of 1 in Py-d_5

Table
^{13}C(100MHz) and ^{1}H(400MHz) NMR spectral data for **1** in Py-d_5

atom	^{13}C(mult)	^{1}H(mult, J(Hz), intgrtn)
1	70.4(d)	4.83(dd, 10.9, 5.7, 1H)
2	32.0(t)	2.85(m, 1H)
		2.00(m, 1H)
3	39.4(t)	1.59(d-like, 12.2, 1H)
		1.38(m, 1H)
4	33.9(s)	
5	53.6(d)	1.53(d, 7.3, 1H)
6	24.1(t)	3.61(dd, 7.3, 13.6, 1H)
		1.37(m, 1H)
7	43.2(d)	2.28(s-like, 1H)
8	44.1(s)	
9	51.9(d)	2.53(d, 8.9, 1H)
10	51.9(s)	
11	71.6(d)	5.53(d, 8.9, 1H)
12	45.5(d)	2.65(br-s, 1H)
13	22.0(t)	1.76(t-like, 12.1, 1H)
		2.62(m, 1H)
14	28.7(t)	2.28(m, 1H)
		1.37(m, 1H)
15	86.2(d)	4.66(d, 3.4, 1H)
16	79.1(s)	
17	72.3(t)	5.77(d, 11.4, 1H)
		5.63(d, 11.4, 1H)
18	26.5(q)	0.72(s, 3H)
19	57.5(t)	2.27(d, 10.9, 1H)
		2.56(1H)
20	68.3(d)	4.15(s, 1H)
NCH2	51.1(t)	2.39(m, 1H)
|		2.55(m, 1H)
CH3	14.0(q)	1.05(t, 7.3, 3H)
C=O	167.3(s)	
|		
Ph	132.1(s)	
	130.0(d)	8.26(d, 7.6, 2H)
	128.5(d)	7.25(dd, 7.6, 7.6, 2H)
	132.6(d)	7.42(dd, 7.6, 7.6, 1H)

H-6β (δ3.61) to C_4, C_7, C_8 and C_{20}; H3-18 (δ0.72) to C_3, C_4, C_5 and C_{19}; H-20 (δ4.15) to C_{10}(C_9) and C_5; H-5 (δ1.53) to C_{18}, C_7, C_9 (C_{10}), C_{19} and C_{20}; N-$C\underline{H}_2CH_3$ (δ2.39) to N-$CH_2\underline{C}H_3$, C_{19} and C_{20}; 12-H (δ2.65) to C_{14}, C_{11} and C_{15}.

It is interesting to note that biogenetically lassiocarpine (1) would be generated from the ring-opening of the epoxy precursors such as gomandonine[7] or yesoxine,[8] and subsequent esterification.

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References and Note

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5) In the HMBC chart inserted in this text, these two cross peaks were not observed. But by the decrement of the thresh-hold value they appeared clearly in the chart.
6) C_{15}-C_{16} glycol system of **1** resisted to the acetonide formation under customary condition. This also indicates that C_{16}-OH group is to be *trans* to C_{15}-OH group.
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