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The Structure of Abscisterol A: A Novel 21-Nor-$\Delta^{17(20)}$-unsaturated Sterol from *Cryptosporiopsis abietina*

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Abstract: The structure of abscisterol A (1), isolated from *C.abietina*, has been determined as 21-nor-24-methylene-5α-cholesta-7,17(20)-*E*-diene-4β,19-epoxy-3β-ol from a series of 1D and 2D NMR experiments and an advanced Mosher method.

The resinous stem canker of *Chamaecyparis obtusa* Sieb. et Zucc., which has long been well-known among Japanese foresters but whose cause was unknown, and recently Kobayashi *et. al.*[1] reported that *Cryptosporiopsis abietina* causes this disease. We isolated some sterols which indicate the abscisic activity against *C. obtusa* leaves[2], from the EtOAc extract of acetone soluble fraction of *C. abietina* mycelium. In this communication, we report the structure elucidation of the main component abscisterol A (**1**). Though a few naturally occurring $\Delta^{17(20)}$-unsaturated sterols have been described[3], all of them possess 21-carbon. Abscisterol A is the first report of nor-21-$\Delta^{17(20)}$-sterol from nature.

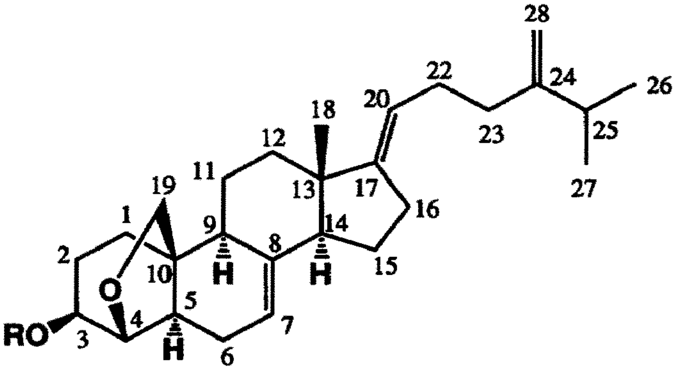

Fig. 1 R=H: Abscisterol A (**1**), R=Ac: **2**

Abscisterol A (**1**)(80.7 mg from 46.5 l of the culture) was obtained as a white amorphous powder, mp 153-156°, $[\alpha]_D^{22}$ -3.3° (*c* 1.1, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1} 3500-3200(-OH), 2900(CH), 1095,1055 (-C-O-C-). The molecular formula of **1** was established as $C_{27}H_{40}O_2$ by EI-HR-MS(m/z 396.3006; calcd. 396.3028). The ^{13}C NMR spectrum of **1** showed 26 signals and the signal ($\delta^{13}C$ 22.0) correlated with 6H doublet (δ^1H 1.05) in the ^{13}C-^1H COSY spectrum, so the carbon signal was assigned as two equivalent methyl groups(C-26, 27). The characteristics of carbon signals were defined by DEPT experiments, indicating presence of three methyls, eleven methylenes, eight methines, and five quaternary carbons. Based on eight degrees of unsaturation and six sp^2 carbons ($\delta^{13}C$ 155.6s, 151.5s, 137.2s, 117.3d, 116.6d, and 107.0t), **1** has three double bonds and five ring system.

Four partial structures shown in Fig. 2 were elucidated by the detailed analysis of ^1H-^1H COSY spectrum. The long range coupling(J=1.5 Hz) was observed between one of the methylene protons(δ^1H 3.84:

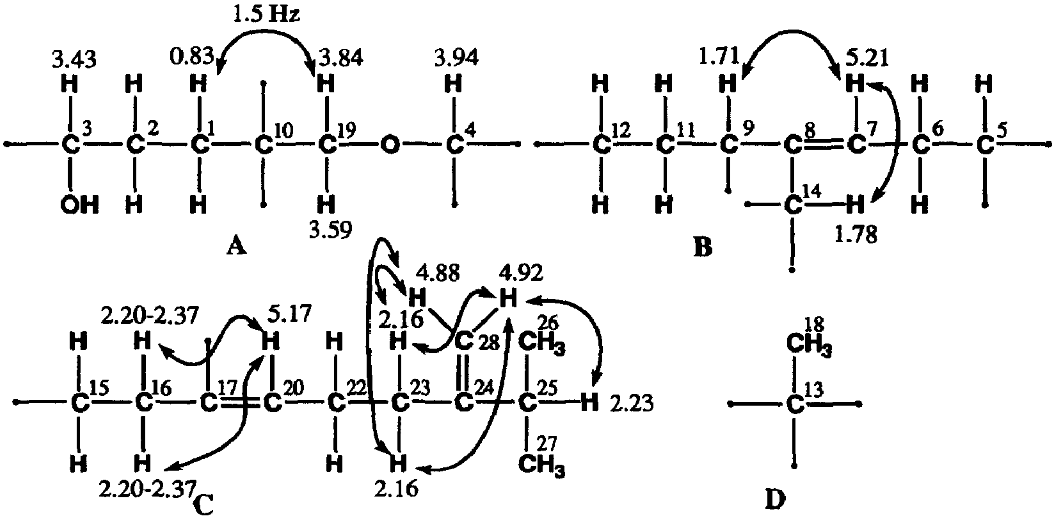

Fig. 2 Partial structures of abscisterol A (1). Arrows indicate long range ^1H-^1H couplings.

Table 1 ^{13}C(125 MHz) and ^1H(500 MHz) NMR spectral data for abscisterol A (1)[a] in C_6D_6

C#	$\delta^{13}C$	δ^1H (*J* Hz)	C#	$\delta^{13}C$	δ^1H (*J* Hz)
1	35.0t	α0.83	13	45.4s	
		β1.50-1.64	14	53.6d	1.78
2	30.4t	α1.94	15	23.2t	1.50-1.64
		β1.50-1.64	16	26.6t	2.20-2.37
3	72.5d	3.43	17	151.5s	
4	87.1d	3.94	18	19.1q	0.64
5	43.98d	1.22(dd 6.8 10)	19	72.4t	H_R 3.59(d 7.8)
6	26.0t	α1.88			H_S 3.84(dd 7.8 1.5)
		β2.05	20	117.3d	5.17
7	116.6d	5.21	21	-	-
8	137.2s		22	34.2t	2.17-2.29
9	42.5d	1.71	23	27.8t	2.16
10	43.99s		24	155.6s	
11	25.3t	α1.50-1.64	25	34.6d	2.23
		β0.89	26 27	22.0q	1.05(d 6.8)
12	36.6t	α1.30(td 13.1 3.8)	28	107.0t	H_Z 4.92(d <1)
		β1.76			H_E 4.88(d <1)

[a] Assignments were based on DEPT, ^1H-^1H COSY, ^{13}C-^1H COSY, COLOC, selected INEPT, and NOESY experiments.

H_S-19) and one of the other methylene protons(δ^1H 0.83: H_α-1) in the partial structure A. Allylic couplings between the olefinic methine proton(δ^1H 5.21: H-7) and two methine protons(δ^1H 1.71: H-9, 1.78: H-14) were detected in the partial structure B. Similarly allylic couplings were detected between each pair of follows, the olefinic methine proton(δ^1H 5.17: H-20) and the methylene protons(δ^1H 2.20-2.37: H-16), the olefinic methylene protons(δ^1H 4.92: H_Z-28, 4.88: H_E-28) and the methylene protons(δ^1H2.16: H-23), and one of the olefinic methylene protons(δ^1H 4.92: H_Z-28) and the methine proton(δ^1H 2.23: H-25), in the partial structure C. The angular methyl proton(δ^1H 0.64: H-18) indicated that presence of the partial structure D. The ^{13}C- ^{1}H COSY spectrum exhibited three oxygenated carbons, one methylene and two methines, so one of two oxygens in the molecular formula of **1** is ascribable to OH group, and the another is ether bond. The position of OH group was determined by the ^{1}H NMR of the mono acetate **2**[4] of **1**, in which the C-3 methine signal(δ^1H 3.43, δ^{13}C 72.5) showed down-field shift(δ^1H 4.80) and sharpening. So the remaining oxygenated methylene(δ^1H 3.84, 3.59, δ^{13}C 72.4) and the methine(δ^1H 3.94, δ^{13}C 87.1) connects via ether bond.

Connectivities of four partial structures were established by COLOC and selective INEPT[5] experiments. In the COLOC, the 18-Me-protons(δ^1H 0.64) have long range heteronuclear couplings with C-12, C-13, C-14 and C-17, and the H-14 methine proton(δ^1H 1.78) correlated with C-15, so the connectivity of partial structure B, C and D were revealed. The H-4 methine proton(δ^1H 3.94) have long range heteronuclear couplings with C-3, C-6 and C-19, and the H-5 methine proton(δ^1H 1.22) correlated with C-3 and C-6, so connectivity of partial structures A and B were revealed. Since the connections of C-5-10-9 cannot be established clearly by 2D NMR experiments because of very close chemical shifts of C-5(δ^{13}C 43.98) and C-10(43.99), these connectivities were established by selective INEPT experiments. Thus, selective INEPT irradiation of H-5 methine proton(δ^1H 1.22) enhanced the signals C-1, C-10, C-19 and C-4, and irradiation of H-9 methine proton(δ^1H 1.71) enhanced the signals C-10 and C-19, so the structure of **1** was determined as shown in Fig. 1.

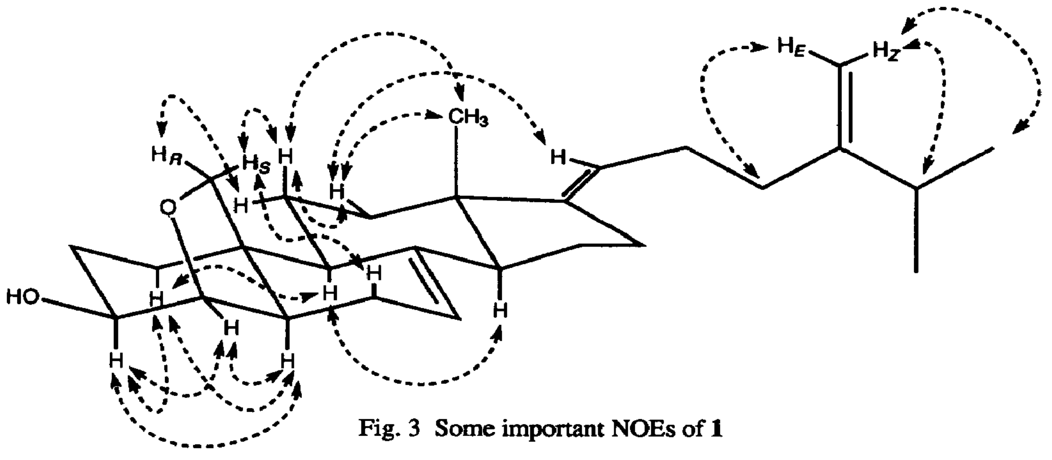

Fig. 3 Some important NOEs of **1**

Additionally some important NOEs were observed by NOESY experiment as shown in Fig. 3. The assignments of H_R-, H_S-19, and H_E-,H_Z-28 were based on this results. The geometry of $\Delta^{17(20)}$ double bond was determined as *E* based on the NOE between H-20 and Hβ-12. Relative configurations were also determined as 3β-OH, 4β,19-epoxy, 5α, 9α, and 14α protons. The absolute configuration of C-3 was established by an advanced Mosher method[6] as *S*(results were shown in Fig. 4). Further, the stereochemistry was confirmed by CD spectrum with 3-oxoabscisterol A (**3**)[7] which indicated positive cotton effect at 293 nm. So the structure of abscisterol A (**1**) was determined as 21-nor-24-methylene-5α-cholesta-7,17(20)-*E*-diene-4β,19-epoxy-3β-ol.

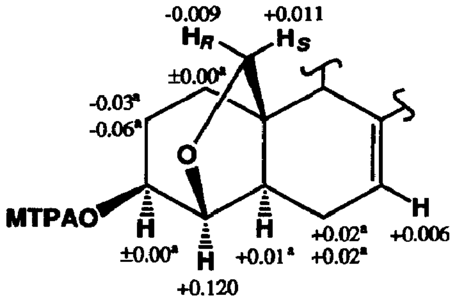

Fig. 4 Δδ values (δ_S-δ_R) were shown in ppm ($CDCl_3$). ^{a}assigned by ^1H-^1H COSY

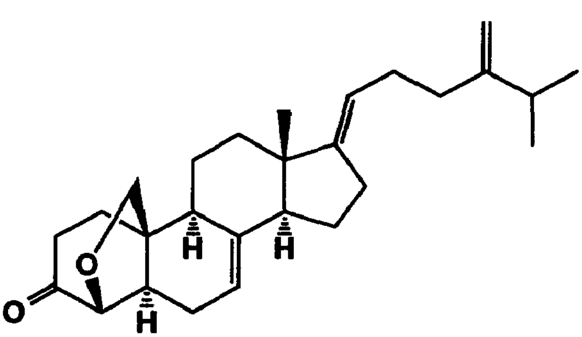

Fig. 5 3-oxoabscisterol A (3)

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2. The abscisic activity was measured as follows: Two cuttings of *C. obtusa* leaves(*c.a.* 2.5 cm, include one or two branches) put into the petri dish which containig 1 ml of test solution, and incubate 7 days at 25°C, 10,000 lux condition. 1 indicated abscisic activity against *C. obtusa* leaves at 10 ppm.
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4. 2: ^1H NMR(C_6D_6, 500 Hz): δ 0.63(3H, s, H-18), 0.80-0.88(2H, m, H_α-1 H_β-11), 1.05(6H,d, *J*=6.8, H-26 27), 1.29(1H, m, H_α-12), 1.32(1H, dd, *J*=6.8 10, H-5), 1.50-1.63(6H, m, H_β-1 H_β-2 H-11 15), 1.68(1H, m, H-9), 1.75(2H, m, H_β-12 H-14), 1.77(3H, s, $C\underline{H}_3$-CO-), 1.85-1.95(2H, m, H_α-2 H_α-6), 2.03(1H, m, H_β-6), 2.15-2.36(7H, m, H-15 16 22 23 25), 3.62(1H, d, *J*=7.8, H_R-19), 3.82(1H, dd, *J*=7.8 1.8, H_S-19), 4.02(1H, s, H-4), 4.80(1H, dd, *J*=8.5 6.7, H-3), 4.89(1H, *J*=1.2, H_E-28), 4.93(1H, d, *J*=1.2, H_Z-28), 5.15-5.20(2H, m, H-7 20)
5. A. Bax, *J. Magn. Reson.*, **1984**, *57*, 314-318.
6. I. Ohtani, T. Kusumi, M. O. Ishitsuka, and H. Kakisawa, *Tetrahedron Lett.*, **1989**, *30*, 3147-3150.
7. 3 was prepared from 1 by Jones oxidation. 3: EI-HR-MS calcd. for $C_{27}H_{38}O_2$ 394.2872, found 394.2910, CD(MeOH) λ_{ext} 314 nm($\Delta\epsilon$ 0.0), 293(+3.0), 266(0.0), 259(-0.11), ^{13}C NMR($CDCl_3$, 125MHz): δ 18.9(C-18), 21.7(C-26 27), 22.8(C-15), 25.47^a(C-11), 25.49^a(C-6), 26.2(C-16), 27.3(C-23), 33.8(C-22), 34.2(C-25), 35.7(C-1), 36.1^b(C-12), 36.4^b(C-2), 42.2(C-9), 45.1^c(C-5), 45.3^c(C-10), 47.2(C-13), 53.1(C-14), 72.5(C-19), 87.3(C-4), 106.3(C-28), 115.4(C-7), 117.1(C-20), 137.3(C-8), 150.8(C-17), 155.8(C-24), 210.9(C-3) ^{a,b,c}values are interchangeable

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