New Methoxytriterpene Dione from the Cuticle of *Picea jezoensis* var. *jezoensis*

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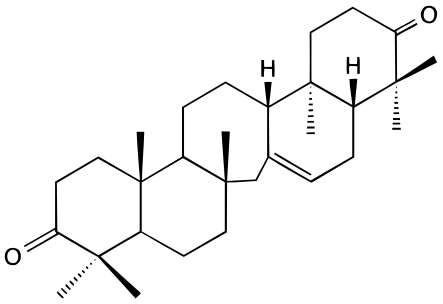
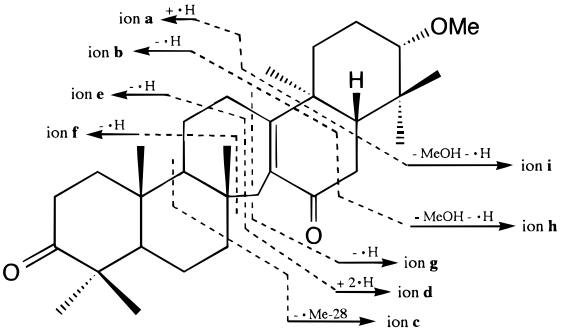
Received August 30, 1996[⊗]

A novel pentacyclic triterpene dione was isolated from the cuticle of *Picea jezoensis* var. *jezoensis* together with the known serrat-14-ene-3,21-dione (**1**), and the structure of this compound was determined as 21α-methoxyserrat-13-ene-3,15-dione (**2**). Detailed NOESY experiments revealed that **2** has a chair form of ring A and a chairlike conformation of ring C, respectively, in $CDCl_3$ solution. Interestingly, single-crystal X-ray analysis indicates that in the solid state **2** has a deformed boat form of ring A, in which the 3-oxo and the 25-methyl groups are arranged in flag-pole positions, and a chairlike form of ring C.

Previously, we reported that the CH_2Cl_2 extract of the cuticle of *Picea jezoensis* (Sieb. et Zucc.) Carr. var. *jezoensis* Mayr. (Pinaceae) contained 21α-hydroxy-3*β*-methoxyserrat-14-en-30-al, 14*β*,15*β*-epoxy-3*β*-methoxyserrat-14-en-21-one, and 14*β*,15*β*-epoxy-3*β*-methoxyserrat-14-en-21*β*-ol, together with eight known triterpenoids.[1,2] Further work on other constituents of the cuticle has now led to the isolation of a new unsaturated triterpene dione (**2**), along with the known compound, serrat-14-ene-3,21-dione (**1**).[3−5] Herein, we describe the structure of **2**.



1



2

The known compound was confirmed as serrat-14-ene-3,21-dione (**1**), which had been synthesized from serratenediol[3] and later isolated from the bark of *Pinus luchuensis* Mayer.[4] Physical and spectral data of **1** were in good agreement with those already reported, while the ^{13}C-NMR spectrum is reported here for the first time. Detailed assignments for the ^{13}C-NMR signals are given in Table 1, together with the ^{1}H-NMR signals.

Compound **2** was assigned the molecular formula of $C_{31}H_{48}O_3$ by HREIMS. The UV and IR spectra exhibited absorption bands characteristic for an α,*β*-unsaturated ketone and a saturated six-membered ring ketone (λ_{max} 230 and 282 nm; ν_{max} 1654 and 1703 cm^{-1}). In the ^{1}H- and ^{13}C-NMR spectra (Table 1), **2** exhibited signals for seven quaternary methyl groups, two methylene groups [δ_H 2.36−2.48 (4H)] vicinal to two different carbonyl carbons [δ_C 199.0 (s) and 217.9 (s)], and an equatorially oriented secondary methoxy group [δ_H 2.76 (1H, dd, J = 11.8, 4.0 Hz, H-21*β*) and 3.39 (3H, s), δ_C 57.6 (q) and 87.4 (d)]. In spite of the absence of any olefin proton signals, **2** showed ^{13}C-NMR signals for two sp^2 quaternary carbons at δ_C 133.0 and 169.8. Thus, it became obvious that **2** must have a tetrasubstituted double bond conjugated to one of two keto groups in the molecule. In addition, the ^{1}H-NMR signals appeared as an AB system at rather low magnetic field at δ_H 1.72 and 2.81 (each 1H, d, J = 14.5 Hz). These signals were attributable to the isolated 27-methylene group in the serratene skeleton.[6] Analysis of all these data suggested that the structure of **2** was an unknown methoxyserratene dione including either the chromophore of a Δ^{13}-en-12-one or a Δ^{13}-en-15-one. The ^{1}H−^{1}H COSY, ^{1}H−^{13}C COSY and COLOC experiments performed on **2** supported the latter structure. The COLOC data provided cross-correlations as shown in Table 1. These correlations indicated **2** to have a gross carbon framework that was presumed to be that of 21-methoxyserrat-13-ene with attached keto groups at C-3 and C-15.

Compound **2** showed nine significant fragment peaks corresponding to ions **a**−**i** between 315 and 121 atomic mass units in its EIMS, along with the parent ion peak (see Experimental Section). Of these, ions **a**−**d** and **g** are characteristic for the fragmentation of a serrat-13-ene skeleton bearing a keto group at C-15. Together with the presence of an equatorial secondary methoxy proton signal, these data indicated compound **2** to be 21α-methoxyserrat-13-ene-3,15-dione.

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[⊗] Abstract published in *Advance ACS Abstracts,* March 1, 1997.

S0163-3864(96)00604-0 CCC: $14.00

Table 1. NMR Data for Compounds **1** and **2**[a]

	δ_H		δ_C		
position	**1**	**2**	**1**	**2**	COLOC data for **2** (C to H)
1	1.49 m	1.54α m	39.5 t	39.2 t	H-5α, H-9α, Me-25
	2.03 m	1.95β m			
2	2.46 m	2.47α m	34.1 t	33.9 t	
	2.48 m	2.48β m			
3			218.2 s	217.9 s	H-5α, Me-23, Me-24
4			47.3 s	47.3 s	Me-23, Me-24
5	1.42 dd (13.8, 2.5)	1.53α m	55.2 d	54.9 d	Me-23, Me-24, Me-25
6	1.46 m	1.48α m	20.1 t	20.1 t	
	1.57 m	1.54β m			
7	1.26 m	1.36α td (12.5, 3.9)	44.2 t	43.0 t	H-5α, H-9α, Me-26
	1.42 m	1.63β m			
8			37.0 s	34.9 s	Me-26
9	0.93 br s	1.14α br s	62.1 d	63.8 d	H-1, H-5α, H-7, H-27, Me-25, Me-26
10			37.8 s	37.9 s	H-11, Me-25
11	1.12 m	1.81β m	25.5 t	20.6 t	
	1.76 m	2.07α m			
12	1.96 m	2.58α dd (14.0, 8.2)	27.7 t	29.7 t	H-9α
	1.22 m	2.06β m			
13	1.81 dd (15.1, 1.3)		56.5 d	169.8 s	H-11, H-17β, H-27, Me-28
14			137.9 s	133.0 s	H-12
15	5.40 dif. t (2.9)		122.3 d	199.0 s	H-17β
16	2.02 m	2.36α dd (12.7, 14.2)	24.5 t	34.6 t	
	2.06 m	2.49β dd (14.2, 3.5)			
17	1.67 dd (12.5, 3.4)	1.63β dd (12.7, 3.5)	51.2 d	50.1 d	Me-28, Me-29, Me-30
18			36.2 s	40.4 s	Me-28
19	1.43 m	1.44β m	38.4 t	34.1 t	H-17β, Me-28
	2.17 ddd (12.9, 5.3, 3.8)	2.03α dt (14.8, 4.9)			
20	2.46 dt (14.2, 3.8)	1.59β m	34.8 t	22.3 t	
	2.76 td (14.2, 5.3)	1.99α m			
21		2.76β dd (11.8, 4.0)	216.9 s	87.4 d	Me-29, Me-30, OMe
22			47.7 s	38.5 s	Me-29, Me-30
23	1.08 s	1.09 s	26.9 q	26.9 q	H-5α, Me-24
24	1.04 s	1.03 s	21.0 q	20.3 q	H-5α, Me-23
25	0.89 s	0.83 s	15.8 q	16.2 q	H-1, H-5α
26	0.87 s	0.69 s	19.3 q	18.7 q	H-7, H-9α, H-27
27	1.84 d (14.6)	1.72α d (14.5)	55.8 t	40.6 t	H-7, H-9α, Me-26
	2.26 d (14.6)	2.81β d (14.5)			
28	0.93 s	1.04 s	13.0 q	17.1 q	H-17β, H-19
29	1.05 s	0.86 s	24.5 q	27.6 q	H-17β, H-21β, Me-30
30	1.09 s	1.00 s	24.5 q	27.6 q	H-17β, H-21β, Me-29
OMe		3.39 s		57.6 q	H 21β

[a] The ^{1}H- and ^{13}C-NMR correlations were based on HETCOR spectra.

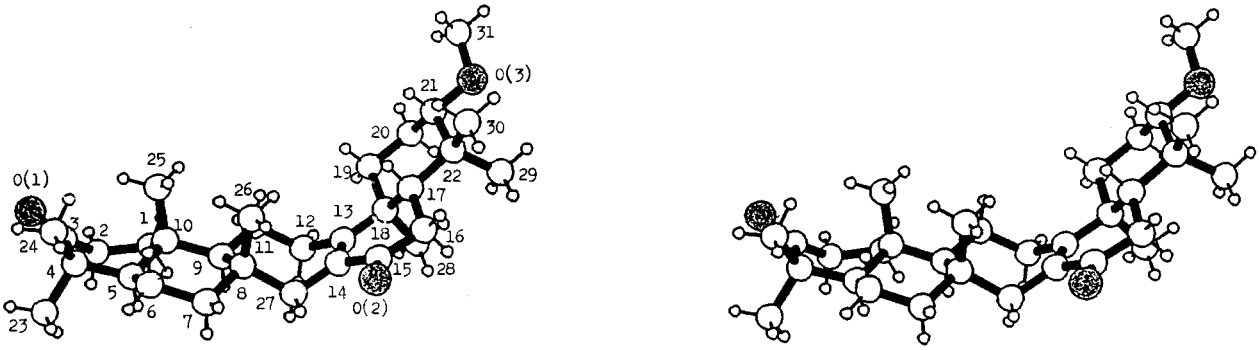

Figure 1. ORTEP diagram of **2**.

Previous workers have examined the CD spectrum and single-crystal X-ray analytical data for serrat-14-ene-3,21-dione (**1**) and reported this molecule to have a deformed boat form of ring A and a chairlike conformation of ring C.[5] Therefore, compound **2** provided a stereochemical uncertainty that needed to be resolved, inasmuch as it contains a Δ^{13}-en-15-one grouping and the same 4,4-dimethyl-3-one system as **1** in the molecule. The single-crystal X-ray analysis furnished an ORTEP diagram for the structure of **2** (Figure 1), in which rings A and C adopt a deformed boat conformation so as to keep the 3-oxo and the 25-methyl groups on the flag-pole positions of the ring and a chairlike form, respectively, when in the solid state. Table 2 shows the list of nonhydrogen atom fractional coordinates for **2**.

On the other hand, the NOESY spectrum of **2** (Figure 2) exhibited correlations for the signals of Me-26 (with H-6β, H-7β, H-11β, and H-27β), H-7α (with H-27α), H-7β (with H-27β), Me-28 (with H-12α, H-16α, and H-20α), H-21β (with H-19β and Me-30), Me-29 (with H-16α and Me-28), Me-30 (with H-17β and H-21β), H-9α (with H-12α), and H-17β (with H-21β and Me-30). These data indicated that ring C of **2** is *trans*-fused with ring B to adopt a chairlike conformation. More significantly, cross interactions were observed for the signals of Me-

Table 2. Non-Hydrogen Atom Fractional Coordinates for Compound **2** (Esd's in parentheses)

atom	*x*	*y*	*z*	*U* (eq)
O(1)	0.1346(7)	0.6121(2)	0.3404(8)	0.105(4)
O(2)	0.0303(5)	0.8425(2)	1.2493(6)	0.088(3)
O(3)	−0.0093(4)	1.0014(1)	0.5813(7)	0.077(3)
C(1)	0.2746(5)	0.7031(2)	0.5077(8)	0.053(3)
C(2)	0.2870(7)	0.6582(2)	0.440(1)	0.071(4)
C(3)	0.1795(7)	0.6314(2)	0.463(1)	0.070(4)
C(4)	0.1275(6)	0.6293(2)	0.6507(9)	0.059(3)
C(5)	0.1573(5)	0.6704(2)	0.7532(7)	0.050(3)
C(6)	0.0732(6)	0.6766(2)	0.9103(8)	0.061(3)
C(7)	0.1116(6)	0.7140(2)	1.0193(7)	0.058(3)
C(8)	0.1134(4)	0.7554(2)	0.9160(6)	0.046(3)
C(9)	0.1950(4)	0.7487(2)	0.7519(6)	0.044(3)
C(10)	0.1657(4)	0.7097(2)	0.6342(6)	0.043(2)
C(11)	0.2147(4)	0.7885(2)	0.6422(7)	0.046(3)
C(12)	0.2819(4)	0.8237(2)	0.7386(8)	0.053(3)
C(13)	0.2012(4)	0.8512(2)	0.8526(8)	0.052(3)
C(14)	0.1522(5)	0.8331(2)	0.9969(7)	0.052(3)
C(15)	0.0773(5)	0.8585(2)	1.1191(8)	0.062(3)
C(16)	0.0632(7)	0.9033(2)	1.0785(9)	0.068(4)
C(17)	0.0637(5)	0.9116(2)	0.8821(8)	0.053(3)
C(18)	0.1846(5)	0.8963(2)	0.8017(7)	0.051(3)
C(19)	0.1725(6)	0.9018(2)	0.5978(8)	0.059(3)
C(20)	0.1390(5)	0.9459(2)	0.5492(8)	0.064(3)
C(21)	0.0203(6)	0.9596(2)	0.6293(9)	0.058(3)
C(22)	0.0181(6)	0.9563(2)	0.8300(8)	0.059(3)
C(23)	−0.0066(7)	0.6172(2)	0.644(1)	0.076(4)
C(24)	0.1967(7)	0.5926(2)	0.740(1)	0.087(5)
C(25)	0.0517(5)	0.7161(2)	0.5235(7)	0.049(3)
C(26)	−0.0152(5)	0.7698(2)	0.8762(7)	0.055(3)
C(27)	0.1710(5)	0.7888(2)	1.0422(7)	0.054(3)
C(28)	0.3000(5)	0.9190(2)	0.865(1)	0.071(4)
C(29)	0.0918(7)	0.9919(2)	0.912(1)	0.083(5)
C(30)	−0.1146(7)	0.9620(2)	0.892(1)	0.081(5)
C(31)	−0.0626(8)	1.0059(3)	0.414(1)	0.089(5)

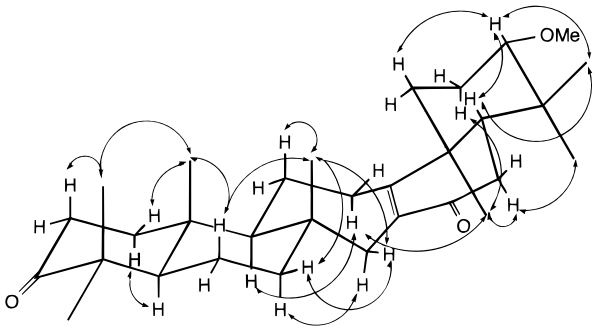

Figure 2. NOESY correlations of compound **2**.

23 (with H-5α), Me-24 (with H-2*β*), Me-25 (with H-1*β*, H-2*β*, and H-11*β*), and H-1α (with H-5α). Contrary to our expectation, the above results indicated that ring A of compound **2** was *trans*-fused with ring B and adopts a chair form in $CDCl_3$, as Me-25 showed extremely clear NOESY enhancements with Me-24 and H-2*β*. In four methylene proton signals appearing between *δ* 2.33 and 2.51, those resonating at *δ* 2.36 (1H, dd, J = 12.7, 14.2 Hz) and 2.49 (1H, dd, J = 14.2, 3.5 Hz) were assigned to H-16α and H-16*β*, respectively, on the basis of the $^1H-^1H$ COSY and $^1H-^{13}C$ COSY spectra. Similarly, the other two methylene proton resonances were observed at *δ* 2.47 and 2.48 (each 1H, m) and attributed to H-2α and H-2*β*, respectively. In addition, methylene signals observed at *δ* 2.58 (1H, dd, J = 14.0 and 8.2 Hz) and 2.06 (1H, m) were assigned, in turn, to H-12α and H-12*β*. A methine proton at *δ* 1.63 (1H, dd, J = 12.7, 3.5 Hz) was assigned to H-17*β*. Although an attempt to prove the exact conformation by analyzing the coupling constants of the H-2α and H-2*β* signals was unsuccessful (because these signals overlapped each other along with those of H-16*β*), the *δ* values and signal patterns of H-2α, H-2*β*, and H-17*β* for **2** were closely similar when compared with those of **1**. Concerning rings A and B, **1** showed the same NOESY correlations as those of **2**, as mentioned above. Hence, **1** must also have a chair form of ring A in $CDCl_3$.

The above results demonstrate that in compounds **1** and **2** ring A changes its conformation according to whether it is in the solid state or in solution. Therefore, in the former state it adopts a defomed boat form, whereas it takes a chair form in the latter state.

Experimental Section

General Experimental Procedures. Melting points were determined on a Ishii hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ using a JASCO DIP-140 digital polarimeter. UV spectra were recorded in EtOH on a Hitachi model 150-20 spectrophotometer. IR spectra were run as KBr disks on a Perkin-Elmer 1720X FTIR spectrophotometer. ^1H- and ^{13}C-NMR spectra were obtained on a JEOL GX-500 spectrometer with standard sequences operating at 500 MHz and 125 MHz, respectively. $CDCl_3$ was used as solvent and TMS as internal standard. All ^{13}C-NMR assignments were based on DEPTGL, 2D $^1H-^1H$ COSY, $^1H-^{13}C$ COSY, COLOC, and NOESY experiments. EIMS and HREIMS were recorded on a Hitachi 4000H double-focusing mass spectrometer (70 eV). Column chromatography was carried out on Si gel (70−230 mesh, Merck). Fractions obtained from column chromatography were monitored by TLC (Si gel 60 HF_{254}, 0.25-mm thick) with visualization under UV (254 and 365 nm). Preparative TLC was carried out on Merck Si gel PF_{254} plates (0.5-mm thick).

Extraction and Isolation. We have already reported that repeated column chromatography of residue **F**, one of six fractions separated from the CH_2Cl_2 extract (365.1 g) of the cuticle of *Picea jezoensis* (Sieb. et Zucc.) Carr. var. *jezoensis* Mayr. (6.0 kg) by preliminary Si gel column chromatography, afforded 14*β*,15*β*-epoxy-3*β*-methoxyserratan-21-one (126 mg) and its 21*β*-ol (107 mg) from the fractions eluted with $CHCl_3$ (fractions 39−42, each fraction: 100 mL) and $CHCl_3$−EtOAc (20:1, fractions 63−67), respectively.[2] At this stage, we had also collected 105 mg of an unexamined yellow gum showing two spots on TLC from the intermediary fractions of 43−61 eluted with $CHCl_3$ in the above separation. Further chromatography of the gummy product on 10% $AgNO_3$-impregnated Si gel (10 g) using *n*-hexane−C_6H_6 (1:1) afforded a crystalline mass from fractions 15−21 (each fraction, 20 mL), which was purified by preparative TLC (solvent, $CHCl_3$−MeOH, 100:1) to afford compound **2** (13 mg). Subsequent column chromatography with the same solvent furnished a crystalline solid from fractions 29−56, which was purified by preparative TLC to give compound **1**: 40 mg, mp 204−206 °C (MeOH−$CHCl_3$), $[\alpha]^{23}_D$ −3.7° (*c* 0.43) [lit.[3] mp 208−210 °C, $[\alpha]_D$ −3.7°]; IR (KBr) ν_{max} 1714 (>C=O), 1637 and 807 (>C=CH−) cm^{-1}; ^1H- and ^{13}C-NMR data, see Table 1; EIMS (70 eV) m/z $[M]^+$ 438, 423, 232, 218, 205, and 203. Physical and spectral data of **1** were in good agreement with those already published for serrat-14-ene-3,21-dione.[3,4]

21α-Methoxyserrat-13-ene-3,15-dione (2): mp 317−319 °C (MeOH−$CHCl_3$); $[\alpha]^{23}_D$ +81.3° (*c* 0.28); HREIMS *m/z* 468.3602 $[M]^+$ ($C_{31}H_{48}O_3$ requires 468.3603); UV (EtOH) λ_{max} (ϵ) 230 (4300) and 282 (7000) nm (C=O and >C=C−C=O); IR (KBr) ν_{max} 2960, 2930, 2850, 1703 (C=O), 1654 (>C=C−C=O), 1465, 1455, 1419 (−CH_2−CO), 1384 and 1363 (*gem*-dimethyl), 1179, 1149, 1106, 1033, 1010, 996, and 982 cm^{-1}; ^1H- and ^{13}C-NMR data, see Table 1; EIMS (70 eV) *m/z* $[M]^+$ 468 (100), [M − Me]$^+$ 453 (8), 439 (29), [M − MeOH]$^+$ 436 (4), [M − Me − MeOH]$^+$ 421 (5), [ion **a**] 315.2320 $[C_{21}H_{31}O_2]^+$ (99), [ion **b**] 299 (4), [ion **c**] 287.2004 $[C_{19}H_{27}O_2]^+$ (19), [ion **d**] 250.1927 $[C_{16}H_{26}O_2]^+$ (100), [ion **e**] 219 (11), [ion **f**] 205 (15), [ion **g**] 203 (15), [ion **h**] 135 (20), [ion **i**] 121 (25).

X-ray Crystallography of Compound 2. A single crystal of **2** was obtained by recrystallization from a mixture of MeOH and $CHCl_3$. Crystal data: $C_{31}H_{48}O_3$, MW 468.72, orthorhombic, space group $P2_12_12_1$, a = 11.067(2) Å, b = 31.969(2) Å, c = 7.586(1) Å, V = 2683.8(5) Å^3, D_x = 1.160 g cm^{-3}, Z = 4. A total of 2459 independent reflection intensities up to $2\theta = 130°$ were measured on a Rigaku four-circle diffractometer with graphite-monochromated CuKα radiation. A total of 1919 reflections with $F_0 > 3\sigma F_0$ were used for the structure analysis by direct method. The non-hydrogen atoms were refined anisotropically by block-diagonal least-squares on a Micro-Vax computer at the Computer Center of Osaka University of Pharmaceutical Sciences. The geometrically ideal positions of H-atoms were calculated in the final refinement with isotropic thermal parameters; their electronic densities were ascertained on a difference Fourier map. The structure of compound **2** was finally refined to R = 0.059 (R_w = 0.085). The atomic scattering factors and anomalous scattering corrections were taken from *International Tables for X-ray Crystallography*.[7] All the crystallographic calculations were performed by the use of a CRYSTAN GM software package.[8] The final non-hydrogen atom fractional coordinates of **2** are listed in Table 2.[9]

Acknowledgments. This work was supported by the Section of Guidance and Spread, National Osaka Forestry Bureau, 1-8-75 Temmabashi, Kita-Ku, Osaka 530, Japan, for which the authors are deeply grateful. Our thanks are also due to Mrs. M. Fujitake of Osaka University of Pharmaceutical Sciences for MS measurements.

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(9) Atomic coordinates, thermal parameters, bond distance and angles observed and calculated structure factors of **2** have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, 12 Union Road, Cambridge CB2 1EZ, UK.

NP960604+