Two New Epoxyserratanes from the Cuticle of *Picea jezoensis*

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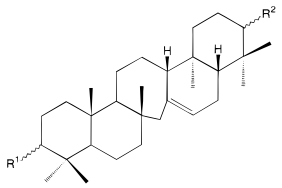
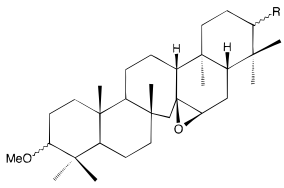
Two new saturated serratane triterpenes were isolated, together with the known compounds 21β-methoxyserrat-14-en-3-one (**1**) and 3β-methoxyserrat-14-en-21-one (**2**), from the cuticle of *Picea jezoensis* Carr. *jezoensis*. Their structures were established as 14β,15β-epoxy-3β-methoxyserratan-21-one (**3**) and 14β,15β-epoxy-3β-methoxyserratan-21β-ol (**4**) on the basis of chemical and spectral evidence.

Recently, we reported that the cuticle of *Picea jezoensis* (Sieb et Zucc.) Carr. *jezoensis* (Mayr.) (Pinaceae) contained 21α-hydroxy-3β-methoxyserrat-14-en-30-al and six known serratene triterpenes.[1] Further investigation of the CH_2Cl_2 extract of the cuticle led to the isolation of two novel exoxyserratanes, **3** and **4**, together with two known serratanes, **1** and **2**. Herein we describe the structure elucidation of compounds **3** and **4**.





1 R^1 = : O, R^2 = β-OMe

2 R^1 = β-OMe, R^2 = : O

5 R^1 = β-OMe, R^2 = β-OH

3 R = : O

4 R = β-OH

4a R = β-OAc

Results and Discussion

One of the two known compounds (**1**) was identified as 21β-methoxyserrat-14-en-3-one by direct comparison with an authentic sample isolated from the stem bark of *Picea jezoensis* Carr. *hondoensis*.[2] The other compound was confirmed to be 3β-methoxyserrat-14-en-21-one (**2**),[3,4] as its physical and spectral data were in good agreement with those already published, except for the ^{13}C-NMR spectrum, which has not been reported previously in the literature. Unambiguous assignments for the ^{1}H- and ^{13}C-NMR signals of **2** are shown in Tables 1 and 2.

Compound **3** exhibited positive purple color on the Liebermann−Burchard test, and the molecular formula was assigned as $C_{31}H_{52}O_3$ from HREIMS. The IR spectrum showed an absorption band characteristic of a six-membered ring ketone at ν max 1710 cm^{-1}. In the ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2), compound **3** revealed signals for seven quaternary methyl groups, one methylene group vicinal to a ketone [δ_H 2.22 and 2.57 (each 1 H, dt, J = 14.2, 4.5 Hz)], an equatorial secondary methoxy group [δ_H 3.36 (3H, s) and 2.63 (1H, dd, J = 12.2, 4.4 Hz, H-3α); δ_C 57.5 (q) and 88.5 (d)], an oxygenated methine group [δ_H 2.89 (1H, t, J = 2.8 Hz); δ_C 59.6 (d)], an oxygenated sp^3 quaternary carbon [δ_C 60.9 (s)], and a keto group [δ_C 216.3 (s)]. Together with the DEPT data, these results indicated compound **3** to be a saturated methoxypentacyclic triterpene ketone bearing an oxide ring.

Table 1. ^{1}H-NMR Spectral Data of Compounds **2**−**4** and **4a** in $CDCl_3$[a]

	compound			
proton	**2**	**3**	**4**	**4a**
H-1	0.88, 1.83m	1.40, 1.94m	1.39, 1.84m	
H-2	1.46, 1.77m	1.49, 1.88m	1.44, 1.78m	
H-3α	2.63dd (12.2, 4.4)	2.63dd (12.2, 4.4)	2.62dd (12.2, 4.4)	2.62dd (12.2, 4.4)
H-5	0.75m	0.74m	0.72m	
H-6	1.46m	1.49m	1.46m	
H-7	1.11, 1.36m	1.14, 1.40m	1.15, 1.39m	
H-9	1.00m	0.74m	0.69m	
H-11	1.09, 1.75m	1.30, 2.01m	1.58, 1.97m	
H-12	1.19, 1.99m	1.08, 1.91m	1.08, 1.91m	
H-13	1.84m	1.50m	1.51m	1.48m
H-15	5.38br s	2.89t (2.8)	2.81t (2.8)	2.83t (2.8)
H-16α	1.68m	2.00m	2.04m	
H-16β	1.05m	1.87m	1.91m	
H-17	1.68brs	1.56dd (13.0, 4.4)	1.45dd (13.0, 4.4)	1.50dd (13.0, 4.4)
H-19	1.35, 2.12m	1.40, 2.15m	1.39, 1.96m	
H-20β	2.26dt (14.2, 5.3)	2.22dt (14.2, 4.5)	1.89m	
H-20α	2.75dt (14.2, 5.3)	2.57dt (14.2, 4.5)	2.02m	
H-21α			3.40t (2.6)	4.63t (2.6)
H-23	0.96	0.95	0.95	0.94
H-24	0.75	0.75	0.74	0.74
H-25	0.80	0.83	0.82	0.82
H-26	0.83	1.08	1.08	1.09
H-27α		0.87d (14.2)	0.86d (14.2)	0.84d (14.2)
H-27β		1.96d (14.2)	1.93d (14.2)	1.92d (14.2)
H-28	0.92	0.95	0.74	0.75
H-29	1.04	1.04	0.90	0.94
H-30	1.09	1.09	0.94	0.84
OMe	3.35	3.36	3.36	3.35
OCOMe				2.04

[a] Values were recorded at 300 MHz, δ in ppm, J (in parentheses) in Hz; assignments from 2D COSY data.

In addition to the absence of one methyl group and the presence of one more methylene group in comparison with those of the usual pentacyclic triterpene skeletons, the presence of a >CHOC< grouping as the third oxygen function suggests that compound **3** is a new methoxyserratanone bearing an epoxy ring. It is conceivable that **3** is derived from an analogous serratene constituent in this plant organ by an enzymatic epoxidation of the double bond. This presumption was verified by analyzing extensive 2D ^{1}H−^{1}H COSY, 2D ^{1}H−^{13}C COSY, and HREIMS data. In the 2D long-

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Table 2. ^{13}C-NMR Spectral Data of Compounds **2**−**4** and **4a** in $CDCl_3$[a]

	compound			
carbon	**2**	**3**	**4**	**4a**
C-1	38.5t	38.6t	38.5t	38.6t
C-2	22.3t	22.3t	22.3t	22.3t
C-3	88.4d	88.5d	88.5d	88.5d
C-4	38.9s	38.9s	38.9s	38.9s
C-5	56.3d	56.2d	56.2d	56.2d
C-6	18.8t	18.4t	18.4t	18.4t
C-7	45.2t	44.8t	44.8t	44.8t
C-8	37.1s	39.0s	39.1s	39.1s
C-9	62.8d	63.1d	63.4d	63.3d
C-10	38.2s	38.1s	38.1s	38.1s
C-11	25.5t	25.5t	25.4t	25.4t
C-12	27.2t	27.5t	27.1t	27.1t
C-13	56.5t	55.7t	56.7t	56.6t
C-14	138.3s	60.9s	61.3s	61.3s
C-15	121.9d	59.6d	59.3d	59.3d
C-16	24.5t	23.5t	22.8t	22.8t
C-17	51.2d	45.2d	38.0d	38.9d
C-18	36.2s	35.5s	35.2s	35.2s
C-19	38.4t	37.9t	31.8t	32.5t
C-20	34.8t	34.6t	25.1t	22.6t
C-21	217.0s	216.3s	75.7d	77.7d
C-22	47.7s	46.8s	37.1s	36.2s
C-23	28.1q	28.1q	28.1q	28.1q
C-24	16.2q	16.1q	16.1q	16.1q
C-25	15.7q	16.2q	16.3q	16.2q
C-26	19.8q	20.3q	20.4q	20.4q
C-27	55.9t	55.2t	55.4t	55.3t
C-28	13.0q	14.7q	14.8q	14.7q
C-29	21.6q	22.8q	22.9q	27.4q
C-30	24.5q	24.8q	27.8q	22.6q
OMe	57.5q	57.5q	57.5q	57.5q
OCOMe				21.3q
OCOMe				170.9s

[a] Values were recorded at 74.5 MHz, δ in ppm; assignments are from DEPT, ^{1}H-^{13}C COSY, and long-range ^{1}H-^{13}C COSY experiments.

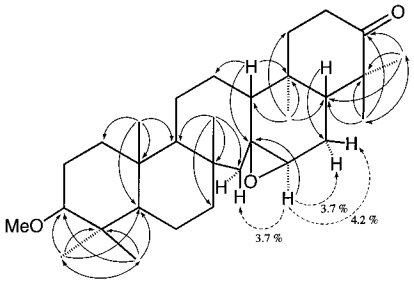


Figure 1. ^{1}H-^{13}C COSY long-range couplings and key NOE differences observed in **3**. —: 2D long-range ^{1}H-^{13}C COSY (HETCOR) data. – – – – –: NOE difference data.

range ^{1}H−^{13}C COSY (HETCOR) experiment, signals of H-13, H-15, H-17 and all the methyl protons of **3** provided ^2J and ^3J cross correlations with those of the related carbons (Figure 1).

Detailed HREIMS analysis brought precious information on the plane structure of **3** (Scheme 1). Fragment ion peaks corresponding to $[M - MeOH]^+$ and $[M - Me - MeOH]^+$ were observed at *m/z* 438.3498 (ion **a**) and 423.3263, respectively, in the high mass number region. Peaks that appeared at *m/z* 287.2364 (ion **b**) and 275.2377 (ion **c**) were assigned to the moiety of A, B, and C rings, with an oxygen atom generated from ion **a** by the fission of the D ring involving an epoxy group. A peak observed at *m/z* 257.2272 was attributed to ion **d** caused by either the elimination of one formaldehyde molecule from ion **b** or the loss of one water molecule from ion **c**. Cleavage of the C ring provided four predominant peaks from the left portion of **3** at *m/z* 248.2133 (base peak, ion **e**), 221.1902 (ion **h**), 216.1875 $[\mathbf{e} - MeOH]^+$ (ion **i**), 203.1794 (ion **k**), and 189.1646 $[\mathbf{h} - MeOH]^+$ (ion **n**), along with three fragment ion peaks attributable to the right portion of **3**, including two oxygen atoms, at *m/z* 235.1681 (ion **f**), 222.1620 (ion **g**), and 207.1738 (ion **j**). A peak at *m/z* 201.1640 was assigned to ion **1**, caused by the further loss of one methyl group from ion **i**.

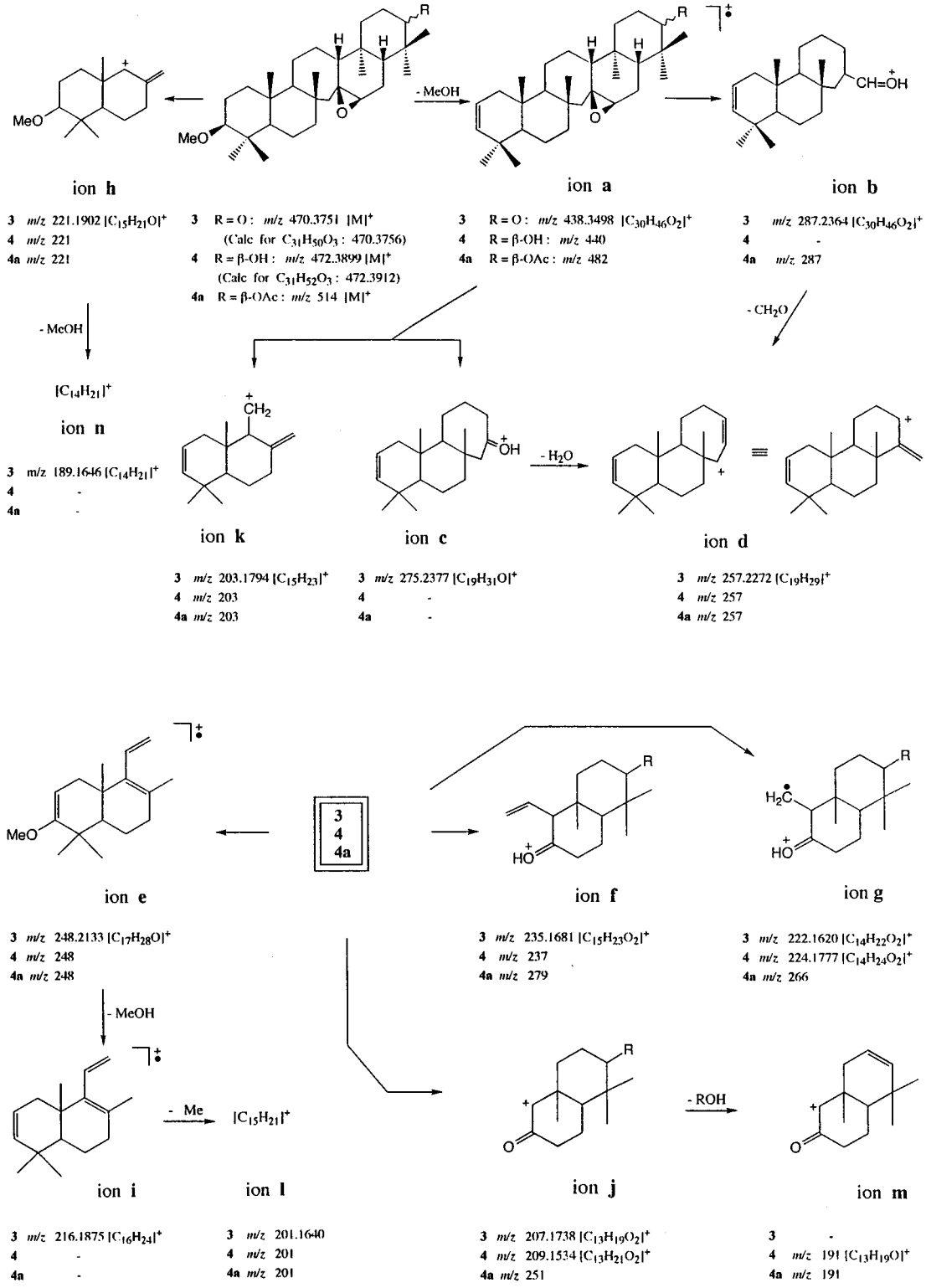
As a whole, the above fragmentations were similar to those of the known 3β-methoxyserratenes.[2,5] The appearance of peaks for ions **b**−**d**, **f**, **g**, and **j** indicated that the epoxy ring was located between the 14 and 15 positions of **3**. Along with the signal pattern of the proton geminal to the methoxy group, these data indicated **3** to be 14ξ,15ξ -epoxy-3β-methoxyserratan-21-one.

The stereochemistry was determined by analyzing the ^{1}H-NMR signal of H-15 and the NOE data, together with the use of Dreiding stereomodels (Figure 2). In the model of **3** having a 14β,15β-epoxy group and a cis-fused crown/half-chair conformation of C/D rings (**3a**), the dihedral angles between H-15α and H-16α and between H-15α and H-16β become almost 60° each. In this form, the ^{1}H-NMR signal of H-15α would split into a triplet due to the presence of vicinal 16-methylene protons. Furthermore, the E ring in **3a** sustains some torsion by the strained D ring, and the dihedral angles between H-19α and H-20α, between H-19α and H-20β, and between H-19β and H-20α adopt angles near 30°, 90°, and 30°, respectively. The patterns of ^{1}H-NMR signals for H-15 and H-20 observed at δ 2.89 (t, J = 2.8 Hz) and δ 2.22 (dt, J = 14.2, 4.5 Hz, H-20α) and 2.57 (dt, J = 14.2, 4.5 Hz, H-20β), respectively, supported structure **3a**. In the model of the 14α,15α-epoxy isomer of **3** involving a trans-fused chair/half-chair conformation of the C/D rings (**3b**), however, the dihedral angles between H-15β and H-16α and between H-15β and H-16β adopt angles of *ca*. 100° and 20°, respectively, and the proton signal of H-15β would split into a doublet of doublets inconsistent with values given in the Experimental Section.

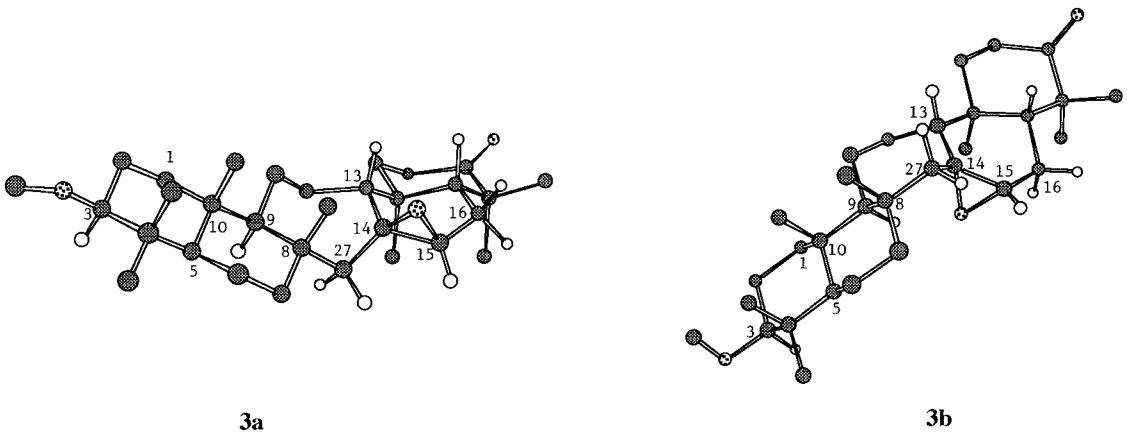
In the NOE difference spectrum of **3** (Figure 1), selective irradiation of the H-15 signal exhibited 3.1%, 4.2%, and 3.7% NOE enhancements for the signals of H-16α, H-16β, and H-27β, respectively, although no NOE has been observed for that of H-27α. All these observations confirm that **3** is 14β,15β-epoxy-3β-methoxyserratan-21-one. Further confirmation of the above structure was achieved by synthesis. Oxidation of 3β-methoxyserrat-14-en-21β-ol (**5**), the most abundant triterpene constituent in this plant source,[1] with CrO_3 in pyridine and subsequent oxidation of the resulting ketone (**2**) with *m*-chloroperbenzoic acid (*m*-CPBA), furnished the epoxy-ketone identical in all respects with compound **3**.

Compound **4** showed positive purple color with the Liebermann−Burchard reagent. The molecular formula was assigned as $C_{31}H_{52}O_3$ by HREIMS. The IR, ^{1}H-NMR, and ^{13}C-NMR spectra (Tables 1 and 2) signals were assigned to seven quaternary methyl groups, to an equatorially oriented secondary methoxy group attributable to the usual C-3 position in the known triterpene skeleton [δ_H 2.62 (1H, dd, J = 12.2, 4.4 Hz, H-3α) and 3.36 (3H, s); δ_C 57.5 (q) and 88.5 (d)], to a

Scheme 1. Mass spectral fragmentation of compounds **3**, **4**, and **4a**.



secondary hydroxyl group [ν max 3450 cm^{-1}; δ_H 3.40 (1H, t, J = 2.6 Hz, H-21α); δ_C 75.7 (d)], to an oxygenated methine proton [δ_H 2.81 (1H, t, J = 2.8 Hz, H-15α); δ_C 59.3 (d)], and to an oxygenated sp^3 quaternary carbon [δ_C 61.3 (s)]. The DEPT spectrum showed that **4** had the same carbon composition as **3**, except for the presence of a secondary hydroxyl group and the absence of a keto group. Acetylation of **4** afforded the corresponding acetate (**4a**) in which the carbinolic methine proton signal was shifted to δ 4.63 (1H, t, J = 2.6 Hz, H-21α), indicating the secondary hydroxyl group in **4** to have an axial orientation. In the EIMS (Scheme 1), compound **4** showed fragment ion peaks at *m/z* 454 [M − H_2O]$^+$, 440 [M − MeOH]$^+$ (ion **a**), 425 [ion **a** − Me]$^+$ and 422 [ion **a** − H_2O]$^+$ in the high mass number region, as well as **4a** at *m/z* 482 [M − MeOH]$^+$ (ion **a**), 454 [M − HOAc]$^+$, 440 [ion **a** − CH_2CO]$^+$, 425 [440 − Me]$^+$, and 422 [M − MeOH − HOAc]$^+$. Except for ions **c** and **i**, peaks corresponding to fragment ions **b**−**l** and **n** were observed in the spectra of **4** and **4a**, together with an additional peak attributable to [ion **j** − H_2O]$^+$ in **4** and [ion **j** − HOAc]$^+$ in **4a** at *m/z* 191 (ion **m**). Close



3a **3b**

Figure 2. Stereoptic views of compounds **3a** and **3b** by computer modeling.

similarity of the above data to those of **3** indicated that **4** is probably the 21β-hydroxy derivative of **3**. Oxidation of **4** with CrO_3 in pyridine yielded the keto-derivative identical in all respects with **3**. Thus, the structure of **4** was established as 14β,15β-epoxy-3β-methoxyserratan-21β-ol.

Compounds **3** and **4** have not been described previously in the literature, although six 14β-hydroxyserratanes, wightianol,[6] tohogenol and tohogeninol,[7] phlegmanol F,[8] inundoside C,[9] and phlegmanol D,[10] have been isolated from several *Lycopodium* species. Three 14β,26-epoxyserratane derivatives have also been isolated from the whole plants of *Primula rosea* (Primulaceae).[11]

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micro-melting-point apparatus and were uncorrected. Optical rotations were measured in $CHCl_3$ using a JASCO DIP 140 digital polarimeter. IR spectra were recorded as KBr disks using a Perkin-Elmer 1720X FTIR spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Varian XL-300 spectrometer with standard pulse sequences operating at 300 MHz and 74.5 MHz, respectively. All NMR spectra were recorded in $CDCl_3$, chemical shift values are reported with ppm relative to TMS, and coupling constants are in Hz. Carbon multiplicities were determined by DEPTGL experiments. All ^{13}C-NMR assignments were based on DEPTGL, ^{1}H−^{1}H COSY, ^{1}H−^{13}C COSY, long-range ^{1}H−^{13}C COSY, and NOE difference experiments. EIMS and HREIMS were run on a Hitachi M-80 mass spectrometer (70 eV). Si gel 60 and alumina 90 (each 70−230 mesh, Merck) were used for column chromatography, as well as Si gel HF_{254} and PF_{254} (Merck) for TLC.

Isolation of Compounds. Detailed fractionation of residues **A**−**E** separated from the CH_2Cl_2 extract (365.1 g) of the dried cuticle of *Picea jezoensis* (Sieb. et Zucc) Carr. *jezoensis* (Mayr.) (6.0 kg) by Si gel column chromatography has been reported previously.[1] In the course of the above investigation, we left detailed examination of the following two eluates unfinished, that is, (a) a gum (10.6 g) collected from the early $CHCl_3$ fractions in the rechromatography of residue **A** and (b) residue **F** (43.6 g), obtained from the fractions eluted between residues **B** and **C** on the preliminary Si gel column chromatography of the extract. Repeated column chromatography of the gum on alumina (500 g) afforded 21β-methoxyserrat-14-en-3-one (**1**) [278 mg; mp 226.5−228 °C (MeOH/$CHCl_3$); $[\alpha]^{23}_D$ −1° (*c* 0.44) [lit.[2] mp 227−229.5 °C, $[\alpha]^{23}_D$ −1° (*c* 0.27)]], identical in all respects (mmp, $[\alpha]_D$, co-TLC, IR, ^{1}H and ^{13}C NMR, and EIMS) with an authentic sample[1] from the fractions 12−16 (each fraction: 100 mL) eluted with *n*-hexane−C_6H_6 (3:1). Successive column chromatography with the same solvent afforded the known 3β-methoxyserrat-14-en-21-one (**2**) [146 mg; mp 268.5−270 °C (MeOH/$CHCl_3$); $[\alpha]^{23}_D$ −29° (*c* 0.33) [lit.[4] mp 272.5−273 °C, $[\alpha]^{23}_D$ −29°]] from fractions 21−36 (each fraction: 100 mL): IR ν max cm^{-1} 1709, 1635, 1410, 860, and 846; ^{1}H- and ^{13}C-NMR see Tables 1 and 2; HREIMS *m/z* $[M]^+$ 454.3815 ($C_{31}H_{50}O_2$). Physical and IR, ^{1}H-NMR, and EIMS data of **2** were in good agreement with those already published. Repeated column chromatography of residue **F** on Si gel (1 kg) afforded a crystalline solid (126 mg) from the fractions eluted with $CHCl_3$ (fractions 39−42, 100-mL fractions), which was purified by preparative TLC (plate: 0.5-mm thick, 20 × 20 cm, solvent: $CHCl_3$−MeOH, 30:1) to give compound **3** (107 mg). Continuous elution of the column with $CHCl_3$−EtOAc (20:1) furnished a crystalline solid, 32 mg, from fractions 63−67, which was purified by preparative TLC (plate: 0.5-mm thick, 20 × 20 cm, solvent: $CHCl_3$−MeOH, 20:1) to give compound **4** (21 mg).

14β,15β-Epoxy-3β-Methoxyserratan-21-one (3): prisms; mp 271.5−274 °C (MeOH/$CHCl_3$); $[\alpha]^{23}_D$ −27° (*c* 0.28); HREIMS *m/z* $[M]^+$ 470.3751 ($C_{31}H_{50}O_3$ requires 470.3756); IR ν max cm^{-1} 2970, 2960, 2853, 1710 (six-membered ring C=O), 1458, 1388, and 1365 (gem dimethyl), 1103, 1048, 1008, and 995; ^{1}H and ^{13}C NMR see Tables 1 and 2; EIMS (70 eV) *m/z* $[M]^+$ 470 (82), $[M - Me]^+$ 455 (18), [ion **a**] 438 (6), 423 (5), 381 (3), [ion **b**] 287 (24), [ion **c**] 275 (6), [ion **d**] 257 (13), [ion **e**] 248 (100), [ion **f**] 235 (66), 233 (29), [ion **g**] 222 (60), [ion **h**] 221 (25), [ion **i**] 216 (38), [ion **j**] 207 (36), [ion **k**] 203 (33), [ion **l**] 201 (57), and [ion **n**] 189 (47).

Synthesis of Compound 3 from 5. (a) A solution of CrO_3 (50 mg) in pyridine (2 mL) was added to a solution of 3β-methoxyserrat-14-en-21β-ol (**5**) (50 mg) in pyridine (2 mL) with stirring at 5 °C, and the mixture was kept at room temperature for 5 h. Workup as usual yielded a crude crystalline mass (55 mg), which was purified by preparative TLC (0.5-mm thick, 20 × 20 cm; $CHCl_3$) to give 3β-methoxyserrat-14-en-21-one (**2**) [45 mg; mp 272−274 °C (MeOH/$CHCl_3$); $[\alpha]^{23}_D$ −29° (*c* 0.25); EIMS *m/z* $[M]^+$ 454], identical in all respects (mmp, $[\alpha]_D$, co-TLC, IR, ^{1}H and ^{13}C NMR, and EIMS) with an

authentic sample isolated above. (b) A solution of 0.000 28 M *m*-CPBA in $CHCl_3$ (5 mL) was gradually added to a solution of synthetic compound **2** (40 mg) in $CHCl_3$ (5 mL) with stirring at room temperature. After being allowed to stand for 4 h, the reaction mixture was washed with 5% Na_2CO_3 and H_2O. Evaporation of the solvent *in vacuo* afforded a residue that was purified by preparative TLC (0.5−mm thick, 20 × 20 cm; $CHCl_3$−MeOH, 30:1) to give 14*β*,15*β*-epoxy-3*β*-methoxyserratan-21-one: 38 mg; mp 272−274 °C (MeOH/$CHCl_3$); $[\alpha]^{23}_D$ −27° (*c* 0.33). The resulting product was identified by direct comparison (mmp, $[\alpha]_D$, co-TLC, IR, ^1H and ^{13}C NMR, and EIMS) with compound **3**.

14*β*,15*β*-Epoxy-3*β*-methoxyserratan-21*β*-ol (4): prisms; mp 251−253 °C (MeOH/$CHCl_3$); $[\alpha]^{23}_D$ −1° (*c* 0.10); HREIMS *m*/*z* $[M]^+$ 472.3899 ($C_{31}H_{52}O_3$ requires 472.3912); IR *ν* max cm^{-1} 3450 (OH), 2961, 2938, 2850, 1458, 1387, and 1363 (*gem*-dimethyl), 1106, 1034, 995, 892, and 823; ^1H and ^{13}C NMR see Tables 1 and 2; EIMS (70 eV) *m*/*z* $[M]^+$ 472 (13), $[M - Me]^+$ 457 (3), $[M - H_2O]^+$ 454 (4), [ion **a**] 440 (1), [ion **a** − Me] 425 (8), [ion **a** − H_2O] 422 (4), 393 (2) [ion **b**] 287 (1), [ion **d**] 257 (100), [ion **e**] 248 (3), [ion **f**] 237 (10), [ion **g**] 224 (39), [ion **h**] 221 (13), [ion **j**] 209 (43), [ion **k**] 203 (10), [ion **l**] 201 (9), [ion **j** − H_2O, ion **m**] 191 (11), [ion **n**] 189 (23), 187 (17), and 136 (100).

Acetylation of Compound 4. Compound **4** (21 mg) was acetylated as usual (Ac_2O/pryidine, each 1 mL) to give the corresponding acetate **4a**: 20 mg; mp 255−258 °C (MeOH/$CHCl_3$); IR *ν* max cm^{-1} 2960, 2938, 2850, 1737, 1460, 1388, 1366, 1245, 1186, 1107, 1034, 996, 889, and 831; ^1H and ^{13}C NMR see Tables 1 and 2; EIMS (70 eV) *m*/*z* $[M]^+$ 514 (46), 499 (18), [ion **a**] 482 (5), [M − HOAc] 454 (19), [ion **a** − CH_2CO] 440 (10), 425 (26), [ion **a** − HOAc] 422 (25), 393 (13), [ion **b**] 287 (7), [ion **f**] 279 (22), [ion **g**] 266 (86), [ion **d**] 257 (20), [ion **j**] 251 (67), [ion **e**] 248 (19), [ion **h**] 221 (18), [ion **k**] 203 (15), [ion **l**] 201 (19), [ion **m**] 191 (24), [ion **n**] 189 (29), 187 (30), and 136 (100).

Oxidation of Compound 4 with CrO_3. A solution of CrO_3 (18 mg) in pyridine (1.5 mL) was gradually added into a solution of compound **4** (18 mg) in pyridine (2 mL) with stirring at 5 °C, and then the mixture was left at room temperature for 5 h. Workup as described above furnished a crystalline solid, 17 mg, which was purified by preparative TLC (0.5-mm thick, 20 × 20 cm, $CHCl_3$−MeOH, 30:1). Recrystallization from MeOH−$CHCl_3$ afforded a keto derivative [15 mg; mp 271−273 °C; $[\alpha]^{23}_D$ −27° (*c* 0.23)], which was identified by direct comparison (mmp, $[\alpha]_D$, co-TLC, IR, ^1H and ^{13}C NMR, and EIMS) with an authentic sample of **3**.

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