Serratene triterpenoids from *Lycopodium clavatum* L. (*Lycopodiaceae*)

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Three serratene triterpenoids, 3β,21α-dihydroxy-26-nor-8,14-sekogammaser-14(27)-en-8-one (**1**), 3β,21α-dihydroxy-8,14-sekogammasera-8(26),14(27)-diene (**2**), and 3α,21β,24-trihydroxyserrat-14-ene (**3**), were isolated from the Siberian chemorace of *Lycopodium clavatum* L. and identified. Compound **1** was isolated from *L. clavatum* L. for the first time.

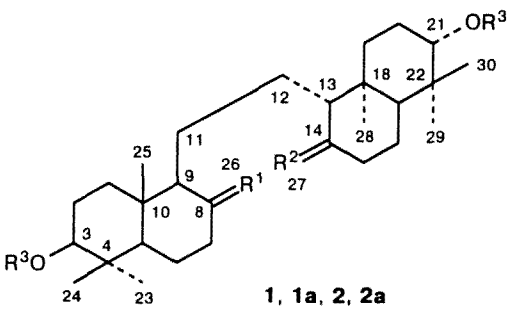
Key words: *Lycopodium*, serratenes, nor- and seko-triterpenoids, ^1H and ^{13}C NMR, mass spectra.

Common club-moss *Lycopodium clavatum* L. (*Lycopodiaceae*) is widely used in folk medicine.[1] Plants of genus *Lycopodium* were shown[2] to contain triterpenoids of the serratene group. The chemical composition of plants is known to depend largely on the area of their growth.[3] Triterpenoids from club-mosses found in our country have not been investigated earlier. This work studies the triterpenoid composition of the Siberian chemorace of *L. clavatum* L.

Results and Discussion

Three individual compounds, 3β,21α-dihydroxy-26-nor-8,14-sekogammaser-14(27)-en-8-one (**1**), 3β,21α-dihydroxy-8,14-sekogammasera-8(26),14(27)-diene (**2**), and 3α,21β,24-trihydroxyserrat-14-ene (**3**), were isolated from the chloroform extract of the aerial part of the Siberian strain of *Lycopodium clavatum* L. gathered in East Siberia. Compound **1** is a minor component. This is likely the reason for its isolation for the first time in spite of the previous detailed study of this species of club-mosses.[2,4]

The structure of compound **1** was confirmed by a combination of NMR, IR-, and mass spectrometry data and also by comparison of its spectral characteristics with the literature data.[5] Thus, 29 signals for carbon atoms in the ^{13}C NMR spectrum and $[M]^+$ 444 point to the nor-triterpenoid structure. The type of fragmentation in the mass spectrum of **1** suggests its onoserrene tetracyclic structure (*cf.* Ref. 5). In fact, the symmetry of the molecule of **1** defines the presence of pairs of signals with similar chemical shifts in the ^{13}C NMR spectrum. The signals for the carbon atoms at the double bond (107.94 and 147.48 ppm), at the carbonyl group (211.82 ppm), and in the α-position to the above-mentioned fragments are exceptions to this regularity. The signals with chemical shifts 78.71 and 79.04 ppm

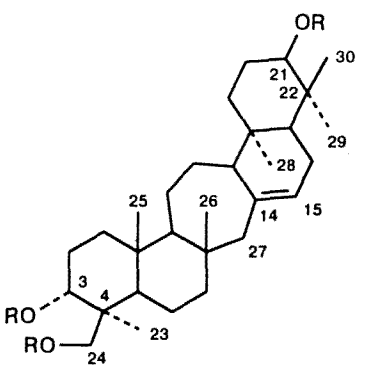


1, **1a**, **2**, **2a**

R^1 = O (**1**, **1a**), CH_2 (**2**, **2a**)

R^2 = CH_2 (**1**, **2**)

R^3 = H (**1**, **2**), Ac (**1a**, **2a**)



3, **3a**

R = H (**3**), Ac (**3a**)

point to the presence of two hydroxylated carbon atoms in the molecule of **1**. The positions of the exomethylene groups at C(8) and C(14) in the onoserin derivatives are

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Table 1. ^{1}H NMR data for compound **1**

Proton	δ (*J*/Hz)
2 H(2)	1.66 (m, H_α); 1.78 (m, H_β)
H(3)	3.22 (dd, *J* = 4.2, 11.7)
H(5)	1.59 (dd, *J* = 2.7, 12.3)
2 H(6)	1.81 (m, H_α); 2.13 (m, H_β)
2 H(7)	2.39 (ddd, H_α, *J* = 6.3, 6.4, 19.0); 2.50 (m, H_β)
H(9)	2.48 (m)
H(11)	1.46 (m, H_α); 2.05 (m, H_β)
2 H(12)	1.10 (m, H_α); 1.96 (m, H_β)
H(13)	1.55 (m)
2 H(20)	1.62 (s, H_α); 1.75 (m, H_β)
H(21)	3.33 (dd, *J* = 4.2, 11.8)
2 H(27)	4.49 (s)
6 Me	0.63 (s); 0.68 (s); 0.74 (s); 0.81 (s); 0.98 (s); 1.08 (s)

known to be governed biogenetically.[2] One of them is oxidized to the carbonyl group in the case of **1**.

In the ^{1}H NMR spectrum of **1**, the protons geminal to the hydroxyl groups are characterized by the chemical shifts at 3.22 ppm (dd, *J* = 4.2 and 11.7 Hz) and 3.33 ppm (dd, *J* = 4.2 and 11.8 Hz). The 2D DQF COSY spectrum revealed H(3) → 2 H(2) and H(21) → 2 H(20) spin systems that suggests the positions of the hydroxyl functions at C(3) and C(21). The values of coupling constants for H(3) and H(21) indicate the equatorial orientation of both hydroxyl groups.

The ^{1}H NMR data for compound **1** reported previously[5] gives little information. Thus, only the signals of two olefin and two hydroxymethine groups are given besides the signals for the protons of methyl groups. This motivated us to examine the 2D DQF COSY spectrum of **1** in more detail. In particular, we established the spin systems of the protons of C and B rings and the chain H(9) → H(11) → H(12) → H(13) that allowed us to assign most of the signals in the ^{1}H NMR spectrum of **1** (Table 1).

Thus, compound **1** was identified as 3β,21α-dihydroxy-26-nor-8,14-sekogammaser-14(27)-en-8-one.[5]

Compounds **2** and **3** are the main components of the triterpenoid fraction of *L. clavatum* L. The physicochemical constants, IR spectra, and ^{1}H and ^{13}C NMR spectra of **2** and **3** and their acetates **2a** and **3a** coincide with those reported for α-onocerin[5] and lycoclavanol[6] and their acetates. However, the optical rotations for **1**, **1a**, and **2** differ significantly from those reported.[5,7,8]

Experimental

Air-dried raw material (492 g) was ehaustively extracted with chloroform. The extract was concentrated, and the residue (21 g) was subsequently treated with hexane and benzene. Column chromatography was performed on silica gel L 40/100 using the following solvent systems: (**A**) chloroform—methanol (98 : 2, hereinafter v/v), (**B**) hexane—acetone (7 : 2), and (**C**) hexane—acetone (7 : 3). TLC was carried out on Silufol plates in systems **A**, **B**, and (**D**) benzene—ethanol (9 : 1). The spots were visualized by treating with 0.5 % vanillin in 50 % orthophosphoric acid.[9]

Repeated column chromatography of the benzene fraction yielded **1** (0.017 g, elution with system **C**), **2** (1.9 g, elution with system **A**), and **3** (0.04 g, elution with system **B**). Acetates **1a**—**3a** were synthesized from compounds **1**—**3**.[8] Compound **1a** was additionally purified by column chromatography in a solvent system **D**. Acetates **2a** and **3a** were crystallized from chloroform—methanol (2 : 1).

Melting points were determined on a Kofler block. Optical rotations were taken on a POLAMAT A polarimeter. IR spectra were recorded in KBr pellets on a Specord UV-VIS spectrometer. Mass spectra (EI, 70 eV) were taken on an LKB-2091 instrument at 310 °C.

NMR spectra of **1** and **1a** were registered in $CDCl_3$ on a Varian VXR 500S spectrometer equipped with a SUN 3/50 computer with a standard VNMR software. Two-dimensional ^{1}H—^{1}H spectra were obtained by COSY experiment. Spectra of **2** and **2a** (in $CDCl_3$) and **3** and **3a** (in C_5D_5N) were registered on Bruker WP-200 (^{1}H, 200.13 MHz) and Jeol FX-90Q (^{13}C, 22.49 MHz) spectrometers at 25 °C with $SiMe_4$ as internal standard.

3β,21α-Dihydroxy-26-nor-8,14-sekogammaser-14(27)-en-8-one (1), m.p. 208—210 °C ($CHCl_3$), $[\alpha]_{546}^{20}$ −26.1° (*c* 0.92, $CHCl_3$). Lit.[5]: m.p. 213—215 °C, $[\alpha]_D^{29}$ −35.1° (*c* 0.23, $CHCl_3$). IR, ν/cm^{-1}: 3040 (=CH); 1710 (C=O); 1650 (C=C); 895 (C=CH_2). MS, *m/z* (I_{rel} (%)): 444 $[M]^+$ (20), 426 (24), 411 (17), 223 (28), 207 (4.7), 135 (100). $C_{29}H_{48}O_3$.

Diacetate of 1 (1a), m.p. 194—196 °C ($CHCl_3$), $[\alpha]_{546}^{20}$ +5.9° (*c* 0.85, $CHCl_3$). Lit.[5]: m.p. 185—187 °C, $[\alpha]_D^{30}$ −11.2° (*c* 0.27, $CHCl_3$). MS, *m/z* (I_{rel} (%)): 528 $[M]^+$ (11), 510 (8), 468 (12), 135 (59), 43 (100). $C_{33}H_{52}O_5$.

α-Onocerin (2), m.p. 206—208 °C ($CHCl_3$—MeOH, 2 : 1), $[\alpha]_{546}^{20}$ −12.2° (*c* 0.82, $CHCl_3$—MeOH, 2 : 1). Lit.[8]: m.p. 202—203 °C ($CHCl_3$—MeOH), $[\alpha]_D$ +18° (*c* 0.287), +1° (*c* 0.92, pyridine). IR, ν/cm^{-1}: 3080 (=CH); 1640 (C=C); 885 (C=CH_2). MS, *m/z* (I_{rel} (%)): 442 $[M]^+$ (13), 409 (11), 229 (10), 203 (18), 135 (100). $C_{30}H_{50}O_2$. ^{1}H and ^{13}C NMR data correspond to those reported.[5]

Diacetate of 2 (2a), m.p. 224—225 °C ($CHCl_3$—MeOH, 2 : 1), $[\alpha]_{546}^{20}$ +37.9° (*c* 0.58, $CHCl_3$). Lit.[7]: m.p. 222—224 °C (MeOH—Me_2CO), $[\alpha]_D$ +29° (*c* 3.84), +28° (*c* 1.74).

Lycoclavanol (3), m.p. 338—340 °C ($CHCl_3$—MeOH, 2 : 1), $[\alpha]_{546}^{20}$ −37.9° (*c* 0.56, $CHCl_3$—MeOH, 2 : 1). Lit.[2]: m.p. 308—310 °C, $[\alpha]_D^{13}$ −23.1° (*c* 0.39, $CHCl_3$—MeOH, 4 : 1). MS, *m/z* (I_{rel} (%)): 458 $[M]^+$ (43), 440 (22), 425 (12), 220 (52), 187 (52), 135 (54), 109 (50), 43 (100). $C_{30}H_{50}O_3$. ^{1}H and ^{13}C NMR data correspond to those reported.[6]

Triacetate of 3 (3a), m.p. 200—202 °C ($CHCl_3$—MeOH, 2 : 1), $[\alpha]_{546}^{20}$ −39.1° (*c* 0.46, $CHCl_3$—MeOH, 2 : 1). Lit.[7]: m.p. 197—198 °C, $[\alpha]^{13}$ −23.1°.

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