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SAXOSTEROL, A NEW DIHYDROXY CHOLESTEROL FROM *NARTHECIUM OSSIFRAGUM*

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Key Word Index—*Narthecium ossifragum*; Liliaceae; dihydroxy cholesterol; saxosterol; saxosteryl esters.

Abstract—A new sterol, (22*R*)-cholest-5-ene-3β,16ξ,22-triol, designated saxosterol, has been isolated from *N. ossifragum*. In the plant saxosterol occurs as steryl esters, being esterified in the 3β-position. The main fatty acids are capric acid (42%) and lauric acid (43%).

INTRODUCTION

The major sterol from the flowers of *Narthecium ossifragum* (L.) Huds. is narthesterol, (22*R*)-cholest-5-ene-3β,22-diol) [1, 2]. In the plant it occurs as 3β-steryl esters, mainly of capric acid and lauric acid [3].

We now wish to report the structure of a new hydroxylated cholesterol from the unsaponifiable fraction of the same plant material. For this sterol we propose the name saxosterol, derived from *vallsaks*, which is the local name of *N. ossifragum* in the districts where the plant was collected. The second syllable in *vallsaks* refers to the shape of the leaves. In Old Norse *sax* is the name of a short, single-edged sword, derived from the Franconian *scramasaxa*.

RESULTS AND DISCUSSION

From the unsaponifiable fraction of *N. ossifragum* a new sterol (**1**) was isolated with mp 182–83°, $[\alpha]_D^{20} - 27°$ ($CHCl_3$). Thin layer chromatography showed the new sterol to be more polar than the previously known (22*R*)-22-hydroxycholesterol from the same plant. The mass spectrum of **1** gave a molecular ion at *m/z* 418, corresponding to $C_{27}H_{46}O_3$. Acetylation with acetyl chloride yielded a triacetate as shown by ^{1}H NMR. Proton NMR further demonstrated the presence of an olefinic proton (δ 5.36) of a trisubstituted double bond.

The mass spectrum of **1** displayed fragments at *m/z* 400 $[M-H_2O]^+$, 382 $[M-2H_2O]^+$, 347 $[M-(C\text{-}23\text{–}C\text{-}27)]^+$, 318, and 300. The ion at *m/z* 318 was due to cleavage of the C-20/C-22 bond, with H transfer to the nucleus. Further loss of water from this fragment gave the base peak at *m/z* 300. The fragmentation pattern thus demonstrated the presence of one hydroxyl group at C-22. This assignment was confirmed by an intense peak at *m/z* 173 in the mass spectrum of the TMSi-derivative of saxosterol. The same dominant ion at *m/z* 173 is found in the mass spectrum of the TMSi-derivative of (22*R*)-22-hydroxycholesterol [4, 5]. An ion at *m/z* 129, characteristic of TMSi-derivatives of Δ^5-3β-sterols [6], showed that one hydroxyl group occupied the 3β-position.

Hydrogenation of **1** gave a saturated sterol **2**. The molecular ion appeared at *m/z* 420, corresponding to $C_{27}H_{48}O_3$. Oxidation of **2** with Jones' reagent [7] gave a triketone (**3**), demonstrating that all three hydroxyls were secondary. The IR spectrum of **3** showed carbonyl bands at 1711 cm^{-1} (3- and 22-ketone) and at 1737 cm^{-1} (5-membered ring ketone). The latter value agrees with those of other steroids with a carbonyl group at C-16 [8], whereas 15-ketosteroids absorb at lower wave numbers [9, 10].

The mass spectrum of compound **3** gave a molecular ion at *m/z* 414, in accordance with $C_{27}H_{42}O_3$. A fragment at *m/z* 358 was ascribed to cleavage of the C-23/C-24 bond with H-transfer (McLafferty rearrangement). Fragments at *m/z* 343 and 315 were due to α-cleavage adjacent to the C-22 keto group [11].

Other strong fragments occurred at *m/z* 273 and 231. The first one was due to cleavage of the C-17/C-20 bond with loss of the methyl group at C-18 [12]. The second fragment was caused by the loss of C-15, C-16, C-17 and the side chain. Corresponding fragmentations have been observed in other 16-ketosteroids [12], while 15-ketosteroids display different fragmentation patterns [10, 12].

These data show that the three hydroxyl groups of saxosterol are situated at C-3, C-16, and C-22. In 1975 Giral and Rivera isolated a sterol from *Calibanus hookerii*, which they named calibagenin [13]. Calibagenin was assigned the structure cholest-5-ene-3β, 16ξ, 22ξ-triol. It has mp 195–196° and $[\alpha]_D^{20} - 56°$ ($CHCl_3$), and is thus clearly different from saxosterol. Hydrogenation and oxidation yielded a triketone with mp 166–167°, which is identical with the mp of the corresponding triketone obtained from saxosterol. The mass spectral and IR carbonyl absorption values of **3** are in good agreement with the corresponding data for the triketone of Giral and Rivera [13]. However, the ^{1}H NMR methyl shifts of **3** differ somewhat from the published values, but a direct comparison could not be made, since no sample of their triketone was available.

Complete acetylation of saxosterol required the use of acetyl chloride, while complete acetylation of calibagenin was achieved with acetic anhydride. This indicates that saxosterol may have the more hindered 16β-configuration, while calibagenin might be the 16α-isomer.

The esters of saxosterol were isolated from the lipid extracts of the plant. Gas chromatography showed that the dominant fatty acids of the esters were capric acid (42%) and lauric acid (43%). The amounts of the more common long chain (C_{16} and C_{18}) fatty acids were very

low (Table 1). The saxosteryl esters displayed the same fatty acid pattern as that reported for the esters of narthesterol [3].

The mass spectrum of the TMSi derivatives of the natural ester mixture had a peak at *m/z* 544, representing loss of one fatty acid from the molecular ion. Peaks at *m/z* 454 and 364 were due to loss of one and two TMSOH groups, respectively, from the *m/z* 544 ion. This demonstrates the presence of two free hydroxyl groups in the esters. The dominant ion at *m/z* 173 showed the presence of a free 22-hydroxyl group in the saxosteryl esters. The mass spectrum lacked the peak at *m/z* 129, which is typical of TMSi-derivatives of Δ^5-3β-sterols [6], thus indicating esterification in the 3β-position. This assignment was supported by the absence of a strong hydroxyl band at 1054 cm^{-1} in the IR spectrum, a band characteristic of free 3β-sterols [14]. Consequently, the saxosteryl esters are monoesters esterified in the 3β-position, with free hydroxyl groups in the 16- and 22-positions.

The unsaponifiable fraction of the lipid extract from 1 kg (fr. wt) of the flowering parts yielded *ca* 0.24 g saxosterol. This is 12% of the amount of narthesterol in the same sample. Free saxosterol was not detected.

EXPERIMENTAL

Flowering parts of *N. ossifragum* were collected near Kristiansund, Norway. The isolation of the saxosteryl esters was performed as previously described for esters of narthesterol [3], except that HPLC was carried out with hexane–methyl *t*-butyl ether (3:2) as mobile phase and UV detection was at 208 nm. The purified esters were hydrolysed with 0.8 M KOH in MeOH. Saxosterol was extracted with Et_2O, and the fatty acids were methylated and analysed as described in ref. [3].

Saxosterol (**1**). Crystallized from MeOH, mp 182–183°, $[\alpha]_D^{20}$ −27° ($CHCl_3$; *c* 0.2). R_f 0.35 on silica gel G with CH_2Cl_2–MeOH (97:3). (Narthesterol had R_f 0.71 in the same system). IR ν_{max}^{KBr} cm^{-1}: 3375, 1054 (s), 1035 (s), 1021 (m), 1008 (m). EIMS (probe) 70 eV *m/z*, (rel.int): 418 $[M]^+$ (1), 400 (3), 382 (2), 347 (3), 329 (6), 318 (3), 301 (28), 300 (100), 285 (33), 282 (25), 271 (48), 267 (37), 253 (12), 232 (15), 214 (15). EIMS (probe, 22.5 eV) of the TMSi-derivative, *m/z* (rel.int): 544 $[M-90]^+$ (1), 432 (1), 417 (11), 213 (2), 174 (16), 173 (100), 129 (9). High resolution MS: 418.3429. $C_{27}H_{46}O_3$ requires 418.3447. ^1H NMR (270 MHz, $CDCl_3$): δ 0.910 (6H, *dd*, *J* = 6.6 Hz, C-26 and C-27), 0.924 (3H, *s*, C-18), 0.972 (3H, *d*, *J* = 6.6 Hz, C-21), 1.018 (3H, *s*, C-19), 3.55 (1H, *m*, C-3), 3.65 (1H, *m*, C-22), 4.35 (1H, *m*, C-16), 5.36 (1H, *d*, *J* = 5.1 Hz, C-6).

Table 1. Relative amounts of the fatty acids of the saxosteryl esters from *N. ossifragum*

Fatty acid	Weight %
8:0	8
10:0	42
12:0	43
14:0	1
16:0	4
18:0	0.5
18:1	1
18:2	0.5

Saxosteryl esters from N. ossifragum. IR ν_{max}^{KBr} cm^{-1}: 3465, 1735, 1720, 1037 (*s*), 1008 (*m*). EIMS (probe, 22.5 eV) of the TMSi-derivatives, *m/z* (rel.int.): 544 (3), 454 (3), 372 (24), 364 (2). 282 (30), 174 (18), 173 (100).

Saxosterol triacetate. Prepared with acetyl chloride in dry pyridine (reaction with Ac_2O gave the diacetate). Crystallized from MeOH, mp 142–143°. $[\alpha]_D^{20}$ +13° ($CHCl_3$; *c* 0.2). IR ν_{max}^{KBr} cm^{-1}:1740, 1734, 1726, 1245. EIMS (probe) 70 eV, *m/z* (rel.int): 484 $[M-HOAc]^+$ (100), 424 $[M-2HOAc]^+$ (2), 364 $[M-3HOAc]^+$ (13), 349 (6), 313 (3), 282 (5), 253 (9), 213 (11). ^1H NMR (270 MHz, $CDCl_3$): δ 0.868 (6H, *dd*, *J* = 7.3 Hz, C-26 and C-27), 0.892 (3H, *s*, C-18), 0.980 (3H, *d*, *J* = 6.6 Hz, C-21), 1.022 (3H, *s*, C-19), 2.03 (6H, *s*, 2 Ac), 2.11 (3H, *s*, 1 Ac).

Dihydrosaxosterol (**2**). Saxosterol (225 mg) was hydrogenated in THF with PtO_2 at 22° and 1 atm pres. for 240 hr giving a mixture of saxosterol–dihydrosaxosterol (2:1). Separation was done by HPLC on a Bondapak C-18 column (7.8 mm × 30 cm) with MeOH–H_2O (87:13) and an RI detector. Dihydrosaxosterol crystallized from MeOH, mp 202–203°. IR ν_{max}^{KBr} cm^{-1}: 3364, 1036 (vs), 1008 (m). EIMS (probe) 70 eV, *m/z* (rel.int.):420 M^+ (1), 402 (4), 331 (32), 302 (100), 287 (47), 273 (78), 255 (10), 234 (11). ^1H NMR (270 MHz, $CDCl_3$): δ 0.81 (3H, *s*, C-18), 0.89 (3H, *s*, C-19), 0.90 (3H, *d*, *J* = 6.0 Hz, C-26), 0.91 (3H, *d*, *J* = 6.0 Hz, C-27), 0.95 (3H, *d*, *J* = 6.6 Hz, C-21), 3.60 (1H, *m*, C-3), 3.65 (1H, *m*, C-22), 4.35 (1H, *m*, C-16), no signal at 5.36.

Cholestane-3,16,22-trione (**3**). Dihydrosaxosterol (35 mg) was dissolved in HOAC–Me_2CO (8:2) (9 ml) and oxidized with 0.45 ml Jones' reagent [7] for 10 min at 4°. After addition of H_2O the products were extracted with Et_2O and washed with 2% $NaHCO_3$ and H_2O. Compound **3** was purified by HPLC using a silica-column with $CHCl_3$–EtOH (99.5:0.5) as mobile phase and RI detection. Crystallization from MeOH–H_2O gave 20 mg **3**, mp 166–167°. IR ν_{max}^{KBr} cm^{-1}: 1737, 1711. EIMS (probe) 70 eV, *m/z* (rel.int.): 414 M^+ (5), 399 (7), 358 (60), 343 (28), 315 (52), 301 (17), 273 (10), 271 (12), 246 (6), 231 (23), 217 (9), 99 (58), 81 (100). ^1H NMR (270 MHz, $CDCl_3$): δ 0.803 (3H, *s*, C-18), 0.916 (6H, *dd*, *J* = 5.9 Hz, C-26 and C-27), 1.041 (3H, *s*, C-19), 1.043 (3H, *d*, *J* = 6.3 Hz, C-21), 2.62 (1H, *d*, *J* = 3.7 Hz, C-20). Lit. values [13]: Mp 166–167°, IR ν_{max} cm^{-1}: 1738, 1713 ^1H NMR: δ0.86, 0.98, 1.08, 1.20, 2.66. MS *m/z*: 414, 399, 358, 343, 288, 273, 271, 217.

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AN ACYLATED SITOSTEROL GLUCOSIDE FROM *ALISMA PLANTAGO-AQUATICA*

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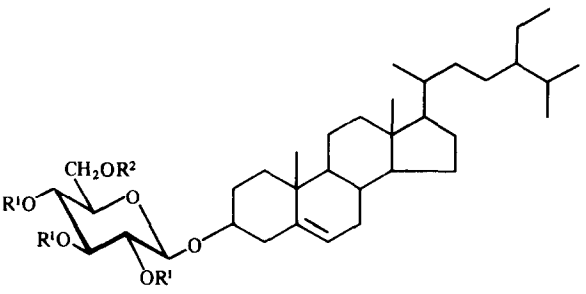
Key Word Index—*Alisma Plantago-aquatica*; Alismataceae; sitosterol-3-*O*-6-stearoyl-β-D-glucopyranoside; sitosterol; methyl stearate.

Abstract—A phytosterol glucoside acylated with stearic acid has been isolated from the methanol extract of the rhizome of *Alisma Plantago-aquatica*, and its structure has been determined as sitosterol-3-*O*-6-stearoyl-β-D-glucopyranoside by spectroscopic data and chemical conversions.

In a previous paper [1] we reported the structures of protostane-type triterpenoids, 16β-methoxy and 16β-hydroxyalisol B monoacetates isolated from the rhizome of *Alisma Plantago-aquatica* L. var. *orientale* Samuels (Alismataceae) [2]. A further study on the constituents of this medicinal plant has now resulted in the isolation of a new sitosterol glucoside acylated with stearic acid.

The IR, ^{1}H and ^{13}C NMR spectra of compound **1** displayed the presence of hydroxy (3400 cm^{-1}) and ester groups, and a sugar moiety, in addition to a saturated fatty acid residue. Methanolysis of **1** afforded two products. The less polar one was identified as methyl stearate by the ^{1}H NMR and mass spectra, whereas the polar product was identified as sitosterol-3-*O*-β-D-glucopyranoside by comparison with spectral data of the authentic sample after a conventional acetylation. This chemical evidence suggested that the stearic acid in **1** should be bonded to a hydroxy group of the glucose moiety in **3**. The ^{1}H NMR spectrum of **1** revealed the two double doublet signals (δ4.25, *dd*, $J=12.2$, 2.5 Hz and 4.52, *dd*, $J=12.2$, 4.0 Hz) which corresponded to the H-6 methylene group in the glucose moiety. Since these signals were not greatly shifted (δ 4.12 and 4.24) on acetyl-



1 R^1 = H, R^2 = $C(=O)(CH_2)_{16}Me$

2 R^1 = Ac, R^2 = $C(=O)(CH_2)_{16}Me$

3 $R^1 = R^2$ = H

4 $R^1 = R^2$ = Ac

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