(1986) *J. Chem. Soc. Perkin Trans.* 1 (in press).
6. Fomum, Z. T., Ayafor, J. F. and Wandji, J. (1985) *Phytochemistry* **24**, 3075.
7. Pearl, I. A. and Beyer, D. L. (1951) *J. Org. Chem.* 216.
8. *Handbook of Chemistry and Physics* (1970–71) 47th edn. The Chemical Rubber Company, Ohio, U.S.A.
9. Devon, T. K. and Scott, A. I. (1975) *Handbook of Naturally Occurring Compounds V.* Academic Press, London.

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(22*R*)-22-HYDROXYCHOLESTERYL ESTERS FROM *NARTHECIUM OSSIFRAGUM*

BØRGE HOLEN and ARNULV STABURSVIK

Department of Chemistry, P.O. Box 30, Agricultural University of Norway, N-1432, As-NLH, Norway

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Key Word Index—*Narthecium ossifragum*; Liliaceae; (22*R*)-22-hydroxycholesteryl esters; fatty acids.

Abstract—The esters of (22*R*)-22-hydroxycholesterol from the flowers of *N. ossifragum* are monoesters, esterified in the 3β-position only. The main components are cholest-5-en-3β,22*R*-diol 3-caprate (47%) and cholest-5-en-3β,22*R*-diol 3-laurate (44%). The sterol has not been found in the free state in the plant.

INTRODUCTION

22-Hydroxycholesterol has been isolated from the flowering parts of *Narthecium ossifragum* as a major component from the unsaponifiable fraction [1, 2]. This sterol, also named narthesterol [3], was subsequently shown to be the 22*R*-epimer, *viz.* cholest-5-en-3β,22*R*-diol [4, 5].

(22*R*)-22-Hydroxycholesterol has not been found in other plants, but has been identified in human meconium [6] and isolated in very small amounts from bovine adrenal glands [7]. (22*R*)-22-Hydroxycholesterol is an intermediate in the biosynthesis of pregnenolone from cholesterol [8].

In the present study the esters of (22*R*)-22-hydroxycholesterol isolated from *N. ossifragum* have been investigated.

RESULTS AND DISCUSSION

The fatty acid composition of the 22-hydroxycholesteryl esters from *N. ossifragum* is shown in Table 1. The predominant fatty acids are capric acid ($C_{10:0}$) and lauric acid ($C_{12:0}$). The total content of these two acids is 91%, while the amount of the more usual long chain fatty acids (C_{16} and C_{18}) is very low, as is the percentage of unsaturated fatty acids.

The IR spectrum of the purified ester mixture displays the characteristic ester carbonyl absorption at 1735 cm^{-1}. The hydroxyl band at 3430 cm^{-1}, with additional hydroxyl bands at 1021 and 1005 cm^{-1}, show that the esters of 22-hydroxycholesterol from *N. ossifragum* are monoesters with a free hydroxyl group. The absence of a strong hydroxyl band at 1054 cm^{-1}, characteristic for 3β-sterols, indicates that the natural esters are esterified in the 3-position. Mass spectra of the TMSi-derivatives of the esters further support the above assignment, since the spectrum shows a dominant peak at *m/z* 173. This peak is due to the fragmentation of the side chain between C-20

Table 1. Relative amounts of the fatty acids of (22*R*)-22-hydroxycholesteryl esters from *N. ossifragum*

Fatty acid	Weight %
8:0	3
10:0	47
12:0	44
14:0	4
16:0	1
18:0	0.5
18:1	tr*
18:2	tr

*tr = trace (< 0.5%).

and C-22 and is typical for the mass spectrum of the TMSi-derivative of (22*R*)-22-hydroxycholesterol [6, 8].

In order to confirm the structure of the natural esters, the synthetic capric acid monoesters of (22*R*)-22-hydroxycholesterol were prepared. Compound **1** had the same R_f value (0.34) as the natural esters, the same hydroxyl-bands in the IR-spectrum, and the mass spectrum had a dominant peak at *m/z* 173. The other monoester (**2**) with R_f 0.15 had a strong alcohol band at 1054 cm^{-1} with a weak band at 1021 cm^{-1}, similar to the IR spectrum of cholesterol [9]. The mass spectrum of **2** had a base peak at *m/z* 155 (fragmentation of the acyl group) and a strong peak (84%) at *m/z* 366 [M − TMSOH − fatty acid]. TLC shows that the 3β-ester is the less polar of the two monoesters, by analogy with TLC of monoesters of 26-hydroxycholesterol [10].

The data above confirm that the (22*R*)-22-hydroxycholesteryl esters isolated from *N. ossifragum* are esterified only in the 3β-position, with a free hydroxyl in the 22*R*-position.

The unsaponifiable fraction from a lipid extract of 1000 g (fr. wt) of the flowering parts contains *ca* 2.1 g of (22*R*)-22-hydroxycholesterol, which represents 43% of the unsaponifiable matter. The presence of free (22*R*)-22-hydroxycholesterol in the plant could not be demonstrated.

EXPERIMENTAL

Silica gel G was used for TLC with $CHCl_3$–EtOH (99.5:0.5), and the sterols were visualized with Ac_2O–H_2SO_4–EtOH (1:1:10). HPLC was carried out on a LiChrosorb Si 60-7 column (Chrompack 9 mm i.d. × 22 cm) with $CHCl_3$–EtOH (99:1), using a Spectra-Physics 3500 B with a Shodex RI detector.

Extraction and isolation. Flowering parts of *N. ossifragum* were collected near Kristiansund, Norway, and the lipids were extracted and purified according to the procedures of ref. [11]. The lipids were separated on a silica gel column and eluted with increasing amounts of Me_2CO in hexane. Elution was monitored by TLC examination of the fractions. Fractions with the esters of (22*R*)-22-hydroxycholesterol were combined and further purified by HPLC where the esters eluted as one asymmetric peak. The esters were hydrolysed with 0.8 M KOH in MeOH. The sterol fraction was isolated and gave one spot on TLC. It was identified as (22*R*)-22-hydroxycholesterol by comparison with authentic material. After neutralization the fatty acids were extracted with CH_2Cl_2 and methylated with BF_3–MeOH (14%). The fatty acid methyl esters were analysed on a glass column (2 mm i.d. × 2.4 m) packed with 10% SP-2330, temp. programming from 60 to 215° at 6°/min. and an N_2 flow of 17.5 ml/min. The peaks were identified by co-chromatography with standards and were quantified from the calibrated FID response. IR spectra of the steryl esters were taken as a film between NaCl plates. The TMSi-ethers were prepared using HMDS–TMCS–pyridine (5:2:10). MS (direct inlet) was performed at 22.5 eV.

Natural mixture of (22R)-22-*hydroxycholesteryl esters from* N. ossifragum. R_f 0.34. IR ν_{max} cm^{-1}: 3430, 1735, 1021 (m), 1005 (s). MS of the TMSi-derivatives *m/z* (rel. int.): 385 (2), 384 (6), 367 (3), 366 (4), 285 (4), 284 (14), 269 (2), 255 (2), 213 (3), 175 (6), 174 (16), 173 (100).

Preparation of caprate esters of (22R)-22-*hydroxycholesterol.* A mixture of (22*R*)-22-hydroxycholesterol (0.4 g) and capric acid anhydride (0.6 g) in 6 ml dry pyridine was kept at room temp. overnight. After addition of H_2O the products were extracted with $CHCl_3$. TLC revealed four compounds with R_f values: 0.80, 0.34 (**1**), 0.15 (**2**) and 0.05 [(22*R*)-22-hydroxycholesterol] which were isolated by HPLC. The compound with R_f 0.80 was identified as the dicaprate ester (226 mg, no hydroxyl band in IR). The other two compounds are the two monoesters.

Cholest-5-en-3β,22R-*diol* 3β-*caprate* (**1**). 146 mg. Crystallized from MeOH, mp 74–75°. R_f 0.34. IR ν_{max} cm^{-1}: 3430, 1735, 1021 (m), 1005, (s). MS of the TMSi-derivative *m/z* (rel. int.): 367 (3), 366 (3), 285 (4), 284 (12), 269 (2), 255 (2), 253 (2), 213 (3), 175 (6), 174 (15), 173 (100).

Cholest-5-en-3β,22R-*diol* 22R-*caprate* (**2**). 27 mg. Crystallized from MeOH, mp 56–57°. R_f 0.15. IR ν_{max} cm^{-1}: 3375, 1735, 1054 (s), 1021 (w). MS of the TMSi-derivative *m/z* (rel. int.): 385 (18), 384 (56), 367 (40), 366 (84), 351 (40), 272 (26), 255 (24), 254 (27), 253 (40), 245 (37), 228 (32), 213 (29), 161 (24), 159 (29), 158 (32), 155 (100).

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REFERENCES

1. Stabursvik, A. (1953) *Acta Chem. Scand.* **7**, 1220.
2. Stabursvik, A. (1959) *A Phytochemical Study of* Narthecium ossifragum (*L.*) *Huds.*, Ser. 2, No. 6, pp. 47–57. Norges Tekn, Vitenskapsakad.
3. Shoppee, C. W., (1964) *Chemistry of the Steroids*, p. 5. Butterworths, London.
4. Burrows, E. P., Hornby, G. M. and Caspi, E. (1969) *J. Org. Chem.* **34**, 103.
5. Mori, H., Shibata, K., Tsuneda, K., Sawai, M. and Tsuda, K. (1968) *Chem. Pharm. Bull.* **16**, 1407.
6. Lavy, U., Burstein, S., Gut, M. and Javitt, N. B. (1977) *J. Lipid Res.* **18**, 232.
7. Dixon, R., Furutachi, T. and Lieberman, S. (1970) *Biophys. Res. Commun.* **40**, 161.
8. Burstein, S., Middleditch, B. S. and Gut, M. (1975) *J. Biol. Chem.* **250**, 9028.
9. Labarrère, J. A., Chipault, J. R. and Lundberg, W. O. (1958) *Analyt. Chem.* **30**, 1466.
10. Teng, J. I., McGehee, M. F. and Smith, L. L. (1981) *J. Steroid Biochem.* **14**, 569.
11. Folch, J., Lees, M. and Sloane Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497.