STARFISH SAPONINS. XXV*. STEROIDAL GLYCOSIDES FROM THE STARFISH *Gomophia watsoni* (*)

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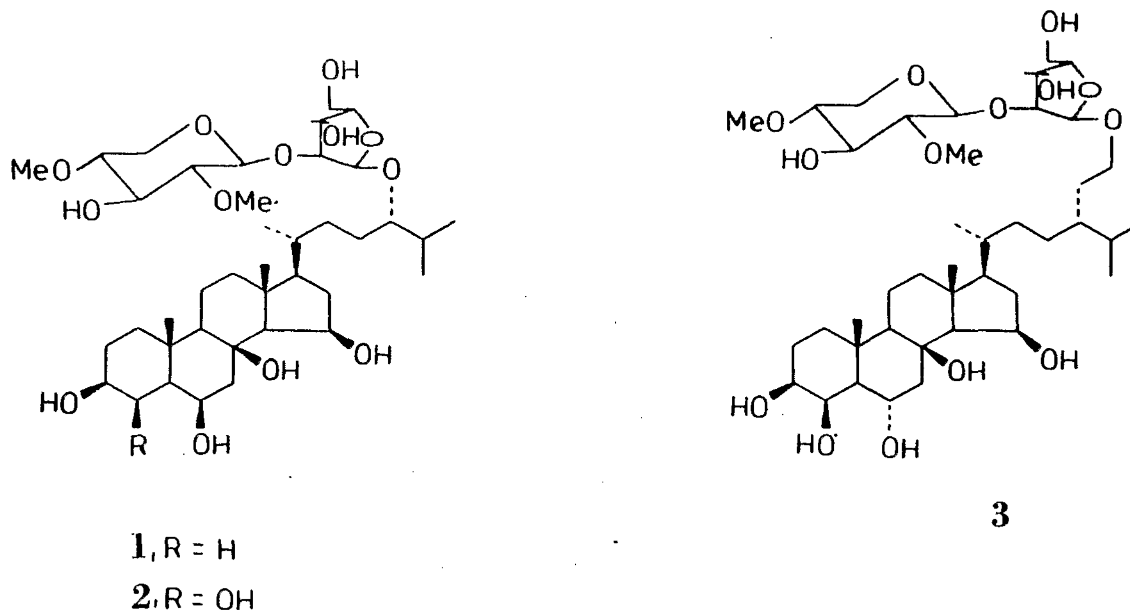
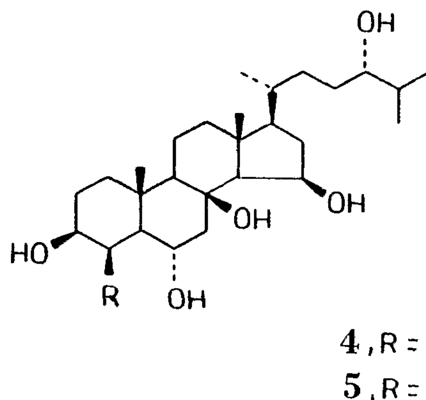
Summary — Two novel steroidal glycosides have been isolated from the starfish *Gomophia watsoni*; they co-occur with one known glycoside and two new polyhydroxysteroids. The structures of the new metabolites, **2**, **3**, **4**, and **5**, were determined on the basis of spectral data.

Steroidal glycosides composed of a polyhydroxylated steroidal aglycone and a carbohydrate portion made up from only one or two monosaccharide units, are a growing subgroup of active compounds among the glycosides isolated from starfishes.





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The first representative of these compounds, the cytotoxic nodososide, has been isolated first from Pacific *Protoreaster nodosus*[1], and later from other species[2]. Further representatives of these substances have been isolated from *Hacelia attenuata*[3,4], *Patiria pectinifera*[5], *Acanthaster planci*[6],

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Halityle regularis[7], and *Echinaster sepositus*[8]. They often co-occurr with polyhydroxysteroids[9,10].

Continuing our study of the biologically active metabolites from starfishes, we have examined the polar extracts of the Pacific starfish *Gomophia watsoni*, collected at Nouméa, New Caledonia. This starfish contained three major steroidal diglycosides and two polyhydroxysteroids. We have recently reported the structure of one of the glycoside constituents: halityloside F, **1**, from the starfish *Halityle regularis*[7]. In this paper we propose structures for the new metabolites, gomophioside A, **2**, gomophioside B, **3**, and the polyhydroxysteroids **4** and **5**.

24-Hydroxysteroids have been found as aglycones of many starfish-derived glycosides[1,3,5-7] in which the sugar moieties are attached at C(24) of the steroid. In particular, **4** and **5** have been ound as aglycones of halityloside E and D, isolated from *H. regularis*[7]. This was the first time that «non glycosidated» 24-hydroxysteroids were isolated from starfishes and the occurrence of **4** and **5** has now allowed the 24*S* configuration to be established by comparison of their ^{13}C NMR spectra with those of (24*S*)- and (24*R*)-24-hydroxycholesterols[11]. Recently we assigned the 24*S* configuration to nodososide, the 24-*O*-glycosidated steroid from *Protoreaster nodosus*, based on the GLC modification of Horeau's method applied to a 24-hydroxy compound derived from nodososide itself[12]. The ^{13}C NMR data for **4** (table 3) give now support to the previous stereochemical assignment.

EXPERIMENTAL

Extraction and isolation

The fresh animals (5.8 kg) collected off Nouméa, New Caledonia, in March 1984, were chopped and soaked in water for 4 h. The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with water (one bed volume) then with methanol. The methanol eluate was taken to dryness to give 2.4 g of glassy material, which was chromatographed on a column of LH-60 (4×60 cm) using 2:1 $MeOH/H_2O$ as eluant. Fractions of 8 ml were collected and checked by TLC on SiO_2 with 60:15:25 $BuOH/AcOH/H_2O$. Fractions 30-42 (545 mg) contained «asterosaponins», steroidal penta- or hexa-glycoside sulphates; fractions 51-100 (530 mg) contained a mixture of steroidal glycosides, polyhydroxysteroids and other compounds, which was submitted to droplet counter-current chromatography (DCCC; 7:13:8 $CHCl_3/MeOH/H_2O$ in the ascending mode at a flow of 15 ml h^{-1}; 4-ml fractions were collected) to give the following fractions:

Fractions No.	Amount, mg	Compounds
190-259	28	**3** and **5**
260-290	8	**4**
304-326	12	mixture unknown
327-350	46	**1** and **2**

Each of the above fractions was then submitted to HPLC on a C_{18} μ-bondapack column (30 cm × 7.8 mm i.d.) with 7:3 $MeOH/H_2O$ (flow rate: 5 ml min^{-1}) to give single compounds: *halityloside F*, **1**, 15.2 mg, retention time (rt) in HPLC, 34 min; $[\alpha]_D = -14$ (c=1, MeOH); FAB-MS, m/z 767 (M+Na), *gomophioside A*, **2**, 3.7 mg, rt 24 min; $[\alpha]_D = -31.3$ (c=0.3, MeOH); FAB-MS, m/z 783 (M+Na), *gomophioside B*, **3**, 3 mg, rt 34 min; $[\alpha]_D = -21.5$ (c=0.3, MeOH); FAB-MS, m/z 811 (M+Na), *(24S)-5α-cholestane-3β,6α,8,15β,24-pentol*, **4**, 3.5 mg, rt 20 min, $[\alpha]_D = +12$ (c=0.3, MeOH). EI-MS, m/z 434 (100%, $M^+ - H_2O$), 416 (75), 401 (50), 398 (25), 373 (15), 371 (30), 353 (40), 287 (30), 277 (40), and *(24S)-5α-cholestane-3β,4β,6α,8,15β,24-hexol*, **5**, 4.2 mg, rt 18 min; $[\alpha]_D = +7.2$ (c=0.4, MeOH); EI-MS, m/z 450 (10%, $M^+ - H_2O$), 432 (35), 414 (40), 396 (30), 287 (100), 285 (90). The ^1H and ^{13}C NMR spectra are in tables 1 and 2.

TABLE 1 - ^1H NMR DATA (CHEMICAL SHIFT, MULTIPLICITY, COUPLING CONSTANTS) IN CD_3OD[a]

H at C	1	2	3	4	5
3	3.65, m	*b*	*b*	3.5, m	3.5, m
4		4.10, m, $W_{1/2}$=7	4.29, m, $W_{1/2}$=7		4.29, m, $W_{1/2}$=7
6	3.87, q, 3	4.29, m, $W_{1/2}$=7	4.19, dt, 4, 10.5	3.73, dt, 4, 10.5	4.19, dt, 4, 10
7β	2.42, dd, 3, 14	2.42, dd, 3,14	2.48, dd, 4, 12	2.40, dd, 4, 12	2.40, d, 4, 12
7α		1.68, dd, 3, 14			
15	4.45, m	4.45, t, 7.5	4.45, m	4.45, t, 7.5	4.45, t, 7.5
16	2.4, m	2.4, m	2.4, m	2.4, m	2.4, m
18	1.30, s	1.30, s	1.30, s	1.30, s	1.30, s
19	1.21, s	1.47, s	1.19, s	1.01, s	1.19, s
21	0.97, d, 6.6	0.97, d, 6.4	0.96, d, 6.4	1.00, d, 7	1.00, d, 7
24	*c*	*c*	—	3.25, m	3.25, m
26, 27	0.94, d, 6.6	0.94, d, 6.8	0.87, d, 6.6	0.92, d, 6.8	0.92, d, 6.8
	0.93, d, 6.6	0.93, d, 6.6	0.90, d, 6.6	0.94, d, 6.8	0.94, d, 6.8

([a]) Assignments by spin decoupling. Sugar proton signals: in **1** and **2**, arabinose; H-1: 5.11, br s; H-2: 4.08, d, J=4 Hz; H-4: 3.98, m; H_2-5: 3.79, dd, J=12.5, 4 Hz - 3.65, dd, J=12.5, 4.8 Hz; 2,4-di-*O*-methylxylose, H-1: 4.44, d, J=7.6 Hz; H-2: 2.90, dd, J=9.0, 7.6 Hz; H-3: 3.42, t, J=9.0 Hz; H-4: 3.20, m; H_2-5: 3.14, t, J=10.6 Hz - 4.02, dd, J=10.6, 4.0 Hz. In the spectrum of compound **3** the arabinose signals were slightly shifted; H-1: 5.02, bs; H-2,3: 4.08, m; H-4: 3.93, m. ([b]) Signal overlapped with sugar peaks (*ca.* δ 3.5 ppm). ([c]) Signal hidden by the solvent.

BORONATE FORMATION FROM **4**

A mixture of **4** (1.5 mg) and phenylboronic anhydride (2 mg) in acetone was kept in a stoppered reaction vial at 90 °C for 5 h. After removal of the solvent, the 8,15-monophenylboronate was purified by passing it through a Pasteur pipet filled with a slurry of silica gel in 9:1 $CHCl_3$/MeOH; TLC on SiO_2:R_f in 80:18:2 $CHCl_3$/MeOH/H_2O 0.85 (**4** had R_f 0.57); ^1H NMR ($CDCl_3$), δ: 7.77 (2 H, d, J=6Hz, aromatic H's), 7.43 (3 H, m, aromatic H's), 4.63 (1 H, t, J= 6.3 Hz, H-15α), 3.90 (1 H, dt, J=4, 11 Hz, H-6β), 3.65 (1 H, m, H-3α), 3.33 (1 H, m, H-24), 2.47 (1 H, m, H-16), 2.31 (1 H, dd, J=13, 4 Hz, H-7β, 1.26 (3 H, s, H_3-18), 1.09 (3 H, s, H_3-19), 0.93 (9 H, apparent t, J=7 Hz, H_3-21, H_3-26, and H_3-27).

TABLE 2 - ^{13}C NMR DATA FOR COMPOUNDS **1** - **5** AT 62.9 Mz, δ (ppm) IN CD_3OD[a]

Carbon No.	1	2	N[b]	3	H[c]	4	5
1	41.4	41.0		39.8		39.5	39.7
2	31.7	26.6		26.2		31.5	26.3
3	72.4	73.2		73.7		72.2	73.7
4	36.4	77.4		69.2		32.4	69.1
5	49.8	51.1		57.4		54.0	57.4
6	74.8	76.3		64.8		67.7	64.8
7	44.3	44.1		49.8		49.8	49.8
8	79.4	79.1		77.5		77.5	77.4
9	57.1	57.4		58.1		57.6	58.0
10	36.8	36.9		38.2		38.0	38.1
11	19.9	19.3		19.3		19.8	19.0
12	43.3	43.1		43.4		43.5	43.4
13	44.4	44.3		44.5		44.5	44.4
14	61.9	61.8		62.9		62.8	62.9
15	71.4	71.4		71.2		71.2	71.2
16	42.4	42.1		42.5		42.4	42.4
17	58.1	58.0		58.6		58.1	58.5
18	16.6	16.6		16.5		16.5	16.5
19	15.7	18.6		16.9		14.1	16.9
20	36.4	36.5	36.3	36.6		36.4	36.4
21	19.1	19.1	19.0	19.2		19.1	19.1
22	33.0	32.9	32.8	34.9	34.8	33.4	33.3
23	28.9	28.6	28.6	28.4	28.8	31.8	31.7
24	84.8	84.5	84.5	42.4	42.6	78.2	78.2
25	31.7	31.6	31.5	30.6	30.6	34.5	34.5
26	18.3	18.3	18.3	19.1	19.1	17.5	17.4
27	18.2	18.2	18.2	20.0	20.0	19.4	19.2
28	—	—	—	31.8	31.8	—	—
29	—	—	—	67.9	69.1	—	—

([a]) Assignments by DEPT pulse sequence. δ_C of sugar carbon atoms in **2**: *arabinose*, C(1): 107.9, C(2): 92.6, C(3): 77.8, C(4): 83.9, C(5): 62.6; *xylose*, C(1): 105.1, C(2): 84.9, C(3): 76.5, C(4): 80.9, C(5): 64.4, OMe: 59.0-61.0 ppm. In the spectrum of **1** the sugar carbon signals coincided within ±0.1 ppm with those of **2**, while in the spectrum of **3** significative deviations were observed for C(2) and C(4) of arabinose, δ_C 91.4 and 84.5, respectively, and C(1) of xylose, δ_C 104.6 ppm. ([b]) **N**=nodososide: (24*S*)-24-*O*-[2-*O*-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl]-5α-cholestane-3β-5,6β,8,15α-24-hexol. The complete ^{13}C NMR spectra were described in references 1 (pyridine-d_5) and 7 (methanol-d_4). ([c]) **H**=halityloside H: 24-ethyl-29-*O*-[2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl]-5α-cholestane-3β, 4β,6α,8,15β,16β,29-heptol. The complete ^{13}C NMR spectrum is described in ref. 7.

INSTRUMENTAL

^1H and ^{13}C NMR spectra were recorded on a Bruker WM-250 instrument. The DEPT experiments were performed using polarization transfer pulses of 90° and 135°; in the former case only CH groups were obtained, while in the latter case positive signals for CH and CH_3 and negative ones for the CH_2 groups were obtained. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. Fast Atom Bombardment (FAB) mass spectra were obtained on an AEI MS 902 mass spectrometer, equipped with a Kratos FAB source. Samples were dissolved in a glycerol matrix and placed on a copper probe tip prior to bombardment with Ar atoms of 2-6 kV energy. Droplet counter-current chromatography (DCCC) was carried out on a DCC-A apparatus manufactured by Tokyo Rikakikai Co., equipped with 250 tubes. HPLC separation was made on a μ-bondapack C_{18} column (7.8 mm × 30 cm) using a differential refractometer detector, model 401, a U6K injector and a solvent-delivery system, M 6000A, all from Waters Associates. Rotations were taken on a Perkin Elmer mod. 141 polarimeter.

RESULTS AND DISCUSSION

Fractionation of the aqueous extracts from *Gomophia watsoni* (5.8 kg, fresh) was carried out by successive chromatography on Amberlite XAD-2 resin and Sephadex LH-60, which allowed separation of the more polar sulphated «asterosaponins» from the non-sulphated polar steroids. Fractions containing the non-sulphated material were purified further by droplet counter-current chromatography (DCCC) and finally by high pressure liquid chromatography (HPLC) over a C_{18} bonded-phase column with 30% aqueous methanol to give five compounds in minute amounts. Halityloside F, **1**, whose structure was reported previously[7], is the major component (15 mg). The new compounds, gomophioside A, **2**, gomophioside B, **3** and the polyhydroxysteroids **4** and **5** were obtained in amounts ranging from 3 to 4 milligrams.

GOMOPHIOSIDE A, (24*S*)-24-*O*-[2,4-di*O*-METHYL-β-D-XYLOPYRANOSYL-(1→2)-α-L-ARABINOFURANOSYL]-5α-CHOLESTANE-3β,4β,6β,8,15β,24-HEXOL, **2**

An examination of its spectral data indicated that **2** contained the same 2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl moiety as **1** and other glycosides from *Halityle regularis*[7]. Of the remaining signals (27 signals) present in the ^{13}C NMR spectrum of **2**, six were assigned to carbons bonded to oxygen (table 2). Thus, gomophioside A, **2**, contains one more hydroxyl group in the aglycone than halityloside F, **1**. This conclusion was also supported by the FAB mass spectrum of **2**, which showed molecular ion species at *m/z* 761-783 (M+H, M+Na), sixteen mass units shifted relative to **1** (**1**, 767, M+Na), and fragmentation with loss of 292 mass units from M+H (*m/z* 469), corresponding to the disaccharide unit, and se-

quential loss of water (*m/z* 451, 433, 415, 397). ^{13}C NMR indicated C(24) to be the site of glycosidation, and also established that **2** differs from **1** by the presence of a sixth hydroxyl group at the 4β-position of the steroid aglycone. The most significant features of the ^{13}C NMR spectrum of gomophioside A, **2**, which suggested the location of the hydroxyl group at C(4β), were the upfield shift exhibited by C(2) (5.1 ppm) and the downfield shifts experienced by C(3) (0.8 ppm), C(5) (1.3 ppm) and C(19) (2.9 ppm) relative to **1**. The hydroxyl β shifts at C(3) and C(5) as well as the hydroxyl γ shifts at C(2) and the hydroxyl δ shift at C(19) are quite close to the shifts observed in 4β-hydroxysteroids [*e.g.* in 5α-cholestane-3β,4β-diol, relative to the parent 5α-cholestane-3β-ol, the β-shifts at C(3) and C(5) are +0.8 and +4.0 ppm, respectively, the γ shift at C(2) is −5.5 ppm and the δ shift at C(19) is +3.5 ppm)[13]. We note a large deviation from additivity for C(4) and C(6) (5.7 and 4.7 ppm downfield, respectively) in agreement with that expected for compounds with 1,3-*syn*-diaxial (4β,6β) hydroxyl groups[13]. It is possible that such 1,3-*syn*-diaxial arrangement also causes the small β-effect observed for C(5) in **2** (1.3 ppm) in comparison to the large one observed in the model 5α-cholestane-3β,4β-diol (+4.0 ppm). It is worth of note that C(11) is affected by the presence of the 4β-hydroxyl group, since its resonance is shifted slightly, but consistently, in **2** relative to **1** (19.3 *vs* 19.9 ppm). Significantly, a similar shift was observed in 5α-cholestane-3β,4β-diol relative to 5α-cholestane-3β-diol (20.6[13] *vs* 21.3 ppm[14]) and in many 4β-hydroxysteroids isolated from starfishes[3,7,9,15]. Comparison of the ^{1}H NMR spectral data of **2** with those of **1** (table 1) confirmed the above conclusion. In particular, the H_3-19 signal was shifted downfield to δ 1.47 [in agreement with the 1,3-diaxial interaction $C(19)H_3/4\beta$-OH], the H-6 signal was also shifted downfield to δ 4.29, and the H-4 was observed as a narrow signal at δ 4.10. The remaining proton signals (table 1) were almost identical with those of halityloside F, **1**. The 24*S* configuration is proposed, because the chemical shifts of the signals assigned to the side-chain carbons were virtually identical to those of the corresponding signals for nodososide (table 2).

GOMOPHIOSIDE B, (24*R*)-24-EHYL-29-*O*-[2,4-DI-*O*-METHYL-β-D-XYLOPYRANOSYL-(1→2)-α-L-ARABINOFURANOSYL]-5α-CHLOLESTANE-3β,4β,6α,8,15β,29-HEXOL, 3

An examination of its spectral data indicated that **3** contains the same disaccharide unit as **1** and **2**. Furthermore, the ^{1}H and ^{13}C NMR spectra indicated that the aglycone of gomophioside B, **3**, share the same steroidal 3β,4β,6α,8,15β-pentahydroxy-tetracyclic nucleus as halityloside D, (24*S*)-24-*O*-[2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl]-5α-cholestane-3β,4β,6α,8,15β,24-hexol, isolated from *Halityle regularis* and attenuatoside B-1, isolated from *Hacelia attenuata*[3b]. The FAB-mass spectrum of **3** showed a molecular ion species at *m/z* 811 (M+Na), shifted twenty-eight mass units relative to halityloside D (*m/z* 783). 24-(β-hydroxyethyl)cholestane aglycones have been already found in several steroidal 29-*O*-glycosides isolated from *Halityle regularis*[7]. In agreement with a 24-(β-hydroxyethyl) side chain, the ^{1}H NMR of **3** showed three methyl doublets at δ 0.96, 0.90 and 0.87 and the ^{13}C NMR showed signals for the side-chain carbons with chemical shifts virtually identical to those of the corresponding signals of the previous steroidal 29-*O*-glycosides (table 2), which were assigned by comparison with a synthetic mixture of 29-hydroxyclionasterol and 29-hydroxysitosterol[16]. ^{13}C NMR data also established the location of the glycosyl residue at C(29).

The 24*R* configuration is compatible with both ^{1}H and ^{13}C NMR data but is not rigorously established. In the 220-MHz spectrum of 29-hydroxyclionasterol (24*R*), the C(26) and C(27) methyl protons appear as a triplet at δ 0.84 because coincidental overlap of the low-field arm of one doublet (δ 0.83) with the high-field arm of the other (δ 0.86). On the other hand, the C(26) and C(27) methyl protons appear as two overlapping doublets at δ 0.84 and 0.85 in the spectrum of 29-hydroxysitosterol (24*S*)[16]. In our spectrum, the isopropyl methyl protons appear as two doublets separated by 0.03 ppm as in the case of the 24*R* isomer. Very recently, the ^{13}C NMR spectra of the (24*R*)-and (24*S*)-24-ethyl-5α-cholest-7-ene-3β,29-diol were measured[17] and significative differences were obtained for the resonances of the isopropyl methyl carbons. In the 24*S* isomer, the C(26) and C(27) appear as very close signals at 19.2 and 19.3 ppm, while in the 24*R* isomer they are split by *ca* 1 ppm (18.6-19.7 ppm). Once again, our values (19.1-20.0 ppm) compared better with those of the 24*R* isomer.

(24*S*)-5α-CHOLESTANE-3β,6α,8,15β,24-PENTOL, 4

In the electron-impact mass spectrum the peak at highest mass observed (*m/z* 434) corresponded to loss of water from the molecular formula $C_{27}H_{48}O_5$ (fully saturated cholestane pentol), which was derived from DEPT ^{13}C NMR. ^{13}C NMR spectra also showed the absence of carbon-carbon double bonds and indicated that of the five hydroxyl groups, four were secondary and one tertiary (DEPT pulse sequence). The ^{1}H NMR spectrum contained several features already observed in the spectrum of halityloside E[7], namely a multiplet

TABLE 3 - ^{13}C NMR CHEMICAL SHIFTS (ppm) OF **4** AND THE MODEL (24*S*)- AND (24*R*)-24-HYDROXYCHOLESTEROLS MEASURED IN $CDCl_3$ [a]

Compounds	C(22)	C(23)	C(24)	C(25)	C(26,27)
Natural **4**	32.1	30.7	77.2	33.2	16.9-19.0
(24*S*)-Hydroxycholesterol [11]	32.1	30.6	77.0	33.0	16.7-18.8
(24*R*)-Hydroxycholesterol [11]	31.9	30.4	76.6	33.4	17.2-18.6

([a]) Further carbon resonances in **4** ($CDCl_3$); C(1): 38.3, C(2): 31.0, C(3): 71.5, C(4): 31.7, C(5): 52.8, C(6): 67.0, C(7): 48.6, C(8): 77.2, C(9): 56.4, C(10): 37.0, C(11): 18.7, C(12): 42.2, C(13): 43.4, C(14): 61.7, C(15): 71.1, C(16): 41.8, C(17): 56.9, C(18): 16.1, C(19): 13.6, C(20): 35.0, C(21): 18.6 ppm.

around δ 3.5, a double triplet at δ 3.73 (J=4, 10.5 Hz), and a triplet at δ 4.45 (J=7.5 Hz), assigned to hydroxy-methine protons at positions 3α,6β and 15α. The one-proton double doublet at δ 2.40 (J=12,4 Hz) coupled with H-6β by 4 Hz was assigned to the equatorial proton at C(7)(7β). The lack of additional coupling to the proton with δ 2.40 [C(7)] indicated an adjacent quaternary centre [C(8)]. Thus, the tertiary hydroxyl group was located there. This was confirmed by the formation of a phenylboronate (8β,15β) when **4** was treated with phenylboronic anhydride. The fifth hydroxyl group was placed at C(24) mainly based on ^{13}C NMR data, and comparison with 24-hydroxycholesterols. The ^{13}C NMR spectra of 24-hydroxycholesterols have been described and characteristic differences between 24*R* and 24*S* isomers recognized [11]. In the spectrum of our sample (taken in $CDCl_3$) the shift values of the side-chain carbons were in close agreement with those reported for (24*S*)-24-hydroxycholesterol as compared with those for the corresponding 24*R* compound (table 3).

(24*S*)-5α-CHOLESTANE-3β,4β,6α,8,15β,24-HEXOL, **5**

This was related to **4** by introduction of one hydroxyl group at position 4β, as indicated by comparison of its spectral data with those of **4** (table 1 and 2).

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