84. Novel Cholic-Acid-Type Sterones of *Deltocyathus magnificus*, a Deep-Water Scleractinian Coral from the Loyalty Islands, SW Pacific

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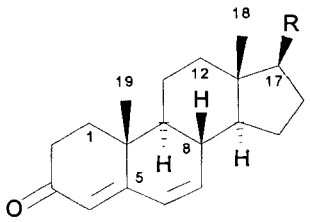
Cholic-acid-type 3-keto steroids **1**–**8**, all isolated from *Deltocyathus magnificus* (**2**–**8** after CH_2N_2 treatment), are the first secondary metabolites obtained from a deep-water scleractinian. Steroids **1**–**3**, **5**, and **7** are new, their main structural oddities being loss of C(24) (see **5**) or hydroxylation in the side chain (see **3** and **7**). Steroids **4**, **6**, and **8**, from the same coral, were previously known from other marine organisms.

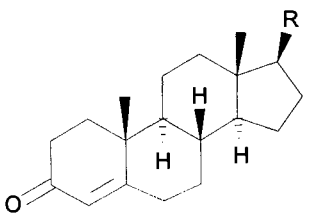
1. Introduction. – Within the Anthozoa, the octocorals have been a privileged target of chemical research. A wealth of information on secondary metabolites resulted from this investigation. In contrast, comparatively little information has thus far been obtained for the hexacorals, especially for the species-rich order Scleractinia. Previously examined Scleractinia were all from shallow water. Secondary metabolites reported from them comprise bioactive tryptophan-derived alkaloids, isolated from various members of the suborder Dendrophylliina [1], polyacetylenes from one species in the suborder Astrocoeniina [2], as well as plant-type triterpenoids [3] and antiviral polysaccharides [4] from a few species in the suborder Faviina.

The whole world of deep-water scleractinians had not yet been touched. Less known to the great public and to chemists, they are nevertheless very common in the World Ocean. Comprising many species (number of the same order as the reef corals) they occur worldwide, from Antarctica to high northern latitudes (*ca.* 70° N at Norway) and down to depths of 6200 m in the Aleutian Trench. This ecologically wide range and taxonomic diversity suggest that there could be chemical diversity of secondary metabolism. As a first approach to this unexplored world, we here report on the isolation of unusual, cholid-acid-type sterones from *Deltocyathus magnificus* MOSELEY, 1876 (suborder Caryophylliina, family Caryophylliidae). Only two of these sterones were already known as natural products, isolated from a shallow-water nudibranch gastropod from British Colombia, probably as metabolized products from a preyed sponge [5].

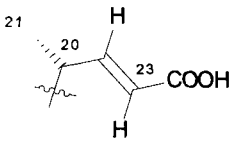
2. Results and Discussion. – The structural analysis of the steroids of *D. magnificus* was centered on the most abundant one, isolated in the natural free carboxylic form **1**. The composition $C_{24}H_{32}O_3$ rests on HR-MS measurements on the molecular ion, while the tetracyclic core was elucidated from typical δ(H) for steroidal Me(18) and Me(19) groups (2*s*), as well as from the observation of a typical α-enonic, shielded H–C(4) and its long-range coupling to C*H*(6)=C*H*(7) (*AB*). This core structure was confirmed by ^{13}C-NMR data (*Table*). MS Data indicating C(17)–C(20) cleavage from the *m*/*z* 269 frag-

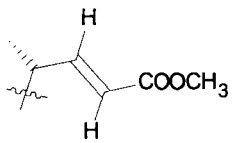


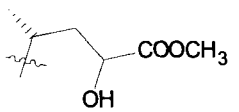
1 R=A **4** R=D
2 R=B **5** R=E
3 R=C

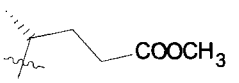


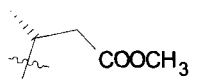
6 R=B **8** R=D
7 R=C

A = 

B = 

C = 

D = 

E = 

ment ion, while the dienone system was also supported from typical UV absorptions. The structure of the truncated side chain was secured from COSY maps (correlation of H–C(23) with H–C(22) and H–C(20) and of H–C(20) with Me(21) (*d*)). Problems were raised instead by ^{13}C-NMR spectra, the resonances for neither C(24) nor C(23) emerging when using either $CDCl_3$ or $CDCl_3/CD_3OD$ mixtures as solvents. It was by recourse to the more sensitive inverse-detection technique that C(23) could be detected at δ *ca.* 119.7 ppm, as correlation map with H–C(23). However, on addition of CD_3COOD, both C(24) and C(23) clearly emerged even in ordinary spectra, which suggests that broadening of signals resulting from intermolecular H-bonding is responsible for failure to detect these C-atoms in $CDCl_3$ or $CDCl_3/CD_3OD$ as solvents. Thus, all C-atoms could be assigned from ^{1}H,^{1}H COSY and HMBC experiments (*Table* and *Exper. Part*). These attributions were confirmed on ester **2** obtained from **1** by treatment with CH_2N_2 (*Table* and *Exper. Part*).

Clues to the structure for the new side-chain-hydroxylated steroid **3** were differences with respect to **1** and **2** in ^{1}H- and ^{13}C-NMR data, in particular the absence of signals for the unsaturated side chain.

The side-chain signals of **3** appear at δ(C) 40.98 (*t*) and 68.08 (*d*), and at δ(H) 4.24 (*ddd*), the latter signal being coupled to those of OH (*d*) and CH_2(22). Further evidence for the OH–C(23) group is deshielding of C(24) and selective-INEPT data (*Exper. Part*); this fits for fragment C on the assumption of intramolecular H-bonding; this is probably also the reason for abundant $M^{+\cdot}$, enough to allow measuring the isotopic composition by HR-MS.

Table. *^{13}C-NMR Data of Steroids Isolated from the Scleractinian Coral* D. magnificus

	1[a])	**1**[d])	**2**[f])	**3**[f])	**4**[f])	**5**[f])	**6**[f])	**7**[f])
C(1)	33.62 (*t*)	33.48 (*t*)	33.96 (*t*)	33.96 (*t*)[g])	33.97 (*t*)[g])	33.96 (*t*)[g])	35.71 (*t*)	35.71 (*t*)
C(2)	33.56 (*t*)	33.48 (*t*)	33.92 (*t*)	33.92 (*t*)[g])	33.92 (*t*)[g])	33.92 (*t*)[g])	33.97 (*t*)	33.99 (*t*)
C(3)	200.60 (*s*)	201.08 (*s*)	199.55 (*s*)	199.60 (*s*)	199.60 (*s*)	199.57 (*s*)	199.53 (*s*)	199.58 (*s*)
C(4)	122.94 (*d*)	122.85 (*d*)	123.64 (*d*)	123.55 (*d*)	123.55 (*d*)	123.60 (*d*)	123.84 (*d*)	123.77 (*d*)
C(5)	165.00 (*s*)	165.41 (*s*)	163.73 (*s*)	163.89 (*s*)	163.92 (*s*)	163.83 (*s*)	171.32 (*s*)	171.51 (*s*)
C(6)	127.59 (*d*)	127.57 (*d*)	127.94 (*d*)	127.84 (*d*)	127.84 (*d*)	127.90 (*d*)	32.88 (*t*)	32.91 (*t*)
C(7)	141.76 (*d*)	142.03 (*d*)	141.08 (*d*)	141.35 (*d*)	141.40 (*d*)	141.21 (*d*)	31.96 (*t*)	32.02 (*t*)
C(8)	37.58 (*d*)	37.55 (*d*)	37.68 (*d*)	37.71 (*d*)	37.73 (*d*)	37.72 (*d*)	35.62 (*d*)	35.60 (*d*)
C(9)	50.51 (*d*)	50.40 (*d*)	50.65 (*d*)	50.65 (*d*)	50.65 (*d*)	50.61 (*d*)	53.76 (*d*)	53.77 (*d*)
C(10)	35.97 (*s*)	35.94 (*s*)	36.07 (*s*)	36.07 (*s*)	36.07 (*s*)	36.07 (*s*)	38.59 (*s*)	38.60 (*s*)
C(11)	20.43 (*t*)	20.39 (*t*)	20.65 (*t*)	20.66 (*t*)	20.67 (*t*)	20.65 (*t*)	21.01 (*t*)	21.02 (*t*)
C(12)	39.16 (*t*)	39.09 (*t*)	39.36 (*t*)	39.57 (*t*)	39.52 (*t*)	39.41 (*t*)	39.45 (*t*)	39.65 (*t*)
C(13)	43.52 (*s*)	43.50 (*s*)	43.71 (*s*)	43.63 (*s*)	43.46 (*s*)	43.54 (*s*)	42.74 (*s*)	42.63 (*s*)
C(14)	53.07 (*d*)	52.98 (*d*)	53.29 (*d*)	56.26 (*d*)	55.69 (*d*)	55.73 (*d*)	55.68 (*d*)	56.38 (*d*)
C(15)	23.48 (*t*)	23.47 (*t*)	23.72 (*t*)	23.69 (*t*)	23.70 (*t*)	23.69 (*t*)	24.18 (*t*)	24.13 (*t*)
C(16)	27.73 (*t*)	27.77 (*t*)	28.04 (*t*)	28.18 (*t*)	28.04 (*t*)	28.12 (*t*)	28.05 (*t*)	28.20 (*t*)
C(17)	54.64 (*d*)	54.47 (*d*)	54.77 (*d*)	53.49 (*d*)	53.42 (*d*)	53.44 (*d*)	54.89 (*d*)	55.93 (*d*)
C(18)	11.87 (*q*)	11.86 (*q*)	12.17 (*q*)	11.96 (*q*)	11.90 (*q*)	11.94 (*q*)	12.22 (*q*)	12.03 (*q*)
C(19)	15.97 (*q*)	15.94 (*q*)	16.31 (*q*)	16.28 (*q*)	16.29 (*q*)	16.30 (*q*)	17.39 (*q*)	17.39 (*q*)
C(20)	39.37 (*d*)	39.49 (*d*)	39.68 (*d*)	32.34 (*d*)	35.35 (*d*)	33.72 (*d*)	39.70 (*d*)	32.34 (*d*)
C(21)	18.96 (*q*)	18.87 (*q*)	19.24 (*q*)	17.97 (*q*)	18.26 (*q*)	19.54 (*q*)	19.22 (*q*)	17.97 (*q*)
C(22)	154.72 (*d*)[b])	155.60 (*d*)[e])	154.52 (*d*)	40.98 (*t*)	31.05 (*t*)[h])	41.35 (*t*)	154.75 (*d*)	41.01 (*t*)
C(23)	[c])	118.74 (*d*)[e])	118.85 (*d*)	68.08 (*d*)	30.94 (*t*)[h])	171.14 (*s*)	118.71 (*d*)	68.12 (*d*)
C(24)	[c])	169.79 (*s*)[e])	167.39 (*s*)	176.52 (*s*)	174.61 (*s*)	–	167.44 (*s*)	176.56 (*s*)
MeO	–	–	51.41 (*q*)	52.53 (*q*)	51.51 (*q*)	51.40 (*q*)	51.39 (*q*)	52.52 (*q*)

[a]) $CDCl_3/CD_3OD$ 21:4; assignment from ^{13}C, DEPT, $^1H,^1H$-COSY, HMQC, and HMBC.
[b]) $w_{1/2} = 8.7$ Hz.
[c]) Not detected.
[d]) $CDCl_3/CD_3OD/CD_3COOD$ 20:4:1.
[e]) $w_{1/2} = 2.0$, 1.9, and 2.2 Hz for C(22), C(23), and C(24), resp.
[f]) $CDCl_3$.
[g])[h]) This data can be interchanged in the same column.

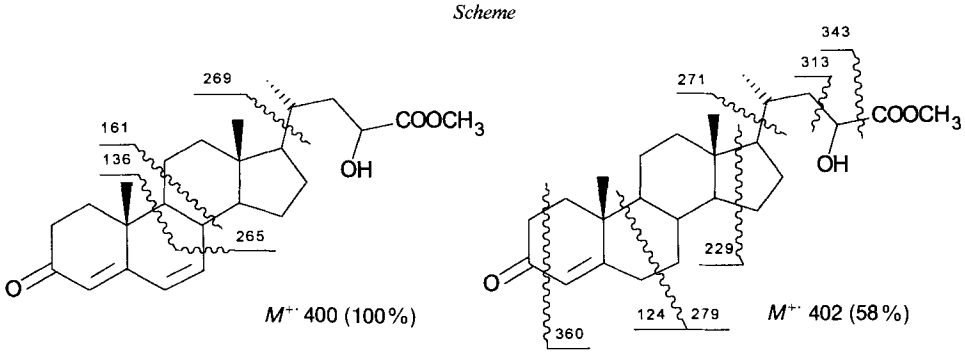
The series of MS fragmentations shown in the *Scheme* furnish additional evidence, in analogy with known α,β-unsaturated 3-keto steroids [6].

NMR Spectra revealed that compound **4** has the same tetracyclic core as **2**. Its saturated side chain was suggested by ^{13}C-NMR data (*t* in place of the 2 *d* of **2**). In agreement, the ester C=O was shifted downfield by 7.2 ppm. MS afforded further support to structure **4**, which was found to correspond to a metabolite already isolated from both human hepatoblastoma cells in culture [7a] and the transformation of ursodeoxycholic acid in cultures of the bacterium *Alcaligenes recti* under aerobic conditions [7b].

The same tetracyclic core and lack of side-chain olefinic ^{13}C-NMR *d*'s were found for the new steroid **5** as well; a single, deshielded CH_2 group (δ(C) 41.35 ppm (*t*) and δ(H) 2.45 and 2.40 ppm) revealed the shortened side chain E. This was confirmed by HR-MS on M^+ 370.

Structures **6** and **8** were assigned from the lack of NMR signals for the C(6)=C(7) system and identity of side-chain signals with the corresponding ones of **2** and **4**, respec-

Scheme



tively. HR-MS supported these conclusions. Steroids **6** and **8** correspond to compounds isolated from the nudibranch gastropod *Aldisa sanguinea cooperi* [5], while steroid **8** also corresponds to a degradation product of cholic acids in anaerobic cultures of the bacterium *Pseudomonas* sp. [8], while steroid **6** was also obtained by chemical synthesis [9].

Finally, the structure for the new steroid **7** was assigned by comparison with **3**, relying on the absence of NMR signals for the C(6)=C(7) system and downfield shift of the ^{13}C-NMR *s* for both C(5) and C(24). MS Fragmentations for **7** (*Scheme*) warrant the same comments as for **3**.

If microorganisms are not involved, the identity of precursor acids of **6** in phylogenetically and ecologically as distant organisms as a deep-water scleractinian coral like *D. magnificus* and a shallow-water nudibranch gastropod like *A. sanguinea cooperi* speaks for convergence. Whether this is towards serviceable molecules cannot be answered since we have not carried out biological assays with our steroids. Neglect of this problem from our side is due to the difficulty of dealing with potential predators of a coral that inhabits deep, remote waters. To further defend our position, we believe that the assays carried out for **8** on goldfish – a freshwater species that has no chance of becoming a predator of *A. sanguinea cooperi* – hardly warrants the assumed [5] antifeedant role for this compound. To have found **4** and **8** also in bacterial cultures [7] [8], and the first one also in hepatoblastoma cells [7], speaks for chemical bias towards chemically favored structures. In this context, hydroxylation as in side chain C and degradation as in side chain E are such structural oddities that justify *per se* having extended the chemical study to deep-water scleractinians: none of these steroids has been found in any other organism than *D. magnificus*.

The biosynthesis of these steroids may be conceived to start from cholesterol as for cholic acids [7] [10], arriving at D-type side chain, from which C-type side chain may originate as a branching point to either A-type or E-type side chain.

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Experimental Part

1. *General.* All evaporations were carried out under reduced pressure. TLC: *Merck* silica gel *60 PF_{254}*. Reversed-phase flash chromatography (FC): *Merck LiChroprep RP-18* (40–63 μm). HPLC: *Merck LiChrosorb RP-18* (7 μm) or *Merck-LiChrosorb Si-60* (7 μm), in both cases with 25 × 1 cm columns; solvent flow 5 ml min^{-1}; UV monitoring at λ 255 nm. Polarimetric data: *JASCO-DP-181* polarimeter. UV (λ_{max} in nm, ε in mol^{-1} 1 cm^{-1}): *Perkin-Elmer-Lambda-3* spectrophotometer. NMR: *Varian-XL-300* (^{13}C at 75.43 MHz, ^{1}H at 299.94 MHz); δ in ppm rel. to $CHCl_3$ (7.26 ppm) or $CHCl_3$ (77.00 ppm) and *J* in Hz; probe temp. 20° and solvent $CDCl_3$ if not otherwise stated; br. indicates submerged signals; NOE means differential NOE, obtained with preirradiation of 6 s and reported as irradiated proton(s)→relaxed proton(s) (% increment); multiplicities and C and H assignments from DEPT [11], $^{1}H,^{1}H$-COSY [12], $^{1}H,^{13}C$-COSY [13], and HMBC [14] (reported as ^{1}H→correlated ^{13}C). MS (*m*/*z* (%)): EI and HR; *Kratos MS80* with home-built acquisition system.

2. *Collection and Isolation. Deltocyathus magnificus* was collected by beam trawl near the Loyalty Islands, SE Pacific during cruise MUSORSTOM 6: sample 526M, stat. CP 413, 20°40.10′S, 167°03.50′E, depth 463 m; sample 527M, stat. CP 415, 20°40.20′S, 167°03.95′E, depth 461 m, both on 15th February 1989. The corals were immediately soaked in 95% EtOH. After a long storage of these samples in the cold and dark, the solvent was evaporated in summer 1995, leaving an aq. residue that was subjected to reversed-phase FC (H_2O/MeOH gradient elution); the eluates with H_2O/MeOH 1:3 and 1:4 gave UV-absorbing TLC spots with R_f comprised between 0.3 and 0.7 (AcOEt) for a mixture of compounds (NMR). A fraction of this mixture was subjected to reversed-phase HPLC (MeCN/H_2O 7:3): **1** as a broad peak at t_R 20 min (7.3 mg). Subsequent eluates were combined with the remaining mixture from the above FC and subjected to reversed-phase HPLC (MeCN/H_2O/AcOH 75:25:2) to give elution peaks at t_R 6.0, 9.4, 10.7, and 13.0 min. These eluates were evaporated separately, the residues dissolved in MeOH (*ca.* 5 ml/mg), treated with excess of CH_2N_2 in Et_2O (*ca.* 20 ml/mg), stirred overnight at r.t. in the dark, and finally subjected to HPLC. The mixture of methyl esters eluted at t_R 6.0 min was subjected to *Si-60* HPLC (hexane/AcOEt 13:7): **7** (t_R 14.5 min, 1.6 mg) and **3** (t_R 17.5 min, 2.0 mg). The mixtures of methyl esters eluted at t_R 10.7 and 13.0 min were subjected separately to *Si-60* HPLC (hexane/AcOEt 4:1): **6** (t_R 14.0 min, 2.3 mg) and **2** (t_R 15.5 min, 2.6 mg) from the first, and **8** (t_R 9.7 min, 1.0 mg) and **4** (t_R 10.5 min, 4.4 mg) from the latter. The mixture of methyl esters eluted at t_R 9.4 min was subjected to *RP-18* HPLC (MeOH): **5** (t_R 5.4 min, 1.1 mg).

3. *(22E)-3-Oxochola-4,6,22-trien-24-oic Acid* (**1**). $[\alpha]^{20}$ (λ/nm) = −34.1 (589), −24.3 (546), +138.7 (435, *c* = 0.37, EtOH). UV (EtOH): 283 (23200). ^{1}H-NMR ($CDCl_3/CD_3OD$ 21:4): 1.91 (*ddd*, J_{gem} = 14.2, $J(1\beta,2\beta)$ = 5.4, $J(1\beta,2\alpha)$ = 2.1, H_β–C(1)); 1.61 (br. *ddd*, $J_{gem} = J(1\alpha,2\beta)$ = 14.2, $J(1\alpha,2\alpha)$ = 5.2, $J(1\alpha,19)$ small, H_α–C(1)); 2.47 (*ddd*, J_{gem} = 17.9, $J(2\beta,1\alpha)$ = 14.2, $J(2\beta,1\beta)$ = 5.4, H_β–C(2)); 2.33 (br. *ddd*, J_{gem} = 17.9, $J(2\alpha,1\alpha)$ = 5.2, $J(2\alpha,1\alpha)$ = 2.1, H_α–C(2)); 5.56 (br. *s*, *J*(4,6) and *J*(4,7) small, H–C(4)); 6.01 (br. *dd*, *J*(6,7) = 9.8, *J*(6,8) = 2.3, *J*(6,4) small, H–C(6)); 6.05 (br. *dd*, *J*(7,6) = 9.8, *J*(7,8) = 1.3, *J*(7,4) small, H–C(7)); 2.12 (br. *dd*, *J*(8,9) = *J*(8,14) = 9.5, *J*(8,6) = 2.3, *J*(8,7) = 1.3, H–C(8)); 1.17 (br., H–C(9)); 1.13, 1.45 (br., 2 H–C(11)); 1.18, 1.95 (br., 2 H–C(12)); 1.18 (br., H–C(14)); 1.16, 1.35 (br. and *ddd*, resp., 2 H–C(15)); 1.68 (br., 2 H–C(16)); 1.20 (br., H–C(17)); 0.70 (*s*, Me(18)); 1.02 (*s*, Me(19)); 2.22 (br., H–C(20)); 1.00 (*d*, *J*(21,20) = 6.7, Me(21)); 6.72 (br. *dd*, *J*(22,23) = 15.5, *J*(22,21) = 9.0, H–C(22)); 5.64 (br. *d*, *J*(23,22) = 15.5, H–C(23)); the last two signals sharpened in $CDCl_3/CD_3OD/CD_3COOD$ 20:4:1. NOE: H–C(4)→H–C(6) (6); Me(18)→H–C(8) (8), H–C(20) (4); Me(19)→H_β–C(1) (10), H_β–C(2) (6), H–C(8) (10). HMBC: H_β–C(1)→C(3), C(5), C(10); H_α–C(1)→C(19); H_β–C(2)→C(3); H_α–C(2)→C(3); H–C(4)→C(2), C(6); H–C(6)→C(4), C(5); H–C(7)→C(5), C(9), C(14); H–C(8)→C(6), C(7), C(9), C(14); H–C(12) at 1.95 ppm→C(9), C(14); Me(18)→C(14), C(17); Me(19)→C(5), C(9); H–C(20)→C(17), C(22); Me(21)→C(17), C(20), C(22); H–C(22)→C(24). MS: 368 (12, M^+), 269 (9, C(17)–C(20) breaking), 175 (10), 161 (6), 28 (100). HR-MS: 368.2333 ± 0.0028 ($C_{24}H_{32}O_3^+$, calc. 368.23514).

4. *Methyl (22E)-3-Oxochola-4,6,22-trien-24-oate* (**2**). $[\alpha]^{20}$ (λ/nm) = −26.9 (589), −22.3 (546), +136.9 (435, *c* = 0.13, EtOH). UV (EtOH): 283 (23800). ^{1}H-NMR: 1.98 (br., H_β–C(1)); 1.71 (br. *ddd*, $J_{gem} = J(1\alpha,2\beta)$ = 14.1, $J(1\alpha,2\alpha)$ = 5.1, $J(1\alpha,19)$ small, H_α–C(1)); 2.56 (*ddd*, J_{gem} = 17.8, $J(2\beta,1\alpha)$ = 14.1, $J(2\beta,1\beta)$ = 5.5, H_β–C(2)); 2.43 (*dddd*, J_{gem} = 17.8, $J(2\alpha,1\alpha)$ = 5.1, $J(2\alpha,1\beta)$ = 2.1, $J(2\alpha,4)$ = 0.9, H_α–C(2)); 5.67 (br. *s*, $J(4,2\alpha)$ = = 0.9, *J*(4,6) and *J*(4,7) small, H–C(4)); 6.09, 6.10 (br. *AB*, J_{AB} = 9.6, H–C(6), H–C(7)); 2.19 (br. *dd*, *J*(8,9) = *J*(8,14) = 10.0, H–C(8)); 1.16–2.07 (br., H–C(9), 2 H–C(11), H_α–C(12), H–C(14), 2 H–C(15), 2 H–C(16), H–C(17)); 2.04 (*ddd*, *J* = 12.8, 3.3, 3.3, H_β–C(12)); 0.79 (*s*, Me(18)); 1.11 (*s*, Me(19)); 2.31 (br., H–C(20)); 1.10 (*d*, *J*(21,20) = 6.7, Me(21)); 6.83 (*dd*, *J*(22,23) = 15.5, *J*(22,21) = 8.9, H–C(22)); 5.76 (*dd*, *J*(23,22) = 15.5, *J*(23,21) = 0.8, H–C(23)); 3.72 (*s*, MeO). MS: 382 (100, M^+), 367 (10, $[M - Me]^+$), 351 (8), 350 (7), 335 (4), 322 (5), 269 (94, C(17)–C(20) break), 247 (7, C(7)–C(8) and C(9)–C(10) break), 229 (3), 227 (10), 187 (26), 175 (83), 161 (39), 147 (30), 145 (31), 136 (46, C(7)–C(8) and C(9)–C(10) break), 133 (50), 107 (46), 95 (87), 81 (50), 28 (66). HR-MS: 382.25073 ± 0.0009 ($C_{25}H_{34}O_3^+$, calc. 382.25079); 269.18972 ± 0.0011 ($C_{19}H_{25}O^+$, calc. 269.19054).

5. *Methyl 23-Hydroxy-3-oxochola-4,6-dien-24-oate* (**3**). $[\alpha]^{20}$ (λ/nm) = −5.5 (589), +11.0 (546), +211.2 (435, c = 0.10, EtOH). UV (EtOH): 283 (21000). ^{1}H-NMR: 1.99 (*ddd*, J_{gem} = 14.0, $J(1\beta,2\beta)$ = 5.4, $J(1\beta,2\alpha)$ = 2.2, H_β–C(1)); 1.69 (br., H_α–C(1)); 2.57 (*ddd*, J_{gem} = 17.9, $J(2\beta,1\alpha)$ = 14.0, $J(2\beta,1\beta)$ = 5.3, H_β–C(2)); 2.44 (*dddd*, J_{gem} = 17.9, $J(2\alpha,1\alpha)$ = 5.4, $J(2\alpha,1\beta)$ = 2.2, $J(2\alpha,4)$ = 0.8, H_α–C(2)); 5.67 (br. *s*, $J(4,2\alpha)$ = 0.8, $J(4,6)$ and $J(4,7)$ small, H–C(4)); 6.09 (br. *dd*, $J(6,7)$ = 9.9, $J(6,8)$ = 2.0, $J(6,4)$ small, H–C(6)); 6.12 (br. *d*, $J(7,6)$ = 9.9, $J(7,4)$ and $J(7,8)$ small, H–C(7)); 2.20 (br. *dd*, $J(8,9)$ = $J(8,14)$ = 9.7, $J(8,6)$ = 2.0, $J(8,7)$ small, H–C(8)); 1.14–1.96 (br., H–C(9), 2 H–C(11), H_α–C(12), H–C(14), 2 H–C(15), 2 H–C(16), H–C(17)); 2.47 (*ddd*, J = 12.9, 3.3, 3.3, H_β–C(12)); 0.79 (*s*, Me(18)); 1.11 (*s*, Me(19)); 1.78 (br., H–C(20)); 1.02 (*d*, J(21,20) = 6.6, Me(21)); 1.66, 1.39 (br., 2 H–C(22)); 4.24 (*ddd*, J(23,22) = 11.0, 2.5, J(23,OH) = 5.9, H–C(23)); 2.58 (*d*, J(OH,23) = 5.9, OH); 3.79 (*s*, MeO). NOE: H–C(6)→H–C(4) (16); H–C(8)→Me(18) (4), Me(19) (4); Me(18)→H–C(8) (10), H–C(20) (7); Me(19)→H–C(8) (13); Me(21)→H_β–C(12) (6), H–C(23) (12). Selective INEPT: on irradiations at both H–C(23) or CH_3O, only correlation with C(24). MS: 400 (100, $M^{+\cdot}$), 385 (11, $[M - Me]^+$), 382 (3, $[M - H_2O]^{+\cdot}$), 341 (5), 311 (5, C(22)–C(23) break), 269 (51, C(17)–C(20) break), 265 (15, C(7)–C(8) and C(9)–C(10) break), 229 (10), 227 (11), 187 (17), 175 (48), 174 (47), 161 (5), 136 (66, C(7)–C(8) and C(9)–C(10) break), 95 (46), 28 (72). HR-MS: 400.26127 ± 0.0011 ($C_{25}H_{36}O_4^{+\cdot}$, calc. 400.26136); 269.19008 ± 0.0011 ($C_{19}H_{25}O^+$, calc. 269.19054); 265.17830 ± 0.0023 ($C_{16}H_{25}O_3^+$, calc. 265.18037); 136.08845 ± 0.0004 ($C_9H_{12}O^{+\cdot}$, calc. 136.08881).

6. *Methyl 3-Oxochola-4,6-dien-24-oate* (**4**). $[\alpha]^{20}$ (λ/nm) = +19.1 (589), +61.8 (546), +523.6 (435, c = 0.11, EtOH). UV (EtOH): 283 (21000). ^{1}H-NMR: 1.95 (br., H_β–C(1)); 1.70 (br. *ddd*, J_{gem} = $J(1\alpha,2\beta)$ = 14.0, $J(1\alpha,2\alpha)$ = 5.0, $J(1\alpha,19)$ small, H_α–C(1)); 2.55 (*ddd*, J_{gem} = 18.0, $J(2\beta,1\alpha)$ = 14.0, $J(2\beta,1\beta)$ = 5.4, H_β–C(2)); 2.43 (*dddd*, J_{gem} = 18.0, $J(2\alpha,1\alpha.)$ = 5.4, $J(2\alpha,1\beta)$ = 2.3, $J(2\alpha,4)$ = 0.8, H_α–C(2)); 5.66 (br. *s*, $J(4,2\alpha)$ = 0.8, $J(4,6)$ and $J(4,7)$ small, H–C(4)); 6.10 (br. *dd*, $J(6,7)$ = 9.8, $J(6,8)$ = 1.9, $J(6,4)$ small, H–C(6)); 6.12 (br. *d*, $J(7,6)$ = 9.8, $J(7,4)$ and $J(7,8)$ small, H–C(7)); 2.18 (br. *dd*, $J(8,9)$ = $J(8,14)$ = 10.0, H–C(8)); 1.07–2.10 (br., H–C(9), 2 H–C(11), 2 H–C(12), H–C(14), 2 H–C(15), 2 H–C(16), H–C(17), 2 H–C(22)); 0.75 (*s*, Me(18)); 1.10 (*s*, Me(19)); 1.44 (br., H–C(20)); 0.93 (*d*, J(21,20) = 6.4, Me(21)); 2.35, 2.23 (*ABCD*, J_{AB} = 15.5, J_{AC} = 10.0, J_{AD} = 5.3, J_{BC} = 6.8, J_{AD} = 9.6, 2 H–C(23)); 3.66 (*s*, MeO). MS: 384 (100, $M^{+\cdot}$), 369 (9, $[M - Me]^+$), 353 (5), 269 (44, C(17)–C(20) break), 249 (21, C(7)–C(8) and C(9)–C(10) break), 229 (11), 227 (9), 187 (11), 175 (45), 174 (38), 161 (40), 136 (68, C(7)–C(8) and C(9)–C(10) break), 95 (47), 28 (67). HR-MS: 384.26613 ± 0.0009 ($C_{25}H_{36}O_3^{+\cdot}$, calc. 384.26644); 249.18337 ± 0.0021 ($C_{16}H_{25}O_3^+$, calc. 249.18545).

7. *Methyl 3-Oxo-24-norchola-4,6-dien-23-oate* (**5**). $[\alpha]^{20}$ (λ/nm) = −10.0 (589), +14.7 (546), +113.5 (435, c = 0.06, EtOH). UV (EtOH): 280 (20000). ^{1}H-NMR: 1.97 (br., H_β–C(1)); 1.68 (br., H_α–C(1)); 2.56 (*ddd*, J_{gem} = 17.8, $J(2\beta,1\alpha)$ = 14.1, $J(2\beta,1\beta)$ = 5.3, H_β–C(2)); 2.43 (br., H_α–C(2)); 5.67 (br. *s*, $J(4,2\alpha)$, $J(4,6)$, and $J(4,7)$ small, H–C(4)); 6.10, 6.11 (br. *AB*, J_{AB} = 9.9, H–C(6), H–C(7)); 2.19 (br. *dd*, $J(8,9)$ = $J(8,14)$ = 9.7, H–C(8)); 1.07–2.10 (br., H–C(9), 2 H–C(11), 2 H–C(12), H–C(14), 2 H–C(15), 2 H–C(16), H–C(17)); 0.80 (*s*, Me(18)); 1.11 (*s*, Me(19)); 1.95 (br., H–C(20)); 1.00 (*d*, J(21,20) = 6.2, Me(21)); 2.45, 2.40 (br., 2 H–C(22)); 3.67 (*s*, MeO). MS: 370 (81, $M^{+\cdot}$), 355 (11, $[M-Me]^+$), 269 (26, C(17)–C(20) break), 235 (26, C(7)–C(8) and C(9)–C(10) break), 227 (15), 187 (13), 175 (37), 174 (34), 161 (76), 136 (100, C(7)–C(8) and C(9)–C(10) break), 129 (26), 127 (16), 95 (74), 28 (61). HR-MS: 370.24974 ± 0.0013 ($C_{24}H_{34}O_3^{+\cdot}$, calc. 370.25079); 269.19054 ± 0.0015 ($C_{19}H_{25}O^+$, calc. 269.19054).

8. *Methyl (22E)-3-Oxochola-4,22-dien-24-oate* (**6**). $[\alpha]^{20}$ (λ/nm) = +55.8 (589), +89.2 (546), +133.3 (435, c = 0.12, EtOH). UV (EtOH): 239 (11800). ^{1}H-NMR: 0.85–2.50 (br., 2 H–C(1), 2 H–C(2), 2 H–C(6), 2 H–C(7), H–C(8), H–C(9), 2 H–C(11), 2 H–C(12), H–C(14), 2 H–C(15), 2 H–C(16), H–C(17), H–C(20)); 5.73 (br. *s*, $J(4,2\alpha)$, $J(4,6)$ and $J(4,7)$ small, H–C(4)); 0.75 (*s*, Me(18)); 1.19 (*s*, Me(19)); 1.09 (*d*, J(21,20) = 6.8, Me(21)); 6.83 (*dd*, J(22,23) = 15.5, J(22,21) = 9.1, H–C(22)); 5.75 (*dd*, J(23,22) = 15.5, J(23,21) = 0.9, H–C(23)); 3.72 (*s*, MeO). HMBC: H–C(4)→C(6), C(10); H–C(22)→C(20), C(21), C(24); H–C(23)→C(20), C(24); MeO→C(24). MS: 384 (58, $M^{+\cdot}$), 369 (8, $[M - Me]^+$), 353 (8), 352 (10), 342 (22, C(1)–C(2) and C(3)–C(4) break), 299 (11), 271 (83, C(17)–C(20) break), 269 (24), 253 (22), 229 (49), 227 (11), 147 (52), 124 (100, C(6)–C(7) and C(9)–C(10) break), 28 (57). HR-MS: 384.26615 ± 0.0013 ($C_{25}H_{36}O_3^{+\cdot}$, calc. 384.26644); 271.20578 ± 0.0010 ($C_{19}H_{27}O^+$), calc. 271.20619).

9. *Methyl 23-Hydroxy-3-oxochol-4-en-24-oate* (**7**). $[\alpha]^{20}$ (λ/nm) = +33.8 (589), +61.3 (546), +91.3 (435, c = 0.08, EtOH). UV (EtOH): 240 (9800). ^{1}H-NMR: 0.86–2.50 (br., 2 H–C(1), 2 H–C(2), 2 H–C(6), 2 H–C(7), H–C(8), H–C(9), 2 H–C(11), 2 H–C(12), H–C(14), 2 H–C(15), 2 H–C(16), H–C(17)); 0.75 (*s*, Me(18)); 1.18 (*s*, Me(19)); 1.74 (br., H–C(20)); 1.02 (*d*, J(21,20) = 6.5, Me(21)); 1.67, 1.37 (br., 2 H–C(22)); 4.23 (*ddd*, J(23,22) = 11.0, 2.4, J(23,OH) = 6.0, H–C(23)); 2.56 (*d*, J(OH,23) = 6.0, OH); 3.78 (*s*, MeO). Selective INEPT: on irradiation at H–C(23), only correlation with C(24). MS: 402 (58, $M^{+\cdot}$), 387 (8, $[M - Me]^+$), 384 (4,

$[M - H_2O]^{+\cdot}$), 360 (12, C(1)–C(2) and C(3)–C(4) break), 345 (7), 343 (6), 313 (42, C(22)–C(23) break), 279 (24, C(6)–C(7) and C(9)–C(10) break), 271 (12, C(17)–C(20) break), 229 (49), 227 (5), 147 (32), 124 (100, C(6)–C(7) and C(9)–C(10) break), 28 (63). HR-MS: 402.22706 ± 0.0010 ($C_{25}H_{38}O_4^{+\cdot}$, calc. 402.27701); 279.19572 ± 0.0012 ($C_{17}H_{27}O_3^+$, calc. 279.19602).

10. *Methyl 3-Oxochol-4-en-24-oate* (**8**). $[\alpha]^{20}$ (λ/nm) = +35.9 (589), +56.6 (546), +106.4 (435, $c = 0.05$, EtOH). UV (EtOH): 238 (9500). ^{1}H-NMR: 0.80–2.50 (br., 2 H–C(1), 2 H–C(2), 2 H–C(6), 2 H–C(7), H–C(8), H–C(9), 2 H–C(11), 2 H–C(12), H–C(14), 2 H–C(15), 2 H–C(16), H–C(17), H–C(20), 2 H–C(22), 2 H–C(23)); 5.72 (br. *s*, J(4,2α), J(4,6), and J(4,7) small, H–C(4)); 0.71 (*s*, Me(18)); 1.18 (*s*, Me(19)); 0.92 (*d*, J(21,20) = 6.6, Me(21)); 3.66 (*s*, MeO). MS: 386 (68, $M^{+\cdot}$), 371 (9, $[M-Me]^+$), 355 (11), 344 (14, C(1)–C(2) and C(3)–C(4) break), 329 (8), 271 (16, C(17)–C(20) break), 263 (24, C(6)–C(7) and C(9)–C(10) break), 229 (53), 227 (6), 187 (12), 147 (30), 124 (100, C(6)–C(7) and C(9)–C(10) break), 28 (72). HR-MS: 386.28217 ± 0.0011 ($C_{25}H_{38}O_3^{+\cdot}$, calc. 386.28209); 263.20116 ± 0.0009 ($C_{17}H_{27}O_2^+$, calc. 263.20110).

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