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Substituted Methyl 5β-Cholan-24-oates

I—^{17}O NMR Spectral Characterization

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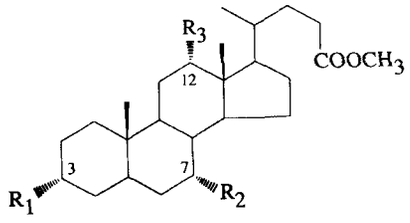
Methyl esters of four common bile acids, 3α-hydroxy-5β-cholan-24-oic (lithocholic) acid, 3α,7α-dihydroxy-5β-cholan-24-oic (chenodeoxycholic) acid, 3α,12α-dihydroxy-5β-cholan-24-oic (deoxycholic) acid and 3α,7α,12α-trihydroxy-5β-cholan-24-oic (cholic) acid, and 14 acetylated, trifluoroacetylated, mesylated and oxo derivatives of methyl 5β-cholan-24-oates were prepared and their ^{17}O NMR spectra recorded. In spite of their relatively high molecular masses and the rigid molecular structure of the steroid skeleton, most of the oxygens included in these structures gave well resolved ^{17}O NMR resonance lines at natural abundance in 0.25–0.5 M acetonitrile solutions at 75 °C. In agreement with the present ^{17}O NMR results, molecular mechanics calculations revealed that a hydroxy substituent located at the 3α-position clearly differs from the hydroxyls at the 7α- and 12α-positions. This is due to the fact that the 3α-hydroxyl possessing only two γ-carbons at antiperiplanar positions is less shielded than the other hydroxyls influenced also by the shielding effects of *γ-gauche* carbons. The spectral deconvolution of the overlapping signals of the 7α- and 12α-hydroxyls is based on a computer-aided method or on chemical substitutions. The oxo groups located at the longitudinal (3-oxo) *vs.* transversal (7- and 12-oxo) axes of the steroid framework show very different quadrupolar relaxation properties and ^{17}O NMR linewidths owing to the strong anisotropy of overall molecular motion. In contrast, the ^{17}O NMR linewidths of all 3α-, 7α- and 12α-hydroxyls are very similar and clearly smaller than those of the corresponding oxo groups, revealing that their quadrupolar relaxation is merely determined by their internal rotation rather than by the overall molecular motion.

KEY WORDS NMR ^{17}O NMR ^{17}O chemical shifts ^{17}O relaxation Methyl 5β-cholan-24-oates Methyl esters of bile acids

INTRODUCTION

^{17}O NMR spectroscopy has been shown to provide a powerful tool in resolving the configurational characteristics of alicyclic ethers and alcohols.[1–6] The ^{17}O NMR chemical shifts of 1000-fold ^{17}O-enriched cholesterol and 31 other steroids have been determined and correlations between ^{17}O NMR shifts and structures have been adduced.[7] However, in the case of large organic molecules the usefulness of ^{17}O NMR spectroscopy at natural abundance is often limited owing to the broadness of the ^{17}O NMR resonance lines combined with the increased molecular size and the decreased rotational correlation time at the site of the oxygen atom.[8] A remarkable enhancement of spectral resolution and sensitivity in ^{17}O NMR experiments at natural abundance has been obtained by working at elevated temperatures and using low-viscosity solvents as reported by Filowitz *et al.*[9] and Gerothanassis.[10] Even proton–oxygen spin–spin couplings of simple alcohols at natural abundance can be determined by measuring the samples in CH_3CN solutions at 75 °C as shown by Chandrasekaran and Boykin.[11] In order to avoid the necessity for an enrichment by tedious syntheses with the costly oxygen-17 isotope,[7] we checked if the above measuring conditions are also suitable for determining the ^{17}O NMR chemical shifts at natural abundance for some common steroids and their easily available derivatives. Therefore, 18 methyl 5β-cholan-24-oate (bile acid) derivatives of general structure **1** containing different types of single- and double-bonded oxygens were prepared and their ^{17}O NMR spectra recorded.



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RESULTS AND DISCUSSION

The ^{17}O NMR chemical shifts at natural abundance and some reference data[7] for methyl-5β-cholan-24-oate derivatives are given in Table 1. The ^{17}O NMR chemical shifts measured in this work are comparable to

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Table 1. ^{17}O NMR chemical shifts of methyl 5β-cholan-24-oate derivatives (1) (ppm from external H_2O) measured for 0.5–1.0 M acetonitrile solutions at 75 °C

Compound	Substitution pattern	R_1	R_2	R_3	δ(^{17}O) (ppm) 3-	7-	12-	COOR	COOR
1	3α-Hydroxy-5β-	OH	H	H	38.3	—	—	139	357
	3α-Hydroxy-5β-[3α,24-$^{17}O_2$]-[a]	OH	H	H	38.86	—	—	—	360
	3α-Hydroxy-5β-[24,24-$^{17}O_2$]-[a]	OH	H	H	—	—	—	141	358
2	3α,7α-Dihydroxy-	OH	OH	H	38.5	23.2	—	139	356
3	3α,12α-Dihydroxy-	OH	H	OH	36.5	18.3	—	140	357
4	3α,7α,12α-Trihydroxy-	OH	OH	OH	37.0	23.7	17.7	139	356
5	3-Oxo-	O	H	H	556	—	—	139	357
	3-Oxo-5β-[3,24-$^{17}O_2$]-[a]	O	H	H	561	—	—	—	360
6	3α-OTFAc-[b]	OTFAc	H	H	187	—	—	139	357
7	3α-Hydroxy-7-oxo-	OH	O	H	37	560	—	140	357
8	3,7-Dioxo-	O	O	H	562	Not obs.	—	139	355
9	3α-Hydroxy-12-oxo	OH	H	O	38	—	Not obs.	139	357
10	3α-OAc-12α-hydroxy-[c]	OAc	H	OH	198	—	18	139	357
11	3α-OAc-12-oxo	OAc	H	O	197	—	Not obs.	139	356
12	3α,12α-Dioxo-	O	H	O	557	—	Not obs.	139	356
13	3α-OAc-7α,12α-dihydroxy-	OAc	OH	OH	194	24	18	139	357
14	3α,7α-DiOAc-12α-hydroxy-	OAc	OAc	OH	193	193	18	139	356
15	3α,7α-DiOAc-12-oxo-	OAc	OAc	O	194	194	560	139	357
16	3α,7α-Dihydroxy-12α-OTFAc-	OH	OH	OTFAc	37	22	169	139	356
17	3α,12α-DiOTFAc-7α-hydroxy-	OTFAc	OH	OTFAc	177[d]	20	171[d]	139	356
18	3α-OMes-7α,12α-dihydroxy-[e]	OMes	OH	OH	176	24	18	139	356
19	3,7,12-Trioxo-	O	O	O	565	Not obs.	Not obs.	139	356

[a] Values taken from Ref. 7.
[b] TFAc = CF_3CO, which overlaps with C=O of cholanoate.
[c] Ac = CH_3CO.
[d] Assignment can be reversed.
[e] Mes = CH_3SO_2.

those reported in the literature[7] owing to the similar measuring conditions used, i.e. acetonitrile solutions at 75 °C. Hence the possible anisotropic effects of the solvent and its hydrogen bonding with the solute molecules are eliminated as fully as possible. Regarding the temperature effects on referencing in ^{17}O NMR spectroscopy,[12] the ^{17}O NMR chemical shift of the external reference (H_2O) is deshielded 2.2 ppm at 30 °C from the value at 75 °C. The chemical shifts in Table 1 are, however, referenced to the external H_2O at 75 °C, thus being directly comparable to the values taken from the literature.[7]

In order to improve the accuracy of the ^{17}O NMR chemical shifts of the natural bile acid methyl esters (**1**–**4**) and to resolve the two strongly overlapping resonance lines of 7α- and 12α-hydroxyls of **4** (see Fig. 1), an integrated software for analysis of NMR spectra on PC named PERCH (Peak Research)[13] is utilized. By this method the ^{17}O NMR chemical shifts of the non-overlapping resonance lines can be reported within the accuracy of 0.25 ppm (digital resolution). For the overlapping lines of 7α- and 12α-hydroxyls of **4** an accuracy of 0.5 ppm is obtained. Without this computer-aided line fitting an accuracy of 1 ppm is achieved.

The ^{17}O NMR chemical shift of 3α-hydroxyl clearly differs from those of the 7α- and 12α-hydroxyls, because the 3α-hydroxyl possessing only two γ-carbons at antiperiplanar (*anti*) orientations is less shielded than the other hydroxyls influenced also by shielding effects of γ-*gauche* carbons.[2–6] The corresponding dihedral angles

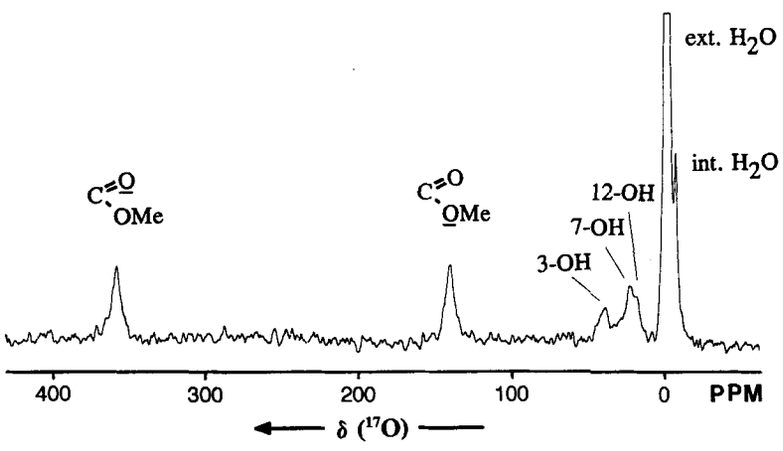

Figure 1. ^{17}O NMR spectrum at natural abundance of methyl cholate (**4**) for a 0.5 M solution in acetonitrile at 75 °C.

obtained by molecular mechanics[14] are θ(3-OH—C-3—C-2—C-1) = 178.9° and θ(3-OH—C-3—C-4—C-5) = 177.5°. For 7α-hydroxyl the dihedral angles are θ(7-OH—C-7—C-6—C-5) = 74.3°, θ(7-OH—C-7—C-8—C-9) = 72.2°, θ(7-OH—C-7—C-8—C-14) = 52.8° and for 12α-hydroxyl θ(12-OH—C-12—C-11—C-9) = 67.4°, θ(12-OH—C-12—C-13—C-14) = 63.2° and θ(12-OH—C-12—C-14—C-18) = 176.2°, respectively.

Hence in the case of the 12α-hydroxy group there exist two shielding γ-carbons near to the *gauche* orientation and one clearly at the *anti* orientation. For the 7α-hydroxyl the all three γ-carbons are further away from the *gauche* orientation than for the hydroxyl in the 12α-position. This model is in agreement with the present data giving for the 7α-hydroxyl less shielded ^{17}O NMR chemical shifts, δ(7-OH) = 23.2 (**2**) and 23.7 ppm (**4**), than for the 12α-hydroxyl, δ(12-OH) = 18.3 (**3**) and 17.7 ppm (**4**). There exist also small differences between the ^{17}O NMR chemical shifts of the hydroxyls at the same position in different compounds. For example, the small variation of the ^{17}O NMR chemical shift of δ(3α-OH) = 38.3 (**1**), 38.5 (**2**), 36.5 (**3**) and 37.0 ppm (**4**) also reveals the influence of some substituent effects. Thus, it is obvious that 12α-OH in **3** and **4** causes small shielding effects on the ^{17}O NMR chemical shifts of 3α-OH in comparison with **1**. However, the origin of these small effects between these two hydroxyls cannot be explained reliably based on the present data alone.

In addition to the computer-based spectral deconvolution, the overlapping ^{17}O NMR spectral lines can also be resolved by chemical substitution reactions. For example, acetylation of a hydroxyl group deshields the ^{17}O NMR spectral line of that oxygen more than 150 ppm. Thus, in methyl 3α-acetyloxy-12-oxo-5β-cholan-24-oate (**11**) the 3α-oxygen resonates at 197 ppm whereas in the unsubstituted compound (**9**) it resonates at 38 ppm. In methyl 3α,7α-diacetyloxy-12-oxo-5β-cholan-24-oate (**15**), however, the 3α- and 7α-oxygens both resonate at 194 ppm. This reveals that the substitution reaction have also their own limitations regarding the ^{17}O NMR spectral simplification.

Similarly as acetylation, trifluoroacetylation can be utilized in resolving the ^{17}O NMR spectra. Cyclohexyl trifluoroacetate used as a model compound gave well resolved ^{17}O NMR lines at δ(C=O) = 351 and δ(—O—) = 182 ppm. A mixture of isomeric 2,6-dimethylcyclohexanols showing ^{17}O NMR spectral lines at 22 ppm (signals of *trans,trans* and *cis,trans* isomers overlap) and −6 ppm (*cis,cis*)[2] gave after trifluoroacetylation signals at δ(C=O) = 347 ppm and δ(—O—) = 172 (*trans,trans*), 174 (*cis,trans*) and 158 ppm (*cis,cis*), respectively.

Trifluoroacetylation of methyl lithocholate (**1**) deshields the ^{17}O NMR chemical shift of 3α-oxygen from 38.3 to 187 ppm. Further, a selective trifluoroacetylation of 12α-hydroxy group of methyl cholate (**4**) can be used as an aid in a spectral resolution. In that case, the 12α-oxygen is transferred to 169 ppm and the overlap with 7α-hydroxyl is avoided. Similarly, the 3α-oxygen of the 3α-mesyl derivative (**18**) is shifted to δ(3α-O) = 176 ppm.

The most serious limitation encountered with the ^{17}O NMR spectral characteristics of the present compounds is the broadness of the resonance lines of double-bonded oxygens anchored at the transversal axis of the steroid skeleton, viz. 7-oxo and 12-oxo substituents. This finding can be explained by considering the different mechanisms responsible for the NMR relaxation of the oxygen-17 nuclei at different positions.[8,15]

First, it has been shown that the relaxation of oxygen-17 nucleus is determined solely by quadrupole interaction.[8,15] Second, the efficiency of this mechanism depends on the reorientation of the principal field-gradient axes relative to a fixed laboratory frame.[15] The relaxation properties of methyl cholate (**4**) and its derivatives containing three oxygen nuclei at the different sites of the steroid skeleton can be used in estimating the elements of the rotational diffusion tensor. Consequently, a more or less complete description of the molecular rotation can be derived. Because precise ^{17}O NMR T_1 and T_2 relaxation time measurements at natural abundance are very time consuming for this kind of molecule, a quantitative analysis of the molecular relaxation and rotation properties is beyond the scope of this work. However, a comparison of ^{17}O NMR linewidths can provide a qualitative picture about the principal factors responsible for the different relaxation characteristics of oxo *vs.* hydroxy oxygens.

A computer-aided deconvolution and line fitting[13] of the ^{17}O NMR spectrum of **4** (Fig. 1) reveals that all the 3α, 7α- and 12α-hydroxyls possess very similar spectral linewidths, $W_{1/2} = 300 \pm 20$ Hz. This suggests that their quadrupolar relaxation is predominantly determined by their internal rotation around the C—O bonds independently on the overall molecular reorientation. The linewidth of the 3α-OH of **4** is in agreement with that of methyl 3α-hydroxy-5β-[3α,24-$^{17}O_2$]cholanoate,[7] $W_{1/2}$(3α-OH) = 300 Hz. On the other hand, the ^{17}O NMR linewidths and relaxation behavior of oxo groups in **5**, **7–9**, **11**, **12**, **15** and **19** anchored tightly in the conformationally rigid molecular framework clearly reflect the anisotropy of molecular rotation. The ^{17}O NMR lines of 3-oxo groups in **5**, **8**, **12** and **19** locating at the longitudinal molecular axis are clearly broadened ($W_{1/2} \approx 650 \pm 50$ Hz) in comparison with the resonance lines of hydroxyls. This linewidth of 650 Hz is broader than those of the ^{17}O-enriched 3-oxo-steroids.[7] Further, the ^{17}O NMR lines of the 7- and 12-oxo groups locating at the transversal molecular axis are very strongly broadened characterized by the linewidths of $W_{1/2} >$ 1500 Hz and only in two samples, **7** and **15**, were these broad resonance peaks separated from the spectral baseline.

A conclusion is that the relaxation of the oxo groups is closely related to the anisotropy of the overall molecular rotation, thus differing from the behaviour of hydroxyls. A quantitative description of the rotational diffusion tensor of this type of molecule, however, can be obtained reliably only by precise relaxation time measurements of strongly ^{17}O-enriched samples.

CONCLUSIONS

^{17}O NMR spectroscopy of methyl 5β-cholan-24-oate (bile acid methyl ester) derivatives at natural abundance is a useful method for differentiating between the

hydroxyl-containing isomers and the degree of substitution and even to some extent the nature of the substituent. A computer-aided spectral deconvolution method improved considerably the knowledge obtained in the case of strongly overlapping 7α-OH and 12α-OH spectral lines of methyl cholate. The only serious limitation encountered in this study was in dealing with the ^{17}O NMR signals of the oxo groups anchored at the transversal rotation axis of the steroid skeleton, viz. at positions 7 and 12, while the 3-oxo group located at the longitudinal rotation axis exhibited easy-to-detect spectral properties. This difference is due to the anisotropy of the overall molecular rotation. In contrast, the ^{17}O NMR relaxation of the hydroxyls is determined predominantly by their internal rotation. The present results reveal unambiguously that ^{17}O NMR spectroscopy of unenriched steroid samples can provide an inexpensive method for the characterization of these physiologically and biologically important compounds.

EXPERIMENTAL

Compounds

3α-Hydroxy-5β-cholan-24-oic acid (lithocholic acid LCA), 3α,7α-dihydroxy-5β-cholan-24-oic acid (chenodeoxycholic acid, CDCA), 3α,12α-dihydroxy-5β-cholan-24-oic acid (deoxycholic acid, DCA) and 3α,7α,12α-trihydroxy-5β-cholan-24-oic adic (cholic acid, CA) were commercial products from Aldrich (purity >98%) and used without further purification. Their ^{13}C NMR spectra measured in DMSO-d_6 were in agreement with the values given in the literature.[16] Methyl lithocholate (MeLC, **1**), methyl chenodeoxycholate (MeCDC, **2**), methyl deoxycholate (MeDC, **3**) and methyl cholate (MeC **4**) were prepared from the corresponding bile acid by acid-catalysed esterification with excess of methanol.[17] The purity of the methyl esters was checked by their melting points, which were in agreement with the literature values.[17] Their ^{13}C NMR chemical shifts were also in excellent agreement with the values reported earlier.[16,18]

Methyl 3-oxo-5β-cholan-24-oate (**5**) was prepared by treating **1** with $K_2Cr_2O_7$ for 72 h at room temperature.[19] The oxidation product was purified with a silica column using chloroform–acetone (95:5) as the eluent. The purity of **5** was checked by its melting point, which was in agreement with the literature value.[17] The previously unreported ^{13}C NMR spectral assignment of **5** was based on the DEPT experiment and a comparison with the known spectra of similar structures.[21]

Methyl 3α-trifluoroacetyloxy-5β-cholan-24-oate (**6**) (new compound) was synthesized from methyl lithocholate (**1**) by treatment with trifluoroaceticanhydride in dry THF at −40 °C.[22] The product was purified with a silica column as described above. The purity of **6** was checked by its melting point (132–134 °C). The molecular mass and structures of **6** were ascertained mass spectrometrically [direct inlet, electron impact (EI) ionization, 35 eV]. Some characteristic *m/z* (intensity, %) values for **6** are 486 (7) $[M]^+$, 437 (6), 372 (100), 357 (22), 344 (27), 329 (49), 315 (10), 257 (32), 230 (14) and 215 (69). The ^{13}C NMR spectrum of **6** is available from E.K. on request.

Methyl 3α-hydroxy-7-oxo-5β-cholan-24-oate (**7**) was prepared from methyl chenodeoxycholate (**2**) by $K_2Cr_2O_7$ oxidation for 24 h at room temperature.[17] The product was purified with a silica column as described above. Its purity was checked by its melting point.[17] As stated in the literature,[17,23] the 7α-hydroxyl was oxidized prior to 3α-hydroxyl. This is ascertained also by the present ^{17}O NMR chemical shifts of **7** (see Table 1) showing a resonance line at 37 ppm typical for 3α-OH. The previously unreported ^{13}C NMR spectral assignment of **7** was based on the DEPT experiment and a comparison with the known spectra of similar structures.[21]

Methyl 3,7-dioxo-5β-cholan-24-oate (**8**) was prepared from methyl 3α-hydroxy-7-oxo-5β-cholan-24-oate (**7**) by a prolonged $K_2Cr_2O_7$ oxidation for 72 h at room temperature.[17] The product was purified column chromatographically similarly to the 3-oxo derivative (**7**). The purity of **8** was checked by its melting point, which was in agreement with the literature values.[24–26] The previously unreported ^{13}C NMR spectral assignment of **8** is obtained by DEPT experiments and by the substituent effects from the related structures.[21]

Methyl 3-hydroxy-12-oxo-5β-cholan-24-oate (**9**) was obtained by limited oxidation of methyl deoxycholate (**3**) with $K_2Cr_2O_7$.[17] The main product was 12-oxo derivative according to the reactivity order in oxidation reactions.[23] The product was purified column chromatographically as described above. Its melting point was in agreement with the literature value.[17] The previously unreported ^{13}C NMR spectral assignment of **9** is obtained by DEPT experiments and by the substituent effects from the related structures.[21]

Methyl 3α-acetyloxy-12α-hydroxy-5β-cholan-24-oate (**10**) (new compound) was synthesized by a limited acetylation of **3**.[18] The purification of **10** was done column chromatographically as described above. The purity of **10** was checked by its melting point (51–53 °C). The molecular mass and structure of **10** were ascertained mass spectrometrically (direct inlet, EI, 35 eV). Some characteristic *m/z* (intensity, %) values for **10** are 448 (very weak) $[M]^+$, 430 (4), $[M-H_2O]^+$, 388 (10), 355 (15), 315 (34) and 255 (100). The ^{13}C NMR spectrum of **10** is available from E.K. on request.

$K_2Cr_2O_7$ oxidation of **10** produced methyl 3α-acetyloxy-12-oxo-5β-cholan-24-oate (**11**). The purification of **11** was performed column chromatographically as described above. The structure of **11** was ascertained by its melting point.[17,27] The full assignment of the ^{13}C NMR spectrum of **11** is based on the DEPT experiments and substituent chemical shifts observed for related compounds.[21]

Methyl 3,12-dioxo-5β-cholan-24-oate (**12**) was prepared by a prolonged $K_2Cr_2O_7$ oxidation of methyl deoxycholate (**3**). The purification of **12** was performed column chromatographically as described above. The purity of **12** was checked by its melting point.[17] The full assignment of the ^{13}C NMR spectrum of **12** is based on the DEPT experiments and substituent chemical shifts observed for related compounds.[21].

Methyl 3α-acetyloxy-7α,12α-dihydroxy-5β-cholan-24-oate (**13**) was prepared by stoichiometrically controlled acetylation of methyl cholate (**4**). It was separated column chromatographically as described above from the reaction mixture containing also a small amount of 3α,7α-diacetyloxy-12α-hydroxy-5β-cholan-24-oate (**14**). The diacetyl derivative (**14**) was prepared in an improved yield with stoichiometrically controlled acetylation using twice the amount of acetic anhydride than in the case of monoacetate. The purities of **13** and **14** were checked by their melting points.[17,28] The full assignments of the ^{13}C NMR spectra of **13** and **14** were obtained by the DEPT experiments and substituent chemical shifts of related compounds.[21]

Methyl 3α,7α-bis(acetyloxy)-12-oxo-5β-cholan-24-oate (**15**) was synthesized by $K_2Cr_2O_7$ oxidation of **14**. It was purified column chromatographically as described above. The purity of **15** was checked by its melting point.[17,27] The ^{13}C NMR spectrum of **15** is available from E.K. on request.

Methyl-3α,7α-dihydroxy-12α-trifluoroacetyloxy-5β-cholan-24-oate (**16**) was prepared from methyl cholate (**4**) by complete trifluoroacetylation and partial hydrolysis according to Bonar-Law *et al.*[22] The purification of **16** was performed column chromatographically as described above. The purity of **16** was checked by its melting point.[22] The ^{13}C NMR spectrum of **16** is available from E.K. on request.

Methyl 3α,12α-bis(trifluoroacetyloxy)-7α-hydroxy-5β-cholan-24-oate (**17**) was obtained by complete trifluoroacetylation of cholic acid, its partial hydrolysis and esterification with methanol. Compound **17** was purified column chromatographically as described above. The melting point of 3α,12α-bis(trifluoroacetyloxy)-7α-hydroxy-5β-cholan-24-oic acid and **17** were in agreement with the values reported in the literature.[22] The ^{13}C NMR spectrum of **17** is available from E.K. on request.

Methyl 3α-methanesulphonyloxy-7α,12α-dihydroxy-5β-cholan-24-oate (**18**) (new compound) was synthesized according to the method reported by Iida and Chang.[29] The purification of **18** was performed column chromatographically as described above. The purity of **18** was checked by its melting point (79–81 °C). The molecular mass and structure of **18** were ascertained mass spectrometrically (direct inlet, EI, 35 eV). Some characteristic *m/z* (intensity, %) values for **18** are 500 (not observed) $[M]^+$, 482 (very weak) $M-H_2O]^+$, 464 (very weak; intensity twice that of the ion *m/z* 464) $[M-2H_2O]^+$, 403 (5), 386 (10), 368 (17), 354 (27), 289 (40) and 253 (100). The ^{13}C NMR spectrum of **18** is available from E.K. on request.

Methyl 3,7,12-trioxo-5β-cholan-24-oate (**19**) (new compound) was prepared by a prolonged oxidation of methycholate (**4**) with

$K_2Cr_2O_7$. The purification of **19** was performed column chromatographically as described above. The melting point (181–183 °C) reported for methyl 3,7,12-trioxocholan-24-oate by Pearson *et al.*[30] was much lower than that obtained now **19**, i.e. 240–241 °C. Unfortunately, Pearson *et al.*[30] did not report what the fusion of the A and B rings was in their compound. Therefore, the molecular mass and structure of **19** were ascertained mass spectrometrically (direct inlet, EI, 35 eV). Some characteristic *m/z* (intensity, %) values for **19** are 416 (24) $[M]^+$, 398 (100) $[M-H_2O]^+$, 385 (12), 367 (9), 343 (22), 301 (34), 283 (71) and 261 (94). The ^{13}C NMR spectrum of **19** is available from E.K. on request.

NMR spectroscopy

All ^{17}O NMR spectra were obtained with a Jeol GSX 270 FT NMR spectrometer working at 36.7 MHz and using a tunable multinuclear 10 mm probehead for 0.25–0.5 M acetonitrile solutions at 75 °C. The spectral width was 36 000 Hz, the number of data points 8000, giving a 9 Hz digital resolution, the flip angle 20 μs (90°), the acquisition time 0.11 s without any pulse delay and 100 000–400 000 scans were accumulated for every spectrum. The FIDs were multiplied by a combined trapezoidal ($T_1 = 0.1\%$, $T_2 = 0.1\%$, $T_3 = 50\%$; corresponding to a left shift in digital filtering) and exponential (50 Hz) windowing in order to eliminate the baseline distortions caused by the acoustic ringing and to enhance the signal-to-noise ratio. All spectra were measured using the proton broadband decoupling (BBD) model and without deuterium lock. All ^{17}O NMR chemical shifts are referenced to the signal of an external H_2O.

Mass spectrometry

All mass spectra were run on a VG AutoSpec high-resolution mass spectrometer using direct inlet and 35 eV EI ionization potential.

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