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New Cyclic Dimers of Cholic Acid

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Summary. Two new cyclic dimers of cholic acid were obtained in the reaction of 3-*O*-acetyl methyl cholate with oxalyl chloride. The oxalates bound the cholate subunits ''side-to-side'' as a result of acylation of 7α and 12α OH groups in the substrate. The selective deprotection of hydroxy groups at C-3 and C-24 proved to be rather difficult and led to various products depending on the reaction conditions.

Keywords. Acylation; Bile acids; Macrocycles; Steroids; Supramolecular Chemistry.

Introduction

The combination of unique construction and unusually specific properties of bile acids makes them nearly perfect building blocks in the design and synthesis of molecular receptors, enzyme models, and transporters, for example, drugs across the phospholipid membranes [1]. Two groups of cyclic structures prepared from bile acids, are cyclocholates [2, 3] and cholaphanes [4, 5].

The syntheses of cyclic supramolecular hosts based on bile acids depended so far exclusively on the formation of bonds among the steroidal units by using the 24-carboxyl group of one of these units, and the 3α hydroxy group of the other (''head to tail'' method). In this paper we report the synthesis of a new type of cyclic dimers of cholic acid in which two steroidal molecules were bound with the less reactive 7α and 12α hydroxy groups.

Results and Discussion

The compounds mentioned above (side-bonded dimers **1** (''*trans*-dimer'') and **2** (''*cis*-dimer'') were obtained by esterification of 3-*O*-acetyl methyl ester of cholic acid (**5**) with oxalyl chloride in pyridine.

In the first experiment, to the solution of **5** an exactly equimolar amount of oxalyl chloride was added. As a result, three less polar products were formed and a

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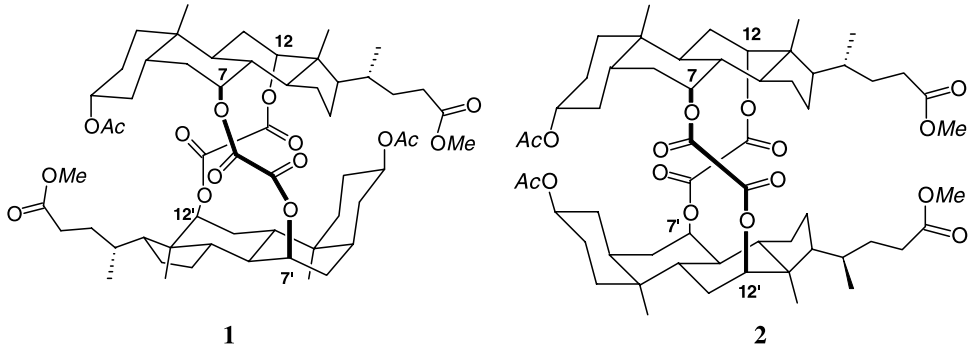
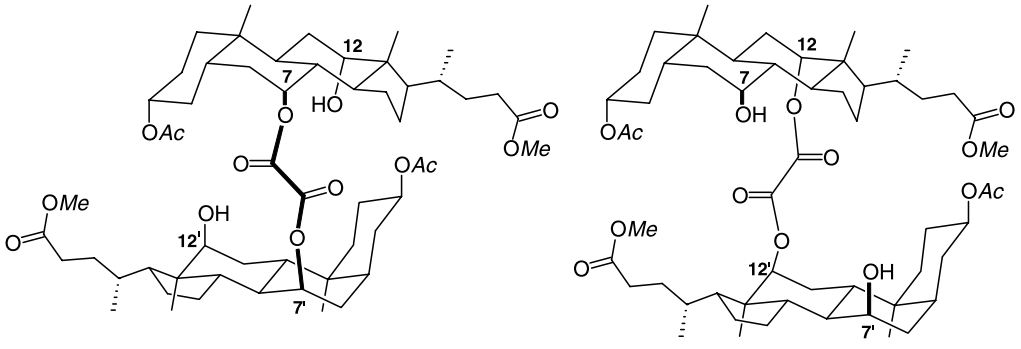


1 **2**

Formulae 1



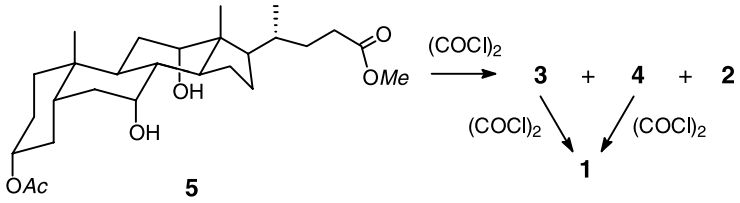
3 **4**

Formulae 2

small amount of the substrate was recovered. Based on the chemical shifts of the C-7 and C-12 protons the following results were obtained: (i) in the case of the least polar product: both 7α and 12α hydroxy groups were esterified and thus one of the expected dimers **1** or **2** was created. This was confirmed by mass spectrometry technique; (ii) in both more polar compounds only one of the hydroxy groups was esterified (the same in both steroidal subunits). Thus, ''non-closed'' dimers **3** and **4** were obtained, in which two molecules of cholic acid were acylated with one molecule of oxalyl chloride.

The structures of **3** and **4** were determined by comparison of their ^{1}H NMR spectra with those of cholic acid and deoxycholic acid, which does not possess the 7α hydroxy group (Scheme 1). The spectrum of **3** (Fig. 1C) shows that only 7α hydroxy groups where acylated contrary to compound **4** (Fig. 1D), which underwent acylation exclusively at 12α-OH groups.

Based on these results it was derived, that three dimeric by-products were formed: two ''non-closed'' dimers **3** and **4**, and a ''cross-dimer'', in which the 7α hydroxy group of the first steroidal subunit and the 12α hydroxy group of the second subunit were acylated. The third product underwent rapid cyclization with the second molecule of oxalyl chloride yielding the least polar product – ''*cis*-dimer'' **2**. In



Scheme 1

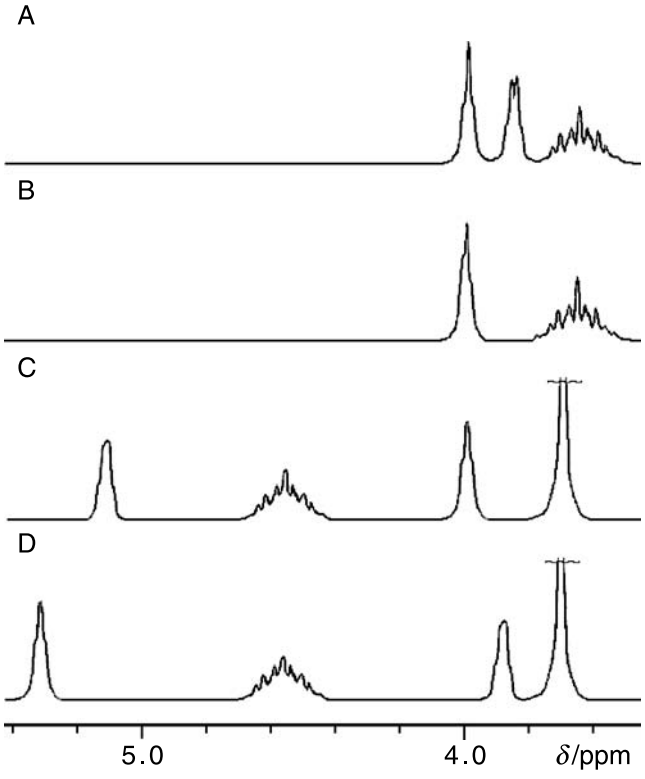


Fig. 1. ^{1}H NMR spectra (chemical shifts range 3.6–5.4 ppm) of cholic acid (A), deoxycholic acid (B), dimer **3** (C), and dimer **4** (D)

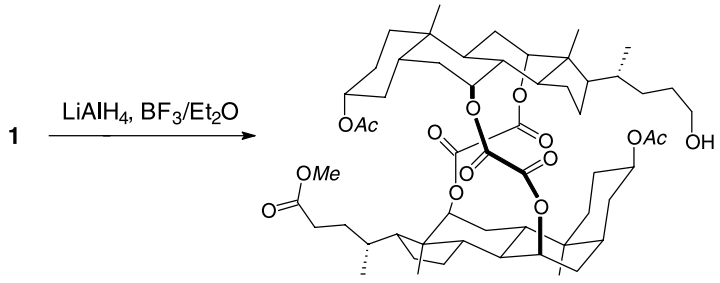
the case of exhaustive acylation with oxalyl chloride a ''*trans*-dimer'' **1** was expected to be formed. This was confirmed in a second experiment (Scheme 1)

The dimers **1** and **2** were subjected to reduction under various conditions in order to obtain compounds with free 3α and 24 hydroxy groups, but none of these experiments was successful: lithium tri-*t*-butoxyaluminum hydride [6] appeared to be inactive, whereas lithium aluminum hydride [7] reduced only the oxalate esters leading thereby to decomposition of the dimers (Table 1).

An attempt to reduce the ester groups in **1** with the $LiAlH_4$–BF_3/Et_2O system [8] was also undertaken, but none of them was reduced to an ether group as expected. A very complex mixture was formed instead in which **6** predominated.

Table 1. Reduction of dimers **1** and **2** with hydrides under various conditions

Substrates	Reducing agent	Reaction conditions	Result
1	$LiAlH(O\text{-}t\text{-}Bu)_3$ (excess)	*THF*, reflux., 12 h	no reaction
1, **2**	$LiAlH_4$ (excess)	*THF*, −70°C, 4 h	no reaction
1, **2**	$LiAlH_4$ (excess)	*THF*, 0°C, 45 min	**5**



6

Scheme 2

It turned out that both oxalate esters remained unchanged and only one of the ester groups in the side chain was reduced to CH_2OH (Scheme 2).

Conclusions

Both cyclic dimers **1** and **2** were readily formed by reaction of **5** with oxalyl chloride. It was proved that selective reduction of ester groups at C-3 and in the side chain in **1** or **2** is rather difficult. The oxalyl esters proved to be the most reactive groups towards $LiAlH_4$ in *THF*, whereas the same reducing agent in the BF_3/Et_2O complex did not reduce the oxalate system, but the COO*Me* in the side chain to CH_2OH, with rather poor yield.

Experimental

Melting points were determined on a *Kofler* apparatus of the *Boëtius* type. NMR spectra were taken with a Bruker AC 200F spectrometer with *TMS* as internal standard. Infrared spectra were recorded on a Nicolet series II Magna-IR 550 FT-IR spectrometer. Mass spectra were obtained with an AMD-604 spectrometer. The reaction products were isolated by column chromatography performed on 70–230 mesh silica gel (J.T. Baker). Thin-layer chromatograms were developed on aluminum TLC sheets precoated with silica gel F_{254} (Merck) and visualized with 50% H_2SO_4 after heating. All solvents were dried and freshly distilled prior to use. Methyl cholate **5** was purchased from Steraloids Inc., and it was used without further purification.

*Reaction of **5** with Oxalyl Chloride – Dimers **2**, **3**, and **4***

To a solution of 408 mg of **5** (0.88 mmol) in 5 cm^3 of anh. pyridine 77 mm^3 (0.9 mmol) of oxalyl chloride (room temp.) were added dropwise with vigrous stirring. After the whole amount of the

chloride was added, the mixture was stirred for additional 0.5 h, poured into acidified H_2O, and products were extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and the solvent was removed. The mixture of the three products was subjected to column chromatography. Pure dimers were eluted consecutively: **2** (benzene/ethyl acetate 83/17, 38 mg, 8%), **3** (benzene/ethyl acetate 8/2, 64 mg, 15%), and **4** (benzene/ethyl acetate 8/2, 72 mg, 17%).

Di[3α-acetoxy-7α,12α-dihydroxy-5β-cholanoic acid methyl ester]7,12′;12,7′-dioxalate (**2**, $C_{58}H_{84}O_{16}$)

Colorless crystals, mp 266–269°C (*n*-hexane/CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 5.30$ (m, 2 12β-H), 5.01 (m, 2 7β-H), 4.51 (m, 2 3β-H), 3.66 (s, 2OCH_3), 1.96 (s, 2CH_3CO), 1.01 (s, 2 19-CH_3), 0.79 (m: s, 2 18-CH_3 and d, 2 21-CH_3) ppm; ^{13}C NMR (50 MHz; $CDCl_3$): $\delta = 174.0$ (2C), 170.9 (2C), 158.2 (2C), 158.0 (2C), 78.9 (2CH), 75.4 (2CH), 74.4 (2CH), 51.5 (2CH_3), 48.9 (2CH), 45.4 (2C), 42.7 (2CH), 41.4 (2CH), 38.3 (2CH), 37.1 (2CH_2), 35.8 (2CH_2), 34.7 (2CH_2), 34.2 (2CH), 31.9 (2CH_2), 31.2 (2CH_2), 30.7 (2CH_2), 30.4 (2CH_2), 30.0 (2CH_2), 29.3 (2C), 28.8 (2CH), 22.7 (2CH_2), 21.6 (2CH_3), 17.6 (2CH_3), 14.1 (2CH_3), 12.0 (2CH_3) ppm; IR ($CHCl_3$): $\bar{\nu} = 1758$, 1730, 1315, 1251, 1186 cm^{-1}; MS (70 eV): $m/z = 1037$ ($M^+ + H$), 949, 609, 535, 519, 430, 370, 253.

Di[3α-acetoxy-7α,12α-dihydroxy-5β-cholanoic acid methyl ester]7,7′-oxalate (**3**, $C_{56}H_{86}O_{14}$)

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 5.09$ (m, 2 7β-H), 4.58 (m, 2 3β-H), 4.01 (m, 2 12β-H), 3.65 (s, 2OCH_3), 1.96 (s, 2CH_3CO), 0.98 (d, $J = 6.2$ Hz, 2 21-CH_3), 0.94 (s, 2 19-CH_3), 0.71 (s, 2 18-CH_3) ppm.

Di[3α-acetoxy-7α,12α-dihydroxy-5β-cholanoic acid methyl ester]12,12′-oxalate (**4**, $C_{56}H_{86}O_{14}$)

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 5.33$ (m, 2 12β-H), 4.53 (m, 2 3β-H), 3.86 (m, 2 7β-H), 3.64 (s, 2OCH_3), 2.01 (s, 2CH_3CO), 0.92 (s, 2 19-CH_3), 0.87 (d, $J = 6.1$ Hz, 2 21-CH_3), 0.78 (s, 2 18-CH_3) ppm.

*Reactions of **3** and **4** with Oxalyl Chloride – Dimer **1***

Dimers **2** and **3** were subjected to the reaction with oxalyl chloride according to the procedure described above with two equivalents of the acylating agent. In both cases the dimer **1** was obtained (elution with benzene/ethyl acetate 85/15) in 55–62% yields.

Di[3α-acetoxy-7α,12α-dihydroxy-5β-cholanoic acid methyl ester]7,7′;12,12′-dioxalate (**1**, $C_{58}H_{84}O_{16}$)

Colorless crystals, mp 240–243°C (*n*-hexane/CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 5.41$ (m, 2 12β-H), 5.17 (m, 2 7β-H), 4.56 (m, 2 3β-H), 3.64 (s, 2OCH_3), 1.99 (s, 2CH_3CO), 1.01 (s, 2 19-CH_3), 0.81 (m: s, 2 18-CH_3 and d, $J = 5.7$ Hz, 2 21-CH_3) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 174.0$ (2C), 170.2 (2C), 158.3 (2C), 156.9 (2C), 77.5 (2CH), 74.4 (2CH), 73.7 (2CH), 51.4 (2CH_3), 48.3 (2CH), 45.3 (2C), 42.6 (2CH), 41.1 (2CH), 37.9 (2CH), 35.4 (2CH_2), 34.7 (2CH_2), 34.3 (2CH), 30.9 (2CH_2), 30.8 (2CH_2), 30.6 (2CH_2), 29.6 (2C), 28.8 (2CH), 27.6 (2CH_2), 26.3 (2CH_2), 26.0 (2CH_2), 22.8 (2CH_3), 22.5 (2CH_2), 21.1 (2CH_3), 17.8 (2CH_3), 11.9 (2CH_3) ppm; IR ($CHCl_3$): $\bar{\nu} = 1758$, 1728, 1314, 1258, 1186 cm^{-1}; MS (70 eV): $m/z = 1037$ ($M^+ + H$), 1019, 964, 946, 610, 536, 430, 370, 253.

*Reduction of **1** with $LiAlH_4$–BF_3/Et_2O – Dimer **6***

Compound **1** (65 mg, 0.063 mmol) was dissolved in 0.23 cm^3 of a solution of BF_3/Et_2O complex (1.89 mmol) in 7 cm^3 of anh. diethyl ether, and added dropwise to a suspension of 29 mg of $LiAlH_4$ (0.765 mmol) in 8 cm^3 of anh. diethyl ether at 0°C. After the addition was completed, the reaction was stirred for 1 h at 0°C and then refluxed for 1 h. Then the excess of reducing agent was carefully quenched with a few drops of H_2O, the precipitate was filtered off, and the reaction mixture was subjected to column chromatography to afford 11 mg of compound **6** (17%) (elution with benzene/ethyl acetate 75/25).

(3α-Acetoxy-7α,12α-dihydroxy-5β-cholanoic acid methyl ester)(5β-cholane-3′α,7′α,12′α,24′-tetraol 3′-acetate)7,7′;12,12′-dioxalate (**6**, $C_{57}H_{84}O_{15}$)

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 5.41$ (m, 2 12β-H), 5.18 (m, 2 7β-H), 4.56 (m, 2 3β-H), 3.64 (s, OCH_3), 3.56 (t, $J = 6.1$ Hz, CH_2-OH), 2.00 (s, CH_3CO), 1.99 (s, CH_3CO), 1.02 (s, 2 19-CH_3), 0.81 (m: s, 2 18-CH_3 and d, 2 21-CH_3) ppm; IR ($CHCl_3$): $\bar{\nu} = 3523$, 1757, 1729, 1315, 1251, 1186 cm^{-1}.

Acknowledgments

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