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MODIFIED BILE ACIDS: PREPARATION OF 7α,12α-DIHYDROXY-3β- AND 7α,12α-DIHYDROXY-3α-(2-HYDROXYETHOXY)-5β-CHOLANIC ACID AND THEIR BIOLOGICAL ACTIVITY

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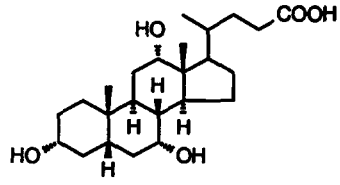
Abstract: *Methodology for the preparation of 7α,12α-dihydroxy-3β- (2) and 7α,12α-dihydroxy-3α-(2-hydroxyethoxy)-5β-cholanic acid (3) is described. Nucleophilic displacement of the 3-mesylate of unprotected cholic acid with ethylene glycol led to the 3β-isomer whereas the 3α-isomer was synthesized via the 7,12-diacetyl protected 3-allyl ether of methyl cholate. Only the 3α-isomer 3 is recognized by the ileal bile acid transport system with affinity comparable to cholic acid.*

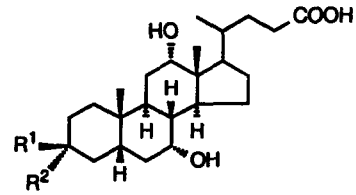
Bile acids play an important physiological role in all mammals. After food ingestion bile acids, the major component in gall bladder bile, are secreted into the small intestine and fulfill various functions during digestion and resorption of fat and cholesterol. Passive diffusion and active transport processes take care of nearly total reabsorption during intestinal passage.[1]

We were interested in the affinity of modified bile acids 2 and 3 to the specific ileal reabsorption system for bile acids and the structural features required for molecular recognition.

Lack of convenient methodology in bile acid chemistry for the synthesis of stereoisomers 2 and 3 prompted us to report our recent results.



1 cholic acid

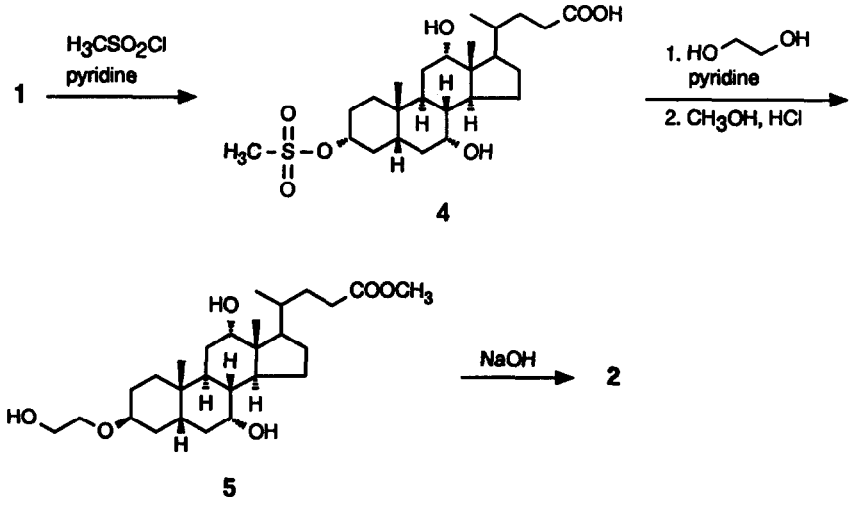


2 $R^1 = OCH_2CH_2OH$, $R^2 = H$
3 $R^1 = H$, $R^2 = OCH_2CH_2OH$

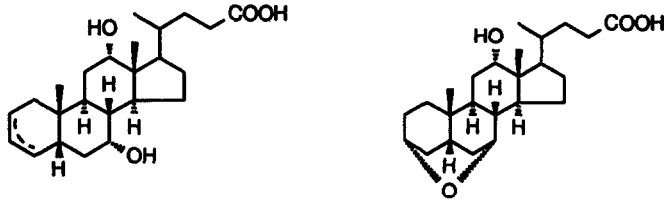
Nucleophilic displacement of mesylate **4** (scheme 1) was expected to proceed with inversion of configuration and seemed to be the most attractive route for large scale preparations of **2**. Cholic acid **1** was treated with 1.2 equiv. methanesulfonyl chloride in pyridine at 0°C for 30 min and at room temperature for 2 h to give mesylate **4** quantitatively after chromatography (silica gel, cyclohexane/ethyl acetate/acetic acid = 5:5:1). Nucleophilic displacement of **4** was affected with excess ethylene glycol in pyridine (5:1) at 100°C for 2 h. After extractive workup the crude mixture containing **6a**, **6b** and **7** as the major side products[2,3] was

esterified with methanol/HCl for simpler purification. Compound[4] 5 was isolated over two steps after chromatography (silica gel, ethyl acetate/methanol = 10:1) in 35-40% yield. The 3-α isomer 3 could not be detected[2] and was synthesized as described below. Hydrolysis of 5 with 1.4 equiv. aqueous 1 N sodium hydroxide in methanol at room temperature for 8 h gave compound[4] 2 in 97% yield.

Alcoholysis of cholesteryl toluenesulfonates with diols proceeds with higher yields and has been described earlier.[5] This might be due to active participation of the 5,6-double bond of the homoallylic system of 5-cholesten-3-yl derivatives in the reaction pathway.



Scheme 1.



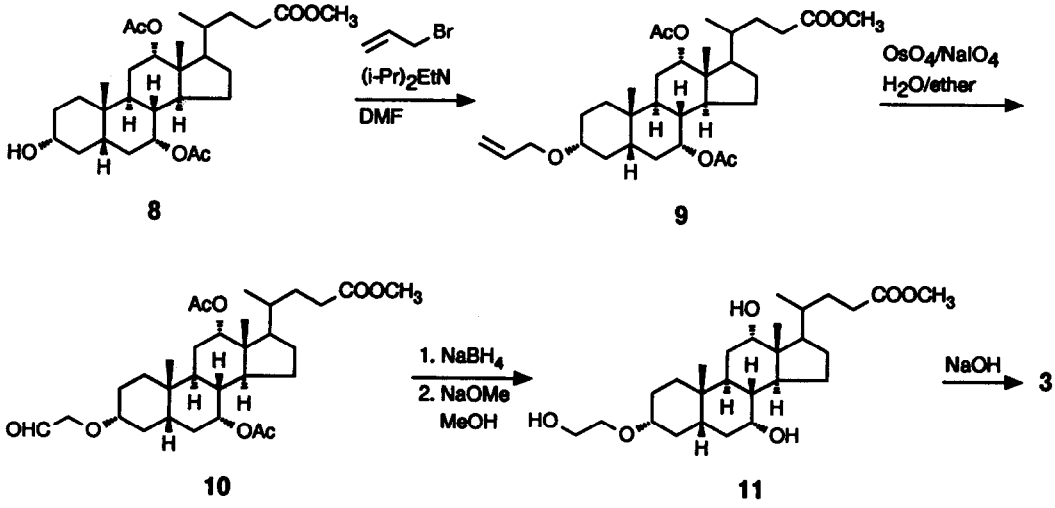
6a Δ^2-olefin

6b Δ^3-olefin

7

For the synthesis of 3 direct monoalkylation of cholic acid or methyl cholate could not be realized under various conditions in reasonable yield. Therefore diacetate[6] 8 (scheme 2) served as the starting material. 8 was refluxed for 8 h in DMF in the presence of 3.0 equiv. N,N-diisopropylethylamine under portionwise addition of excess allyl bromide. 9 was obtained in 81% yield after chromatography (silica gel, cyclohexane/ethyl acetate = 3:1). Cleavage of the double bond to aldehyde **10** went smoothly with cat. osmium tetroxide and 2 equiv. sodium periodate in water/ethyl ether = 1:1 at room temperature for 8 h, 81%

yield after chromatography (silica gel, cyclohexane/ethyl acetate = 3:1). Sodium borohydride reduction of aldehyde **10** in THF/methanol = 4:1 at 0°C and subsequent stirring at reflux of the crude reduction product with 3.0 equiv. sodium methoxide in dry methanol for 3 h gave compound[4] **11** in 84% yield over two steps after chromatography (silica gel, ethyl acetate). Compund[4] **3** was obtained after hydrolysis of **11** with 1.4 equiv. 1 N aqueous sodium hydroxide in methanol at room temperature for 8 h in 91% yield.



Scheme 2.

The synthetic methodology described above for **2** and **3** can be used generally to prepare a variety of new modified bile acids.[7]

Interaction of **2** and **3** with the specific ileal bile acid transport system was studied by inhibition of Na^+-dependent [^{3}H]taurocholate uptake into ileal brush border membrane vesicles (rabbit).[9] Only the 3α-isomer **3** is recognized by the transport system with IC_{25} = 9 μM and IC_{50} = 19 μM compared to cholic acid (IC_{25} = 9 μM, IC_{50} = 17 μM). The 3β-isomer **2** showed only very weak affinity (IC_{25} = 42 μM) and could not inhibit Na^+-dependent taurocholate uptake.

Acknowledgments: We wish to thank Dr. Fehlhaber and Dr. Kogler for analytical support, F. Girbig, U. Gutjahr and S. Kowalewski for performing biological experiments.

REFERENCES AND NOTES

1. F. A. Wilson, Am. J. Physiol. 241, G83-G92 (1981); L. Lack, Environmental Health Perspectives 33, 79-90 (1979).

2. The reaction mixture has been analyzed by HPLC after derivatization of the 24-C carboxylates as 4-nitrobenzylesters: A sample of the reaction mixture has been worked up in the usual way (HCl/ethyl acetate). After evaporation of the combined organic phases the crude product was treated with 2.3 equiv. 4-nitrobenzyl bromide using Adogen 464 as catalyst in saturated aqueous sodium hydrogen carbonate solution at room temp. for 24 h.[8] After extractive workup (HCl/ethyl acetate) the organic phase was analyzed by HPLC (LiChrosorb RP-18, 5 µm, Merck; acetonitrile/water = 63:37; 1.75 ml/min; 254 nm).

3. **6a, 6b and 7** have been characterized as methylesters and 4-nitrobenzylesters. The free acids **6a, 6b** and **7** have been described by C. H. Brieskorn, H. Monsandi, Arch. Pharm. 314, 118-127 (1981).

4. Characteristic analytical data:
   2 amorphous solid, mp 190-192°C; ^{1}H-NMR 270 MHz (DMSO-d_6) δ 0.60 (s, 3H), 0.82 (s, 3H), 0.90-2.45 (m, 24H), 0.93 (d, J=4.0Hz, 3H), 3.31 (m, 2H), 3.48 (m, 3H), 3.62 (m, 1H), 3.78 (m, 1H), 4.00 (d, J=2.7Hz, 1H), 4.08 (d, J=2.7Hz, 1H), 4.45 (t, 1H), 11.90 (s, 1H);

   3 amorphous solid, mp 183-185°C; ^{1}H-NMR 270 MHz (DMSO-d_6) δ 0.60 (s, 3H), 0.83 (s, 3H), 0.90-2.30 (m, 24H), 0.92 (d, J=4.0Hz, 3H), 3.05 (m, 1H), 3.3-3.5 (m, 4H), 3.62 (m, 1H), 3.79 (m, 1H), 4.01 (d, J=2.7Hz, 1H), 4.10 (d, J=2.7Hz, 1H), 4.50 (m, 1H), 11.91 (s, 1H);

   5 amorphous solid, mp 159-160°C; ^{1}H-NMR 270 MHz ($CDCl_3$) δ 0.70 (s, 3H), 0.92 (s, 3H), 0.99 (d, J=4.0Hz, 3H), 1.10-2.45 (m, 27H), 3.48 (t, J=3.2Hz, 1H), 3.59 (m, 1H), 3.66 (s, 3H), 3.70 (m, 2H), 3.86 (m, 1H), 3.98 (m, 1H);

   11 amorphous solid, mp 104-106°C; ^{1}H-NMR 270 MHz ($CDCl_3$) δ 0.69 (s, 3H), 0.90 (s, 3H), 0.95-2.45 (m, 27H), 1.00 (d, J=4.0Hz, 3H), 3.16 (m, 1H), 3.59 (t, J=3.0Hz, 2H), 3.68 (s, 3H), 3.69 (m, 2H), 3.86 (m, 1H), 3.98 (m, 1H).

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6. For the synthesis of **8** methyl cholate was transformed quantitatively to triacetoxy methyl cholate with 4 equiv. acetic anhydride in pyridine using 1.0 equiv. 4-dimethylaminopyridine[10] at 0°C for 30 min and room temperature for 3 h. The 3-acetate was removed selectively with 2.0 equiv. sodium methoxide in dry methanol at room temperature for 2 h to give **8** in >95% yield after filtration through silca gel (cyclohexane/ethyl acetate = 2:1).

7. Publications in preparation.

8. V. Bocchi, G. Casnati, A. Dossena, R. Marchelli, Synthesis 957-961 (1979).

9. G. Burckhardt, W. Kramer, G. Kurz, F. A. Wilson, J. Biol. Chem. 258, 3618-3622 (1983); W. Kramer, G. Burckhardt, F. A. Wilson, G. Kurz, J. Biol. Chem. 258, 3623-3628 (1983).

10. G. Höfle, W. Steglich, Synthesis 619-621 (1972).

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