SYNTHESIS OF BILE ACID ANALOGS: 7-ALKYLATED CHENODEOXYCHOLIC ACIDS

Mizuho Une,[a] Katsumi Yamanaga,[a] Erwin H. Mosbach,[b] Syoji Kuroki,[b] and Takahiko Hoshita[a]

[a]Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima 734, Japan; [b]Department of Surgery, Beth Israel Medical Center, New York, NY 10003, USA

Corresponding author: Mizuho Une
Received October 1, 1987
Revised August 22, 1988

ABSTRACT

This paper describes a method for the preparation of 7-alkylated chenodeoxycholic acids from 3α-hydroxy-7-oxo-5β-cholanoic acid. The synthetic procedure is based upon a Grignard reaction between the keto bile acid and an alkyl magnesium halide. Under the conditions employed, the introduction of alkyl groups is highly stereoselective. Only 7β-alkylated epimers are obtained. The overall yield is several-fold higher than that obtained by the previous method, which involved the preparation of an oxazoline intermediate.

INTRODUCTION

In order to develop new and more effective cholelitholytic agents, our laboratories undertook the synthesis of 7-methyl-substituted analogs of the naturally occurring dihydroxy and trihydroxy bile acids (1,2). It is known from studies in animal models that such bile acid analogs are resistant to 7-dehydroxylation by the intestinal bacterial flora, are readily absorbed from the intestine, and participate in the enterohepatic circulation (3,4). To evaluate the gallstone-dissolving properties of the new compounds in the prairie dog model of cholesterol cholelithiasis, relatively large amounts of the 7-methyl chenodeoxycholic acid (7-Me-CDA) were required. The procedure reported previously

required six steps to synthesize 7-Me-CDA from 7-keto-lithocholic acid (7-KLA), and the overall yield was less than 10%. The present report deals with a modification of the previous procedure. The present method for the preparation of 7-Me-CDA involves the direct alkylation of methyl-7-KLA followed by alkaline hydrolysis. Yields of the order of 50% are readily obtainable. In addition, two new 7-alkyl substituted bile acid analogs, 7-ethyl- and 7-n-propyl-chenodeoxycholic acids (7-Et-CDA and 7-Pr-CDA), were also synthesized by the improved method.

MATERIALS AND METHODS

Melting points were determined with a Kofler hot-stage apparatus (Thomas Scientific, Swedesboro, NJ) and are uncorrected. Proton magnetic resonance (PMR) spectra were obtained in pyridine-d_5 solution at 100 MHz on a JEOL (Peabody, MA) JNM-PS-100 spectrometer. Chemical shifts are given in δ ppm with tetramethylsilane as an internal standard. Thin-layer chromatography was carried out on silica gel G plates (Merck, Darmstadt, West Germany) using a 10% solution of phosphomolybdic acid in ethanol as the detection reagent. Gas-liquid chromatography was performed on a Shimadzu (Columbia, MD) GC-6A gas chromatograph using a glass column [2 m x 3 mm inner diameter (i.d.)] packed with 3% OV-17 on 80/100 mesh Gas Chrom Q. All retention times are given relative to the trimethylsilyl (TMS) ether of methyl cholate. Gas-liquid chromatography-mass spectrometry (GC-MS) was carried out on a Shimadzu model GCMS-QP-1000 gas chromatograph-mass spectrometer. The following operating conditions were employed: column, OV-1 (12 m x 0.25 mm i.d.); injection port temperature, 270°C; column oven temperature, 230-260°C, 2°C/min; separator temperature, 250°C; ionizing source temperature, 250°C; flow rate of helium carrier gas, 40 mL/min; ionizing voltage, 70 eV; ionizing current, 60 μA.

3α,7α-Dihydroxy-7β-methyl-5β-cholanoic acid (IIa) (Figure 1). To a solution of the methyl ester of 7-KLA (4.04 g, 10 mmol) in 100 mL of dry ether there was added dropwise with stirring an ethereal solution of methyl magnesium iodide (30 mmol). The stirring was continued at room temperature for 2 h. At the end of this period, the reaction mixture was poured into 200 mL of water, acidified with 1 N HCl, and extracted twice with 200 mL of ether.

The combined ether extracts were washed with water, dried over anhydrous Na_2SO_4, filtered, and the solvent was removed in vacuo. The residue (4.0 g) was chromatographed on a silica gel column (100 g). Elution with increasing proportions of ethyl acetate in benzene yielded three fractions. Fraction 1, elution with benzene-ethyl acetate, 7:3, v/v, resulted in the recovery of 848 mg of the starting material, methyl 7-KLA. Fraction 2, elution with benzene-ethyl acetate, 6:4 and 4:6, v/v, after alkaline hydrolysis (5% methanolic KOH, refluxing for 1 h) yielded 2.03 g of 3α,7α-dihydroxy-7β-methyl-5β-cholanoic acid (7-Me-CDA) (IIa), melting point (mp) 96-99°C (from methanol-water), PMR (δ ppm): 0.79 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 1.05 (3H, d, J=6 Hz, 21-CH_3), 1.32 (3H, s, 7β-CH_3), 3.86 (1H, m, 3β-H). Chromatographic behavior and spectral data of this sample of 7-Me-CDA were completely identical with that of those prepared by the procedure published previously [single spot upon TLC with solvent systems A-1 and N-1 (Table 1) and single peaks upon GLC on OV-1 and OV-17 (Table 1)] (1). Fraction 3, elution with benzene-ethyl acetate, 3:7 and 1:9, v/v, yielded 737 mg of another product which was probably 7β,24,-24-trimethyl-5β-cholane-3α,7α,24-triol (IIIa). PMR (δ ppm): 0.71 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 0.99 (3H, d, J=6 Hz, 21-CH_3), 1.36 (9H, s, 7-CH_3, and 24-$(CH_3)_2$), 3.67 (1H, m, 3β-H); MS (m/z) (relative intensity): 131 (79), 143 (100), 257 (30).

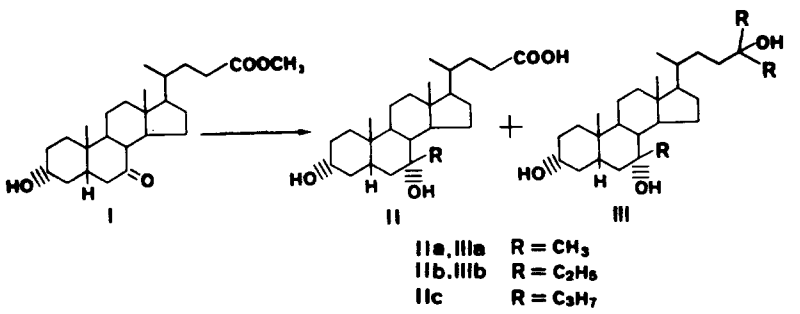

Figure 1. Synthesis of 7-alkylated bile acids.

3α,7α-Dihydroxy-7β-ethyl-5β-cholanoic acid (IIb). Methyl 7-KLA (4.04 g, 10 mmol) was treated with ethyl magnesium bromide (30 mmol) in the same manner as described above. The reaction products were chromatographed on a column of silica gel (100 g), using increasing portions of ethyl acetate in benzene. Elution with benzene-ethyl acetate, 7:3 and 5:5, v/v, yielded methyl 7-KLA and the methyl ester of the desired product, 7-Et-CDA (IIb), respectively. Alkaline hydrolysis of the latter ester with 5%

methanolic KOH afforded 1.68 g of the corresponding free acid, 7-Et-CDA (IIb), melting point 102-103°C (from methanol-water) and single spot upon TLC with solvent systems A-1 and N-1 (Table 1) and single peaks upon GLC on OV-1 and OV-17 (Table 1), PMR (δ ppm): 0.74 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 0.98 (3H, t, J=6 Hz, -CH_2CH_3), 0.99 (3H, d, J=6 Hz 21-CH_3), 3.73 (1H, m, 3β-H). Elution with benzene-ethyl acetate, 3:7, v/v, yielded 430 mg of another product which probably had the structure 7β,24,24-triethyl-5β-cholane-3α,7α,24-triol (IIIb). PMR (δ ppm): 0.74 (3H, s, 18-CH_3), 0.91 (3H, s, 19-CH_3), 1.01 (3H, d, J 6 Hz, 21-CH_3), 1.02 (9H, s, 7-CH_2CH_3 and 24-$(CH_2CH_3)_2$), 3.73 (1H, m, 3β-H). MS (m/z) (relative intensity): 649 (95), 559 (51), 469 (15), 379 (4), 271 (81), 159 (100), 157 (79).

3α,7α-Dihydroxy-7β-n-propyl-5β-cholanoic acid (IIc). The methyl ester of 7-KLA (4.04 g, 10 mmol) was treated with n-propyl magnesium bromide (30 mmol) in the same manner as described for the preparation of 7-Me-CDA. The reaction products were chromatographed on a column of silica gel (100 g) using increasing proportions of ethyl acetate in benzene to give three fractions. Fraction 1 (benzene-ethyl acetate 8:2, v/v), fraction 2 (benzene-ethyl acetate, 6:4, v/v), and fraction 3 (benzene-ethyl acetate 4:6, v/v) yielded 2.64 g of methyl 7-KLA, 840 mg of methyl 7-Pr-CDA, and 640 mg of methyl chenodeoxycholate, respectively. Further elution with benzene-ethyl acetate, 2:8, and 1:9, v/v, gave no material. Alkaline hydrolysis of the ester obtained from fraction 2 gave 810 mg of 7-Pr-CDA (IIc), melting point 102-103°C (from methanol-water) and single spot upon TLC with solvent systems A-1 and N-1 (Table 1) and single peaks upon GLC on OV-1 and OV-17 (Table 1), PMR (δ ppm): 0.73 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 0.97 (3H, t, J=6 Hz, 7-$CH_2CH_2CH_3$), 1.00 (3H, d, J 6=Hz, 21-CH_3), 3.69 (1H, m, 3β-H).

RESULTS AND DISCUSSION

The method for the preparation of 7-methyl-substituted bile acids reported previously was based on the Grignard reaction between methyl magnesium iodide and 7-keto-bile acid oxazolines (1,2). It was assumed that since the conventional Grignard reagent reacts not only with the 7-keto group but also with the carboxyl moiety of the molecule, the latter group had to be protected by formation of an oxazoline derivative. Introduction

of the oxazoline group to protect the carboxyl function can be carried out in high yield. However, the removal of the oxazoline ring subsequent to the Grignard reaction presents a problem. In order to regenerate the carboxylic acid function, the oxazoline derivative had to be treated with dilute acid. The 7-methylated product possesses a tertiary hydroxyl group which is easily dehydrated during the acid hydrolysis. Therefore, the overall yield of 7-Me-CDA from 7-KLA did not exceed 10%.

Cahiez and co-workers (5,6) have reported that organomanganese derivatives, prepared from manganese chloride and organomagnesium derivatives, react efficiently with ketones but do not attack carboxylic esters.

During the course of an investigation of the preparation of 7-Me-CDA (IIa) according to the method reported by Cahiez et al, we found that methyl magnesium iodide, like the corresponding manganese derivative, preferentially reacts with the 7-keto group of methyl 7-KLA (I) under milder conditions (low reaction temperature and low ratio of 7-KLA to Grignard reagent). Hence, 7-Me-CDA could be prepared in better than 50% yield from 7-KLA without the preparation of the oxazoline intermediate, although lesser amounts of the 7,24,24-trimethylated derivative were also formed as a by-product. It should further be noted that in the present procedure, the introduction of the 7-methyl group is highly stereoselective. Only the 7β-methyl-substituted derivative, 7-Me-CDA, was

formed and negligible amounts of its 7α-methyl epimer were obtained. The predominant formation of the 7β-alkylated epimer seems to be reasonable probably because the bending of ring A shields the α-side and the Grignard reagent predominantly approaches to 7-keto group from the β-side.

By this improved method, two new 7-alkylated bile acids, 7-Et-CDA (IIb) and 7-Pr-CDA (IIc), were synthesized. In the preparation of 7-Pr-CDA (IIc), the yield was lower than with 7-Me-CDA or 7-Et-CDA. Further, the yield of the 7,24,24-trisubstituted derivative was quite low. Instead, considerable amounts of chenodeoxycholate were formed by reduction of 7-keto group of 7-KLA with the Grignard reagent. The difference in the activity between n-propyl magnesium bromide and methyl or ethyl magnesium halide cannot yet be fully explained. Probably the chain length of the alkyl group introduced is related to yields of 7-alkylated bile acid analogs. The β-orientation of the newly introduced 7-alkyl groups of 7-Et-CDA and 7-Pr-CDA was tentatively assigned by PMR. The chemical shifts of 19-CH_3 of 7-Et-CDA and 7-Pr-CDA, which are at δ 0.94 and 0.92, respectively, are almost the same as that (δ 0.97) of 19-CH_3 of 7-Me-CDA but not different from that (δ 1.09) of 19-CH_3 of 7-methyl-ursodeoxycholic acid. This result strongly suggests that the orientation of C-7 alkyl groups in these compounds is the same as that of 7-Me-CDA.

Chromatographic and mass spectral data of 7-alkylated chenodeoxycholic acids are shown in Tables 1 and 2. The mass spectra of both 7-Et-CDA (IIb) and 7-Pr-CDA (IIc) were almost identical with that of 7-Me-CDA (IIa) except for an upfield shift 14 and 28

Table 1. Chromatographic Properties of Bile Acid Analogs

Compound	CDA	IIa	IIb	IIc
GLC (RRT)[a]				
OV-1	0.94	1.06	1.24	1.32
OV-17	1.11	1.20	1.38	1.40
TLC (Rf values)[b]				
A-1	0.48	0.54	0.55	0.57
N-1	0.22	0.24	0.27	0.29

The Roman numerals (IIa-IIc) refer to the bile acid analogs described in Figure 1. CDA, 3α,7α-dihydroxy-5β-cholanoic acid.
[a]Retention times relative to methyl ester-TMS ether derivative of cholic acid.
[b]Solvent systems: A-1 (free acids), isooctane-ethyl acetate-acetic acid 10:10:2 (v/v/v), N-1 (methyl esters), benzene-acetone 6:4 (v/v).

Table 2. Mass Spectral Data of Synthetic Bile Acid Analogs

Ion	m/z	IIa	m/z	IIb	m/z	IIc
M	564	-	578	-	592	-
M - C7-alkyl	549	13	549	1	549	24
M - (90 + C7-alkyl)	459	18	459	23	459	52
M - (90 x 2)	384	28	398	36	412	79
M - (90 x 2 + C7-alkyl)	369	4	369	21	369	45
Fragment A[a]	257	96	271	100	285	100
Fragment B[b]	143	100	157	86	171	74

All bile acid analogs were analyzed as their methyl ester-TMS ether derivatives. The Roman numerals (IIa-IIc) refer to the bile acid analogs described in Figure 1.
[a]Fragment A = C3-C7 + 2 x OTMS + C7-alkyl.
[b]Fragment B = C5-C7 + OTMS + C7-alkyl.

mass units for fragments containing the C-7 position. The mass spectra of the methyl ester-TMS ether derivative of 7-Me-CDA showed the predominant ions at m/z 143 and m/z 257. These ions were also present as the major peaks in the mass spectra of the corresponding derivatives of 3α,7α,12α-trihydroxy-7β-methyl- and 3α,7β,12α-trihydroxy-7α-methyl-5β-cholanoic acids (2). Therefore, it can be assumed that the fragment ions are characteristic of the 7-methylated analogs of 3,7-bistrimethylsilanoxy bile acids (2) (see Table 2). The prominent ions at m/z 157 and 271 and at m/z 171 and 285 present in the mass spectra of the methyl ester-TMS ether derivatives of 7-Et-CDA and 7-Pr-CDA, respectively, seem to correspond to the m/z 143 and 257 ions present in those of the corresponding derivatives of 7-methyl bile acid analogs. Thus, the introduction of the 7-alkyl group into the bile acid molecule has a strong directive effect on the mass fragmentation.

Availability of these new bile acid analogs will make it possible to investigate their physiological properties and therapeutic possibilities.

ACKNOWLEDGMENTS

This work was supported in part by USPHS grant HL-24061 (EHM) from the National Heart, Lung and Blood Institute.

APPENDIX

The following trivial names and abbreviations have been used in this paper: chenodeoxycholic acid (CDA), 3α,7α-dihydroxy-5β-cholanoic acid; 7-methyl-chenodeoxycholic acid (7-Me-CDA), 3α,7α-

dihydroxy-7β-methyl-5β-cholanoic acid; 7-methyl-ursodeoxycholic acid (7-Me-UDA), 3α,7β-dihydroxy-7α-methyl-5β-cholanoic acid; 7-keto-lithocholic acid (7-KLA), 3α-hydroxy-7-oxo-5β-cholanoic acid; 7-ethyl-chenodeoxycholic acid (7-Et-CDA), 3α,7α-dihydroxy-7β-ethyl-5β-cholanoic acid; 7-n-propyl-chenodeoxycholic acid (7-Pr-CDA), 3α,7α-dihydroxy-7β-n-propyl-5β-cholanoic acid.

REFERENCES

1. Une M, Cohen BI, and Mosbach EH (1984). New bile acid analogs: 3α,7α-dihydroxy-7β-methyl-5β-cholanoic acid, 3α,7β-dihydroxy-7α-methyl-5β-cholanoic acid, and 3α-hydroxy-7ξ-methyl-5β-cholanoic acid. J LIPID RES **25**:407-410.
2. Kuroki S, Une M, and Mosbach EH (1985). Synthesis of potential cholelitholytic agents: 3α,7α,12α-trihydroxy-7β-methyl-5β-cholanoic acid, 3α,7β,12α-trihydroxy-7α-methyl-5β-cholanoic acid, and 3α,12α-didroxy-7ξ-methyl-5β-cholanoic acid. J LIPID RES **26**:1205-1211.
3. Une M, Singhal AK, McSherry CK, May-Donath P, and Mosbach EH (1985). Metabolism of 3α,7α-dihydroxy-7β-methyl-5β-cholanoic acid and 3α,7β-dihydroxy-7α-methyl-5β-cholanoic acid in hamsters. BIOCHIM BIOPHYS ACTA **833**:196-202.
4. Kuroki S, Mosbach EH, Cohen BI, and McSherry CK (1987). Metabolism of the bile acid analogues, 7β-methyl-cholic acid and 7α-methyl-ursocholic acid. GASTROENTEROLOGY **92**:876-884.
5. Cahiez G, Masuda A, Bernard D, and Normant JF (1976). Reactivite des derives organo-manganeux. I - Action sur les chlorures d'acides. Synthese de cetones. TETRAHEDRON LETT 3155-3156.
6. Cahiez G and Normant JF (1977). Reactivity of organomanganese(II) reagents. Reaction with carbonyl compounds, selective preparation of ketols from keto-aldehydes. TETRAHEDRON LETT 3383-3384.