Preparation and characterization of 3-monohydroxylated bile acids of different side chain length and configuration at C-3. Novel approach to the synthesis of 24-norlithocholic acid

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Abstract A series of 3-monohydroxylated bile acids, in unlabeled and radioactive form, of varying side chain length and configuration at C-3 has been synthesized and rigorously characterized. They include: 3α- and 3β-hydroxy-5β-androstane-17β-carboxylic acids (C_{20}); 3α- and 3β-hydroxy-5β-pregnan-21-oic acids (C_{21}); 3α- and 3β-hydroxy-23,24-bisnor-5β-cholan-22-oic acids (C_{22}); 3α- and 3β-hydroxy-24-nor-5β-cholan-23-oic acids (C_{23}, norlithocholic and isonorlithocholic acids); and 3β-hydroxy-5β-cholan-24-oic acid (C_{24}, isolithocholic acid). A novel approach to the degradation of lithocholic acid acetate to 24-norlithocholic acid is described. This degradation involves the photochemical modification of a Hunsdiecker reaction and Kornblum oxidation of the intermediate 23-bromide. The availability of these compounds makes it possible to study the metabolism and biological effects of short chain bile acids. — **Radomińska-Pyrek, A., T. Huynh, R. Lester, and J. St. Pyrek.** Preparation and characterization of 3-monohydroxylated bile acids of different side chain length and configuration at C-3. Novel approach to the synthesis of 24-norlithocholic acid. *J. Lipid Res.* 1986. **27:** 102–113.

Supplementary key words mass spectrometry • nuclear magnetic resonance • side chain synthesis

In addition to C_{24} bile acids (BA), which are quantitatively the most significant group of steroidal carboxylic acids, BA with both longer and shorter side chains are also encountered in mammalian sources (1). These minor components are categorized either as the direct cogeners of C_{24} BA or as more distantly related in their metabolic origin. The former group includes C_{27} precursors of C_{24} BA (2) as well as C_{23} acids produced by the side chain α-oxidation (3). To the latter group belong C_{20} and C_{21} acids formed by the oxidative catabolism of steroidal hormones (4, 5) as well as C_{22} acids which result from the microbial side chain degradation (6, 7).

Our recent investigations reiterated the previously suspected biological significance of short chain BA homologues. Thus, human meconium was found to be an abundant source of C_{20} (8), C_{21} (9 and unpublished results) and C_{22} (9) components. C_{20} and C_{22} acids were detected in both normal and cholestatic serum (10). The efficient metabolism of C_{20} acids, primarily expressed in their glucuronidation, was demonstrated for the rat liver both in vivo (11) and in vitro (see below).

Especially relevant is the detection of C_{20} and C_{21} components. Their presence in meconium indicates that the oxidative catabolism of 3-oxygenated pregnanes is equally significant and evidently metabolically analogous to that established for corticosteroids (12). On the other hand, the detection of C_{22} acids demonstrates that the intestinal flora is responsible not only for the 7-dehydroxylation and deconjugation (13) but also for the side chain degradation leading to the unique group of secondary BA with 22 carbon atoms.

In continuation of the above investigations, the detailed study of the specificity of the microsomal glucuronidation of short chain BA with respect to side chain length and C-3 configuration was performed. For this purpose we required, in both non-labeled and radioactive form, the complete series of C_{20}, C_{21}, C_{22}, C_{23} and C_{24} BA with both 3α (equatorial) and 3β (axial) hydroxyl groups, 5β-hydrogen, and the natural side chain configuration at C-20. Since only a few of these compounds (C_{20} and C_{24})

Abbreviations: BA, bile acids; GLC, gas-liquid chromatography; TLC, thin-layer chromatography; GLC-MS, gas-liquid chromatography-mass spectrometry; HPLC, high performance liquid chromatography; INEPT, insensitive nuclei enhanced by polarization transfer; IR, infrared spectroscopy; R_t, retention time; R_v, retention volume; ^{13}C FT NMR, Fourier transform carbon-13 nuclear magnetic resonance; ^1H NMR, proton nuclear magnetic resonance; MU, methylene unit; s.c., side chain; UV, ultraviolet spectroscopy.

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are available from commercial sources, it was necessary to synthesize the majority of these compounds from other steroidal substrates. The description of the synthetic procedures and characterization of the products is reported in this account. An accompanying publication describes the syntheses, both chemical and enzymatic, of the corresponding 3-O-β- and carboxyl-attached glucuronides (14). A preliminary account of this work has been presented (15).

MATERIALS AND METHODS

Chemicals and solvents

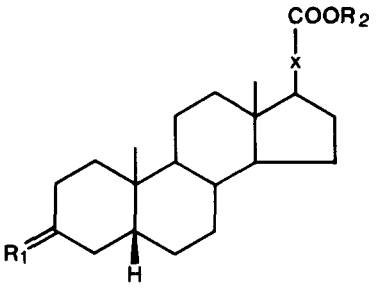
3β-Hydroxy-17-oxoandrost-5-ene (6h) (**Fig. 1**) was purchased from Sigma Chemical Co. (St. Louis, MO). The sodium salt of lithocholic acid (5a) was obtained from Calbiochem-Behring Corp. (La Jolla, CA) or from Sigma Chemical Co. and was converted to the free acid before use. 3β-Hydroxybisnorchol-5-en-22-oic and 3α- and 3β-hydroxy-5β-androstan-17β-carboxylic acids (1a, 1f) as well as all standard C_{24} BA were purchased from Steraloids, Inc. (Wilton, NH). [Carboxyl-^{14}C]lithocholic acid (59 mCi/mmol) was obtained from The Radiochemical Center (Amersham, England). Tritiated sodium borohydride (110 mCi/mmol) was from New England Nuclear. All BA were checked for chemical and radiochemical purity by thin-layer chromatography (TLC) as methyl esters in solvent system A (see section on TLC; plates developed three times) and by gas-liquid chromatography (GLC) as methyl ester-acetates as described below. All standard chemical reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Gas-liquid chromatography

Gas-liquid chromatography (GLC) was carried out with Hewlett-Packard gas chromatographs, Models 5880A and 5738, equipped with flame ionization detectors, and capillary split/splitless and falling needle injectors. The GLC analyses were performed on fused silica capillary columns; DB-1 and DB-5, 0.25 mm i.d., 0.1 μm; 10 m or 15 m, 0.25 mm i.d., 0.1 μm (J & W Scientific Inc., Rancho Cordova, CA). The column temperature and the gas flow rate varied and are specified with each separation. BA were analyzed by GLC after conversion to the corresponding methyl ester-acetates. Methyl esters were obtained by treatment of the BA in methanol–diethyl ether 1:1 (v/v) with an excess of distilled ethereal diazomethane (15 min at room temperature), followed by evaporation under nitrogen. Acetylation was effected by heating at 65°C for 12 hr with acetic anhydride–pyridine 1:1 (v/v). The residue obtained after evaporation under nitrogen was dissolved and chromatographed in benzene on a silica gel column (2 × 0.5 cm). Solvents A and B (see below) were used in order to follow the acetylation and sample purification by TLC.

High performance liquid chromatography

Preparative high performance liquid chromatography (HPLC) was carried out using a single piston mini-pump (LDC, Miton Roy Co., Riviera Beach, FL), a 0.5-ml loop injector, and a refractive index detector Model 771 (Micromeritics, Norcross, GA). A Partisil 10 M9/50 column, 0.5 m, 1 cm i.d. (Whatman Chemical Separation Inc., Clifton, NY) with ethyl acetate–hexane 85:15 (v/v) as eluent at a flow rate of 4 ml/min was used for separation of BA esters; a 600-RP C_{18} reversed phase column (Alltech, Deerfield, IL) with 0.25 M KH_2PO_4–acetonitrile 6:4 (v/v) as eluent at a flow rate of 2 ml/min was used for separation of free BA.



Compound	X =	Side Chain Configuration
1 (C_{20})	–	
2 (C_{21})	$-CH_2-$	
3 (C_{22})	$-CH(CH_3)-$	[20S]
4 (C_{23})	$-CH(CH_3)CH_2-$	[20R]
5 (C_{24})	$-CH(CH_3)CH_2CH_2-$	[20R]
	R^1 =	R^2 =
a	α OH, β H	H
b	α OAc, β H	H
c	α OH, β H	Me
d	α OAc, β H	Me
e	α OH, β H	Et
f	β OH, α H	H
g	β OH, α H	Me
h	β OH, α H	Et
i	β OAc, α H	Me
j	β $OOCC_6H_5$, α H	Me
k	β $OOCC_6H_5$, α H	Et
m	O	H
n	O	Me
o	O	Et

Fig. 1. Structures of monohydroxylated bile acids, their derivatives and substrates utilized for their syntheses.

Thin-layer chromatography

Analytical thin-layer chromatography (TLC) was carried out on precoated silica gel plates (Whatman Chemical Separation Inc.). Solvent systems used for analytical TLC separations were: solvent A, benzene–ethyl acetate 85:15 (v/v), for separation of BA methyl esters; solvent B, benzene–acetone 95:5 (v/v), for separation of methyl ester-acetates of BA; solvent C, benzene–acetone 3:1 (v/v), acidified with 0.05% (v/v) CH_3COOH, for separation of free 3α- and 3β-hydroxylated BA. The compounds were visualized as dark blue spots by spraying the developed plates with Krowicki's reagent (1% phosphomolybdic acid and 0.5% ceric sulfate in 10% sulfuric acid) (16) and heating at 105°C. Preparative TLC plates were visualized by spraying with water.

Mass spectrometry

Electron impact mass spectra were run on Finnigan Model 3300 and Shimadzu QP1000 mass spectrometers. For capillary GLC–MS analyses the column was introduced directly to the ion source as described previously (8).

NMR spectrometry

Fourier transform ^1H (at 89.60 MHz) and ^{13}C (at 22.5 MHz) NMR spectra were measured in $CDCl_3$ using a Joel FX90Q instrument. ^{13}C-NMR spectra were obtained with 5000 Hz sweep width and 8k (real) data points. Carbon substitution was determined utilizing INEPT spectra (17, 18).

SYNTHESES

[3β-^{3}H]-3α- and [3α-^{3}H]-3β-hydroxy-5β-androstane-17β-carboxylic acids (1a, 1f)

The preparation of [3-^{3}H]-labeled 3α- and 3β-hydroxy epimers was carried out by reduction of methyl 3-oxo-5β-androstane-17β-carboxylate (1n) with an excess of NaB^3H_4 (110 mCi/mmol) essentially as described before (11). The reduction yielded methyl [3β-^{3}H]-3α-hydroxy-5β-androstane-17β-carboxylate (1c) (87%) and methyl [3α-^{3}H]-3β-hydroxy-5β-androstane-17β-carboxylate (1g) (12%, by capillary GLC of corresponding methyl ester-acetates) which could be separated by HPLC. Alkaline hydrolysis in methanol–water–KOH 80:10:10 (v/v/w) at 65°C for 20 hr followed by evaporation, dilution with water, acidification, and extraction with ethyl acetate yielded free acids 1a and 1f, respectively, with specific radioactivity of 100 mCi/mmol.

3α- and 3β-hydroxy-5β-pregnan-21-oic acids (2a, 2f) (Fig. 2)

Ethyl 3β-hydroxypregna-5,17(20)-dien-21-oate (7h, see Fig. 2)

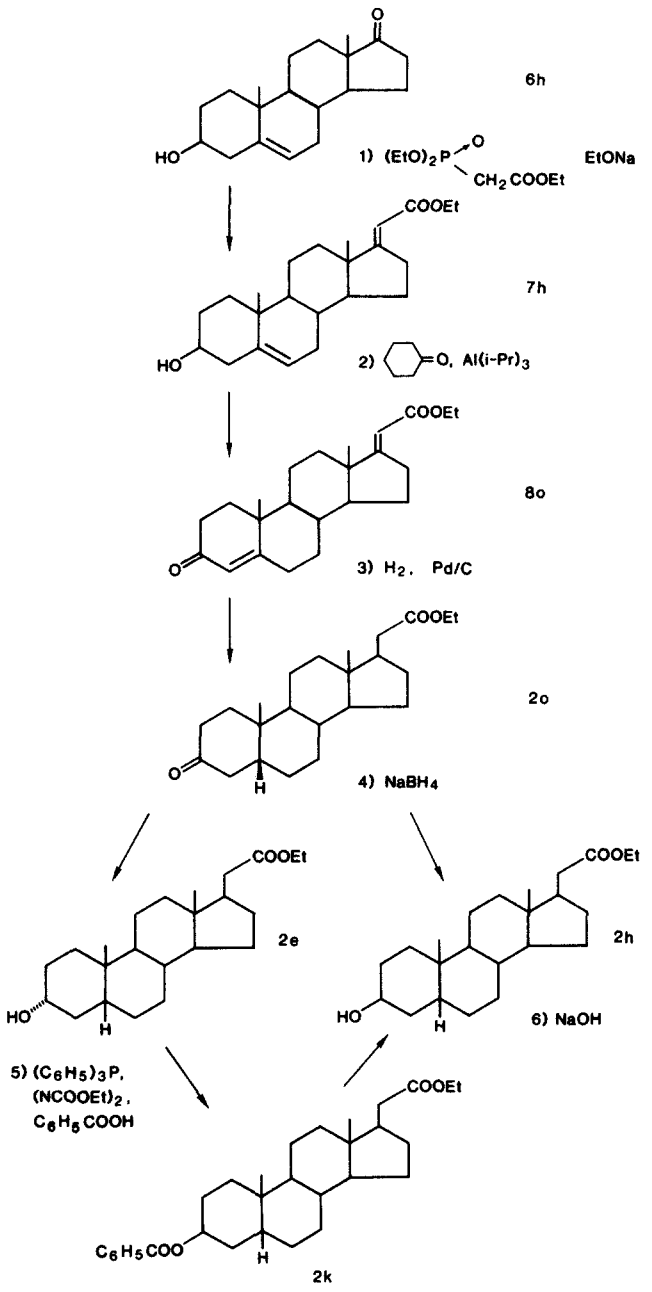


Fig. 2. Synthesis of 3α- and 3β-hydroxy-5β-pregnan-21-oic acids 2a and 2f.

was prepared (89% yield) from 3β-hydroxy-17-oxoandrost-5-ene (6h) (25 g) by the Wittig-Horner reaction in ethanol as described before (9, 19, 20).

The above hydroxy ester (7h) (9.49 g, 26.5 mmol) was oxidized *to ethyl 3-oxopregna-4,17(20)-dien-21-oate* (8o), using the procedure described for the oxidation of cholesterol (21), in toluene (500 ml) and cyclohexanone (80 ml) by treatment for 3 hr at reflux temperature with aluminum isopropoxide (0.5 g) dissolved in toluene (100 ml). Repeated evaporation with water removed most of the cyclohexanone. The residue was extracted with

methylene chloride (3 × 250 ml), filtered, and the extract was dried and evaporated. The solid residue was dissolved in hot hexane (500 ml), filtered, and allowed to crystallize at −20°C. The crude product (8.0 g) was recrystallized to give two crops of the 3-ketone (8o): 6.4 g (>99% pure by capillary GLC) and 1.5 g (>95% pure). The total yield of ethyl 3-oxopregna-4,17(20)-dien-21-oate (8o) was 84%; mp 180–181°C; IR: ν_{max} (CCl_4) 1705 (α,β-unsaturated ester), 1675, 1650, 1615 (α,β-unsaturated ketone), 1190 cm^{-1}; UV: λ_{max} (acetonitrile) 230 nm (ϵ = 29,000); MS: (20 eV, probe) m/z (relative intensity) 356 (12, M^+), 341 (2, M–Me), 310 (9, M–EtOH), 295 (3, M–Me–EtOH), 283 (72, M–COOEt), 268 (59), 232 (M–ring A fragment "124"), 186 (27, M–EtOH–124), 159 (100), 158 (50), 124 (42).

Ethyl 3-oxo-5β-pregnan-21-oate (2o) was prepared from the above compound 8o (4.9 g, 13.7 mmol) dissolved in ethanol (300 ml) by hydrogenation in the presence of 10% palladium–carbon (1.0 g). The residue obtained upon evaporation was treated with an excess of Jones' reagent (22) in acetone (100 ml) for 15 min at 15°C (in order to reoxidize the small amount of 3-hydroxy BA formed during hydrogenation). The resultant mixture of 5β- and 5α-epimers (8:2, containing a trace (<2%) of corresponding 17α isomer, as determined by capillary GLC and GLC–MS) was recrystallized four times from ethanol to give homogenous ethyl 3-oxo-5β-pregnan-21-oate (2o) (2.0 g, 43%); mp 92–94°C; IR: ν_{max} (CCl_4) 1725, 1180, 1160 (ester), 1710 cm^{-1} (ketone); MS: (20 eV, probe) m/z (relative intensity) 360 (19, M^+), 345 (11, M–Me), 342 (20, M–H_2O), 327 (16, M–Me–H_2O), 314 (27, M–EtOH), 290 (100, M–70, part of ring A), 288 (28), 272 (25), 246 (75, M–s.c. and C-16–C-17), 244 (65, M–EtOH–ring A part), 231 (25), 213 (67), 176 (47).

Ethyl 3-oxo-5β-pregnan-21-oate (2o) (102 mg, 0.3 mmol), dissolved in 2-propanol (4 ml), was treated with a solution of $NaBH_4$ (15 mg, 0.4 mmol) in water (0.2 ml) at room temperature for 30 min. Separation and purification of the resulting epimeric 3-alcohols (3α:3β, 8:2) was performed by HPLC.

Ethyl 3α-hydroxy-5β-pregnan-21-oate (2e): pure product by GLC (77.6 mg, 0.228 mmol, 76% yield). TLC: R_f = 0.53 (solvent A); MS: (20 eV, probe) m/z (relative intensity) 362 (2, M^+), 344 (100, m–H_2O), 329 (61, M–H_2O–Me), 317 (4, M–EtO), 316 (4, M–EtOH), 298 (4, M–EtOH–H_2O), 290 (12, M–C-1–C-4 fragment), 283 (5, M–H_2O–EtOH–Me), 256 (9, M–H_2O–s.c.–H, McLafferty product), 248 (10, M–s.c.–C-16–C-17 fragment), 241 (7, "256"–Me), 234 (9), 230 (10), 215 (80).

Ethyl 3β-hydroxy-5β-pregnan-21-oate (2h): pure product by GLC (12. 1 mg, 0.035 mmol, 12% yield). TLC: R_f = 0.59 (solvent A).

Alkaline hydrolysis of the esters 2e and 2h yielded the corresponding free acids purified by recrystallization from aqueous acetone: 2a mp 217–218°C; 2f mp 217.5–219°C (mp depressed when mixed together).

[3β-^{3}H]-3α-hydroxy-5β-pregnan-21-oic acid (2a)

This preparation was analogous to the reaction described above except that NaB^3H_4 was used for the reduction of ethyl 3-oxo-5β-pregnan-21-oate (2o) (12.3 mg). After HPLC purification, ethyl 3α-hydroxy-5β-pregnan-21-oate (2e) (8.1 mg, pure by capillary GLC) was obtained with a specific activity of 31 mCi/mmol. The alkaline hydrolysis of the above ethyl ester produced the acid 2a. TLC: R_f = 0.09 (solvent C).

[3α-^{3}H]-3β-hydroxy-5β-pregnan-21-oic acid (2g)

Ethyl [3β-^{3}H]-3α-hydroxy-pregnan-21-oate (2e) (2.4 mg, 7.2 μmol) in benzene (0.5 ml) was converted to the corresponding 3β-benzoate 2k by the Mitsunobu method (23) as follows (Fig. 2). A mixture of 3α-hydroxy BA ester, triphenyl phosphine (4.6 mg, 17.5 μmol), and benzoic acid (2.1 mg, 17.7 μmol) was treated with excess diethyl azodicarboxylate and kept for 12 hr at room temperature. The reaction mixture was then diluted with hexane and passed through a column (2 × 0.25 cm) of alumina activity III. Elution with acetone–benzene 2:8 (v/v) yielded pure ethyl 3β-benzoyloxy-5β-pregnan-21-oate (2k) (GLC). Its alkaline hydrolysis produced [3α-^{3}H]-3β-hydroxy-5β-pregnan-21-oic acid (2f) (1.9 mg, 31 mCi/mmol). TLC: R_f = 0.18 (solvent C).

(20S)-3α- and 3β-hydroxy-23,24-bisnor-5β-cholan-22-oic acid (3a, 3f)

The two 3-epimeric bisnorcholan-22-oic acids 3a and 3f were synthesized from the commercially available (20S)-3β-hydroxy-23,24-bisnorchol-5-en-22-oic acid. This synthesis, analogous to that described for C_{21} acids, involved the following intermediates.

Methyl-(20S)-3-oxo-23,24-bisnor-chol-4-en-22-oate (yield 82%): mp 166–168°C; IR: ν_{max} (Nujol) 1730, 1180 (ester), 1660, 1610 cm^{-1} (α,β-unsaturated ketone); UV: λ_{max} (acetonitrile) 237 nm; ϵ = 17,000; MS: (20 eV, probe) m/z (relative intensity) 358 (37, M^+), 343 (6, M–Me), 340 (1, M–H_2O), 326 (5, M–MeOH), 316 (10, M–ketene), 301 (4, M–ketene–Me), 299 (4, M–COOMe), 298 (4, M–HCOOMe), 283 (1, M–HCOOMe–Me), 273 (5), 271 (6, M–s.c.), 235 (14, M–ring A–C-6 fragment–H), 234 (14, M–ring A–C-6 fragment–2H), ... 124 (100, ring A–C-6 fragment +2H; ^{1}H-NMR (CCl_4) δ: 5.60 bs (H-4), 3.58 s (OMe), 1.19 s (19-H_3), 1.15 d (J = 6, 21-H_3), 0.715 s (18-H_3).

Methyl (20S)-3-oxo-23,24-bisnor-5β-cholan-22-oate (3n) (yield: 57%): mp 163–164°C; IR: ν_{max} (CCl_4) 1730, 1170 (ester) 1705 cm^{-1} (ketone); MS: (20 eV, probe) m/z (relative intensity) 360 (100, M^+), 345 (35, M–Me), 342 (12, M–H_2O), 328 (57, M–MeOH), 313 (4, M–MeOH–Me), 301

(10, M-COOMe), 300 (13, M-HCOOMe), 295 (6, M-MeOH-Me-H_2O), 290 (69, M-C-2-C-4 fragment), 258 (28, "290"-MeOH), 257 (15), 255 (10, M-s.c.-H_2O), 246 (35, M-s.c.-C-16-C-17 fragment), 231 (31, "246"-Me), 213 (35, "246"-H_2O-Me), 176 (40, "246"-C-1-C-4 fragment), 161 (30), 122 (30).

Methyl (20S)-3α-hydroxy-23,24-bisnor-5β-cholan-22-oate (3c) purified by HPLC (major product, yield 80%): mp 160–162°C; TLC: R_f = 0.56 (solvent A); GLC: DB-1, 15 m, 250°C, 5 PSI N_2, R_t = 5.93 min; MS: (20 eV, probe) m/z (relative intensity) 362 (2, M^+), 344 (70, M-H_2O), 329 (25, M-Me-H_2O), 312 (3, M-H_2O-MeOH), 297 (4, M-H_2O-MeOH-Me), 290 (19, M-C-1-C-4 fragment), 257 (10, M-H_2O-s.c.), 248 (9, M-s.c.-C-16-C-17 fragment), 230 (18), 215 (100).

Methyl (20S)-3β-hydroxy-23,24-bisnor-5β-cholan-22-oate (3g) purified by HPLC (minor product): mp 120–122°C; TLC: R_f = 0.65 (solvent A); GLC (as above): R_t = 5.88 min; MS: (20 eV, probe) m/z (relative intensity) 362 (20, M^+), 347 (21, M-Me), 344 (95, M-H_2O), 329 (29, M-Me-H_2O), 312 (2, M-H_2O-MeOH), 297 (5, M-H_2O-MeOH-Me), 290 (16, M-part of ring A), 285 (6, M-Me-H_2O-COOMe), 257 (7, M-H_2O-s.c.), 248 (15, M-s.c.-C-16-C-17 fragment), 236 (12), 233 (41), 230 (5), 215 (100).

(20S)-[3-^{3}H]-3α- and 3β-hydroxy-23,24-bisnor-5β-cholan-22-oic acids (3a, 3f)

Methyl 3-oxo-23,24-bisnor-5β-cholan-22-oate (3n, 20 mg) in methylene chloride (0.1 ml) and 2-propanol (0.5 ml) was treated successively with one drop of 1% $NaBH_4$ in water, 2.5 mCi of NaB^3H_4 (110 mCi/mmol), and an excess of $NaBH_4$. The reaction was acidified with acetic acid, evaporated, and diluted with water (1 ml). The mixture of products extracted with ethyl ether was separated by preparative TLC (solvent B, three developments) to give more polar methyl 3α-hydroxy-23,24-bisnor-5β-cholan-22-oate (3c, 12.2 mg, specific activity 41.5 mCi/mmol) and less polar methyl 3β-hydroxy-23,24-bisnor-5β-cholan-22-oate (3g, 3.6 mg, specific activity 40.0 mCi/mmol). Hydrolysis to free acids was performed as described above for C_{20} methyl esters.

(20R)-3α- and 3β-hydroxy-24-nor-5β-cholan-23-oic acids (4a, 4f) (Fig. 3)

The following reaction sequence was utilized for the synthesis of norlithocholic (4a) and isonorlithocholic (4b) acids (**Fig. 3**).

Lithocholic acid acetate (5b). Lithocholic acid (5a, Sigma; contaminated (24) with cholan-24-oic acid, as determined by TLC, GLC-MS of methyl ester)[2] was recrystallized twice from ethanol-hexane to give pure lithocholic acid, mp 185–186.5°C, reported mp 185–186°C (25). This acid (21.54 g, 57.2 mmol) was acetylated in methylene chloride (50 ml), acetic anhydride (75 ml), and acetic acid (140 ml).

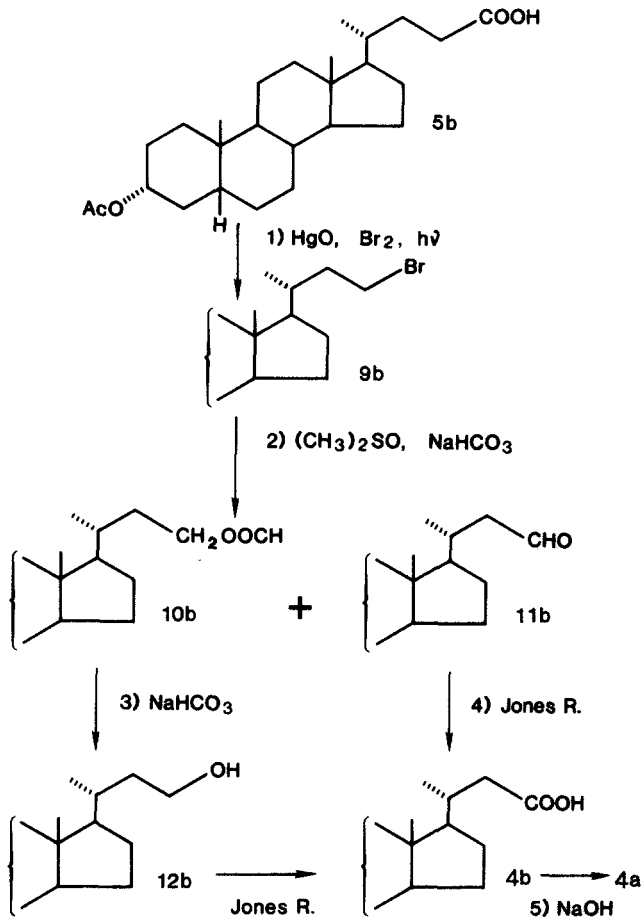


Fig. 3. Synthesis of 3α- and 3β-hydroxy-24-nor-5β-cholan-23-oic acids 4a and 4f by the side chain degradation of lithocholic acid acetate 5b.

On the next day, water (100 ml) was slowly added to decompose the mixed anhydride. Two crops of lithocholic acid acetate 5b were obtained, 23.49 g, 98.1% total yield; mp 167–168°C and 165–166°C, respectively, reported mp 168–169°C (25); IR: ν_{max} (CCl_4) 2400–2900 broad, 1725, 1700, 1250 cm^{-1}, ^{13}C NMR, **Table 1.**

3α-Acetoxy-23-bromo-24-nor-5β-cholan (9b). Lithocholic acid acetate (5b, 23.13 g, 55.3 mmol) and red mercuric oxide (21.0 g) in carbon tetrachloride (250 ml) were refluxed under a nitrogen atmosphere with irradiation by a

[2] In our experience, and in agreement with Budai and Javitt (24), commercial lithocholic acid is usually highly contaminated with a less polar compound. For this impurity, the authors found a relative retention time of 0.36 and a molecular weight of 132. We identified this component as 5β-cholan-24-oic acid by the direct comparison with authentic free cholanoic acid (by TLC) and its methyl ester (GLC, GLC-MS M^+ at 374, base peak at 217). The presence of 5β-cholanoic acid in commercial lithocholic acid might result from the probable method of its preparation, i.e., the Wolf-Kishner reduction of a partially oxidized cholic or chenodeoxycholic acid. The presence of 3,7,12-trioxo- or 3,7-dioxo-5β-cholanoic acids as an inpurity of the intermediate explains the formation of the fully deoxygenated product (compare ref. 26).

TABLE 1. ^{13}C NMR data of 24-nor-5β-cholane derivatives listed in Fig. 3

C#	C_{20} 3α-OAc -COOH 1b	C_{24} 3α-OAc -COOH 5b	C_{23} 3α-OAc -COOH 4b	C_{23} 3α-OAc $-CH_2Br$ 9b	C_{23} 3α-OAc -CHO 11b	C_{23} 3α-OAc $-CH_2OOCH$ 10b	C_{23} 3α-OAc $-CH_2OH$ 12b	C_{23} 3α-OH -COOMe 4c	C_{23} 3β-OH -COOMe 4g
1	35.15	35.13	35.10	35.04	35.13	35.10	34.94	35.45	30.03
2	26.43	26.43	26.34	26.32	26.37	26.37	26.24	30.65	28.35
3	74.39	74.58	74.47	74.31	74.44	74.42	74.34	71.95	67.24
4	32.31	32.36	32.31	32.25	32.33	32.31	32.14	36.56	33.66
5	41.93	42.01	41.95	41.87	41.95	41.95	41.79	42.20	36.67
6	26.99	27.13	27.05	26.99	27.08	27.05	26.94	27.27	26.72
7	26.70	26.72	26.67	26.62	26.72	26.70	26.51	26.51	26.34
8	36.05	35.91	35.86	35.78	35.88	35.85	35.69	35.97	35.75
9	40.57	40.55	40.49	40.41	40.49	40.49	40.36	40.55	39.84
10	34.75	34.69	34.64	34.56	34.67	34.63	34.48	34.67	35.23
11	20.71	20.92	20.87	20.82	20.87	20.87	20.73	20.90	21.17
12	38.76	40.25	40.08	40.17	40.11	40.19	40.08	40.17	40.22
13	44.47	42.85	42.88	42.82	42.96	42.85	42.67	42.93	42.96
14	56.18	56.61	56.59	56.51	56.18	56.56	56.51	56.64	56.75
15	24.53	24.26	24.18	24.15	24.23	24.20	24.10	24.28	24.26
16	23.55	28.24	28.32	28.19	28.59	28.32	28.27	28.35	28.35
17	55.37	56.10	56.10	56.13	56.18	56.34	56.45	56.26	56.29
18	13.38	12.15	12.09	12.01	12.09	12.01	11.90	12.15	12.17
19	23.36	23.42	23.34	23.31	23.39	23.36	23.23	23.44	23.96
20	180.08	35.40	33.64	35.04	31.68	33.12	32.80	33.88	33.88
21	–	18.35	19.54	18.11	20.06	18.68	18.78	19.62	19.60
22	–	30.87	41.39	39.32	50.98	34.56	38.84	41.55	41.55
23	–	31.14	179.61	31.87	203.54	62.30	60.52	174.19	174.14
24	–	180.34	–	–	–	–	–	–	–
$\underline{C}H_3COO$	21.49	21.52	21.44	21.41	21.49	21.47	21.30	–	–
$CH_3\underline{C}OO$	170.81	170.92	170.81	170.48	170.73	170.67	170.56	–	–
$\underline{C}H_3O$-	–	–	–	–	–	–	–	51.41	51.38
H$\underline{C}$OO-						161.24	–		

All spectra were obtained in $CDCl_3$ at 22.5 MHz. δ values are referenced to the central line of $CDCl_3$ δ = 77.10.

500 W tungsten lamp. To this solution bromine (14 g) was slowly added (according to the procedure described in Ref. 27). After 1.5 hr the reaction mixture was filtered to remove inorganic material and the solution was washed with 10% HCl, brine, and saturated $NaHCO_3$. Three crops of bromide 9b were obtained by recrystallization from methylene chloride, hexane, and acetone, respectively, total yield 20.03 g (79.5% mp 190–192°C). MS: (20 eV, probe) m/z (relative intensity) 394, 392 (4.5, M-AcOH), 379, 377 (1.5, M-AcOH-Me), 346, 348 (1.5, M-C-1-C-4 fragment), 313 (2, M-AcOH-Br), 257 (7, M-AcOH-s.c.), 230 (25, M-AcOH-s.c. and C-16, 17), 216 (45, M-AcOH-C-15...17), 215 (100, M-AcOH-C15...17-H, and "230"-Me), 201 (11, "216"-Me). NMR (270 MHz, $CDCl_3$): δ 4.72 tt (J = 11.6 and 4.2 Hz, 3β-H), 3.49 td (J = 9.5 and 4.2 Hz) and 3.36 tt (J = 9.5 and 8.0 Hz) 23-H_2, 2.03 s (OAc), 0.94 d (21-H_3), 0.92 s (19-H_3), 0.66 s (18-H_3).

3α-Acetoxy-23-formyloxy-24-nor-5β-cholane (10b). The above bromide 9b (1.096 g, 2.79 mmol) in dimethyl sulfoxide (15 ml) and dimethyl ether of diethylene glycol (5 ml) was heated at 130°C for 20 hr in the presence of sodium bicarbonate (0.5 g). After dilution with water (250 ml), the mixture of at least three products (TLC, solvent A) was extracted with methylene chloride (4 × 100 ml). The residue (0.890 g) obtained by long drying under vacuum (0.05 mm Hg) was recrystallized from acetone to give crude formate 10b (0.293 g); after three additional recrystallizations, an analytical sample was obtained: mp 167–169°C; MS: (70 eV, probe) m/z (relative intensity) 418 (6, M^+), 400 (1, M-H_2O), 358 (100, M-AcOH), 343 (20, M-AcOH-Me), 230 (40), 215 (87). ^1H NMR δ: 8.06 s (OOCH), 4.73 m (3β-H), 4.20 m (23-H_2), 2.04 s (Ac), 0.95 d (21-H_3), 0.94 s (19-H_3), 0.67 s (18-H_3). ^{13}C NMR in Table 1.

3α-Acetoxy-23-hydroxy-24-nor-5β-cholane (12b). The combined mother liquors from the second, third, and fourth crystallization of the formate 10b were evaporated (0.2 g), dissolved in acetone (15 ml) and methanol (15 ml), and treated with a saturated aqueous solution of sodium bicarbonate (2 ml) at room temperature for 24 hr. Subsequently, the mixture was partially evaporated to give a crystalline product which was purified by preparative TLC in solvent B. 3α-Acetoxy-23-hydroxy-24-nor-5β-cholane 12b was obtained as the major component

(88 mg), mp 104.5–105.0°C; MS: (70 eV, probe) m/z (relative intensity) 390 (0.3, M^+), 372 (0.5, M–H_2O), 360 (0.3, M–CH_2O), 357 (0.3, M–H_2O–Me), 345 (0.3, M–"CH_2CH_2OH"), 344 (0.3, M–"CH_3CH_2OH"), 330 (83, M–AcOH), 315 (18, M–AcOH–Me), 300 (4, M–AcOH–CH_2O), 276 (7), 257 (17, M–AcOH–s.c.), 230 (23), 215 (72), ... 43 (100). ^1H NMR δ: 4.67 m (3β-H), 3.59 m (23-H_2), 0.90 s (21-H_3), 0.88 s (19-H_3), 0.62 s (18-H_3). ^{13}C NMR in Table 1.

3α-Acetoxy-24-nor-5β-cholan-23-al (11b). Aldehyde 11b was obtained as the minor component from the above preparative TLC separation (6 mg). MS (70 eV, GLC–MS) m/z 388 (0.6, M^+), 373 (0.2, M–Me), 370 (0.1, M–H_2O), 355 (0.1, M–H_2O–Me), 344 (6, McLafferty process: M–CH_2=CHOH), 328 (17, M–AcOH), 313 (6, M–AcOH–Me), 284 (25, M–CH_2=CHOH–AcOH), 230 (15), 215 (40), 43 (100); ^1H NMR δ: 9.76 m (23-H), 4.73 m (3β-H), 2.0–2.5 m (22-H_2), 2.05 s (Ac), 1.00 d (21-H_3), 0.94 s (19-H_3), 0.70 s (18-H_3). ^{13}C NMR in Table 1.

3α-Acetoxy-24-nor-5β-cholan-23-oic acid (4b). The mother liquor obtained from the first recrystallization described above was evaporated, dissolved in acetone (200 ml), ethanol (100 ml), and saturated sodium bicarbonate (~10 ml), followed by water to obtain a clear solution. After 48 hr at room temperature, the mixture was partially evaporated to give a crystalline precipitate composed mostly of 3α-acetoxy-23-hydroxy-24-nor-5β-cholane 12b (TLC). This product, without purification, was dissolved in acetone (100 ml) and was treated with an excess of Jones' reagent (22) for 20 hr at room temperature. Subsequently 2-propanol (1 ml) and water (100 ml) were added. Partial evaporation yielded 3α-acetoxy-24-nor-5β-cholan-23-oic acid 4b (0.46 g), recrystallized from hexane, mp 120–122°C after additional recrystallization from aqueous acetone, reported mp 177–178°C (26) and 179–180°C (28) (mp difference probably due to solvate); MS: (70 eV, probe) m/z (relative intensity) 404 (0.4, M^+), 386 (0.5 M–H_2O), 344 (64, M–AcOH), 329 (14, M–AcOH–Me), 290 (6, M–s.c., and C-16–C-17 fragment), 230 (19), 215 (53), 43 (100). ^1H NMR δ: 9.50 br (COOH), 4.72 m (3β-H), 2.48 d (22-H_2), 2.03 s (OAc), 1.03 d (21-H_3), 0.93 s (19-H_3), 0.69 s (18-H_3); ^{13}C NMR in Table 1.

3α-Hydroxy-24-nor-5β-cholan-23-oic acid (4a) was obtained by alkaline hydrolysis of the above acetate 4b. It was recrystallized from aqueous acetone; mp 186–187°C; reported mp 185–186°C (26), 186–187°C (28), and 181–182°C (29). MS: (70 eV, probe) m/z (relative intensity) 362 (0.4 M^+), 344 (13, M–H_2O), 329 (5, M–H_2O–Me), 302 (1.5, McLafferty process: M–AcOH from s.c.), 248 (1.4, M–s.c.–C-16–C-17), 230 (4), 215 (28), ... 41 (100); 20 eV: 362 (1), 344 (32), 329 (11), 302 (1), 290 (4), 248 (6), 230 (21), 215 (100). Methyl ester 4c: ^1H NMR δ: 3.67 s (OMe), 3.60 m (3α-H), 2.40 d (22-H_2), 0.97 d, (J = 7, 21-H_3), 0.92 s (19-H_3), 0.67 s (18-H_3), ^{13}C NMR in Table 1. Methyl ester acetate 4d was characterized by GLC (Fig. 5) and GLC–MS: m/z (relative intensity) 358 (8, M–AcOH), 343 (4, M–AcOH–Me), 304 (4, M–AcOH–C-1–C-4 fragment), 285 (14, M–AcOH–CH_2COOMe), 248 (8, M–AcOH–CH_3COOMe, McLafferty process), 257 (21, M–AcOH–s.c.), 230 (28), 15 (100).

3β-Hydroxy-24-nor-5β-cholan-23-oic acid (4f)

The above compound was prepared from methyl ester 4c according to the procedure described for 2f. The intermediate methyl 3β-benzoyloxy-24-nor-5β-cholan-23-oate (4j) displayed the following spectral properties: MS: (20 eV, probe) m/z (relative intensity) 449 (0.3, M–OMe), 431 (0.2, M–OMe–H_2O), 358 (79, M–C_6H_5COOH), 343 (25, M–C_6H_5COOH–Me), 257 (6, M–C_6H_5COOH–s.c.), 230 (14), 215 (46), ... 105 (100, $C_6H_5CO^+$); ^1H NMR δ: 7.2–8.2 (5H benzoate), 5.33 bs (3α-H), 3.65 s (OMe), 2.40 d (22-H_2), 1.00 s (19-Me), 0.95 d (21-H_3), 0.70 s (18-H_3). Its alkaline hydrolysis afforded the free acid 4g, mp 178–179°C from aqueous acetone. MS: (70 eV, probe) m/z (relative intensity, comp. 3α-isomer 4a) 362 (4, M^+), 347 (4, M–Me), 344 (16, M–H_2O), 329 (18, M–H_2O–Me), 248 (4), 233 (13), 230 (2), 215 (19), ... 41 (100); 20 eV: 362 (17), 347 (19), 344 (69), 329 (72), 248 (21), 233 (69), 230 (10), 215 (100).

Methyl ester 4g, MS: (20 eV, probe) m/z (relative intensity) 376 (11, M^+), 361 (15, M–Me), 358 (34, M–H_2O), 343 (20, M–H_2O–Me), 248 (13), 233 (49), 230 (7), 215 (73), 149 (100); ^1H NMR δ: 4.10 bs (3α-H), 3.66 s (OMe), 2.40 d (J = 10 Hz, 22-H_2), 0.97 d (21-H_3), 0.97 s (19-H_3), 0.69 s (18-H_3); ^{13}C NMR in Table 1.

[3β-^{3}H]-3α- and [3α-^{3}H]-3β-hydroxy-24-nor-5β-cholan-23-oic acids (4a and 4f)

Norlithocholic acid (4a) (7.8 mg, 21.6 μmol) in acetone (1.5 ml) was treated at 0°C with Jones' reagent (22). The mixture was brought to room temperature, diluted with water (1.5 ml), and partially evaporated to give 3-oxo-24-nor-5β-cholan-23-oic acid (4m, 6.9 mg, 89%). This intermediate was dissolved in 0.1 N NaOH (1.5 ml) and treated with NaB^3H_4 (217 mCi/mmol) as described before for the preparation of 3a and 3f. The reaction mixture was acidified with diluted HCl and extracted with ethyl acetate. Preparative TLC in solvent C afforded 4a (4.6 mg, 66%, specific activity 63 mCi/mmol) and 4f (1.8 mg, 26%, specific activity 64 mCi/mmol).

3β-Hydroxy-5β-cholan-24-oic acid (isolithocholic acid (5f) and [24-^{14}C] (5f)

Methyl lithocholate 5c was esterified with benzoic acid using the Mitsunobu procedure (23) to give methyl 3β-benzoyloxy-5β-cholan-24-oate (5j), mp 102–103°C and 128–128.5°C (88% yield, from methanol). IR: ν_{max} (Nujol) 1740, 1715, 1600 cm^{-1}; UV λ_{max} (acetonitrile) 235 nm; ε = 14500; MS: (20 eV, probe) m/z (relative intensity) 372 (100, M–C_6H_5COOH), 357 (24, M–C_6H_5COOH–Me),

318 (8, M–ring A part), 257 (65, M–C_6H_5COOH–s.c.), 230 (22), 215 (55), 122 (60), 105 (90). Isolithocholic acid (5f) was obtained by alkaline hydrolysis: mp 176–177.5°C from aqueous acetone, reported mp 176–177°C (25). Methyl isolithocholate (5g): mp 111–112°C, reported 113–114.5°C (25). MS: (20 eV, probe) m/z (relative intensity) 390 (29, M^+), 375 (21, M–Me), 372 (59, $M-H_2O$), 357 (33, $M-H_2O$–Me), 318 (10, M–part of ring A), 257 (7, $M-H_2O$–s.c.), 248 (14, ABC ion), 233 (48, ABC ion–H_2O), 215 (76), 108 (100).

Using the same reaction for [24-^{14}C]-methyl lithocholate (5c) as the substrate (59 mCi/mmol), labeled isolithocholic acid (5f) with specific activity 56.3 mCi/mmol was obtained.

RESULTS AND DISCUSSION

Preparation of radioactive BA

All radioactive BA, with the exception of lithocholic and isolithocholic acids (5a, 5f), were prepared by the reduction of the corresponding 3-ketones with NaB^3H_4. This reaction results in the prevalent formation of an equatorial-3α-isomer. The axial-3β-alcohol, formed with 10–20% yield, was efficiently separated and purified either by preparative TLC (multiply developed) or HPLC. Alternatively, the following efficient two-step sequence was utilized for the preparation of 3β-hydroxylated BA with 5β-hydrogen. The 3α-hydroxylated BA methyl ester was benzoylated with concomitant C-3 inversion using the Mitsunobu reaction (23, compare also 30), and the 3β-benzoate was subsequently hydrolyzed to the desired 3β-hydroxy BA. In this sequence the 3-^{3}H label is retained. This approach was utilized for the preparation of C_{20}, C_{21}, C_{23}, and C_{24} "3β,5β"-BA.

Preparation of C_{20}–C_{24} monohydroxylated BA

The two C_{20} acids 1a and 1f and their derivatives are commercially available. Alternatively, the corresponding 3-ketone 1m was prepared by a Wittig reaction between 3-ethylenedioxy-17-oxo-5β-androstane and methoxymethylene triphenylphosphorane ($(C_6H_5)_3P{=}CHOMe$) followed by hydrolysis and oxidation. Since the crucial reaction is based on a known procedure (31), the description of this synthesis is not included in the present report.

The two C_{21} 3-hydroxy-5β-pregnan-21-oic acids 2a and 2f were obtained according to the reaction sequence outlined in Fig. 2. The side chain was attached to 3β-hydroxy-17-oxoandrost-5-ene 6h as the substrate utilizing the Wittig-Horner reaction with triethylphosphonoacetate ($(C_2H_5)_2P(O)CH_2COOC_2H_5$) (19, 20). The C_{21} product 7h was subsequently submitted to the Oppenauer oxidation (21) which yielded the α,β-unsaturated ketone 8o. Catalytical reduction afforded the desired 5β isomer 2o as the major product. The second product of hydrogenation with 5α-configuration was removed by fractional crystallization. The application of alkaline conditions for the hydrogenation of 3-oxo-4-ene system in 8o greatly improved the ratio of 5β/5α as was reported for other steroidal compounds (32, 33).

An analogous procedure was employed for the synthesis of the C_{22} acid 3a. In this case, however, the starting material, 3β-hydroxy-23,24-bisnorchol-5-en-22-oic acid, was commercially available and the transformation was limited to the Oppenauer oxidation and hydrogenation of the resulting intermediate α,β-unsaturated ketone. The alkaline conditions were not used for the hydrogenation in order to avoid side chain racemization (19). Despite the higher proportion of 5α-isomer, the desired 3-ketone 3n was easily purified by recrystallization.

An alternative synthesis of C_{22} acids based on a novel side chain construction will be reported separately.

The construction of side chain of C_{23} BA utilizing the Wittig-Horner reaction of pregnenolone (3β-hydroxy-20-oxo-pregn-5-ene) and triethylphosphonoacetate (20) was originally attempted. The hydrogenation of the intermediate with $\Delta^{20-(22)}$ double bond, however, resulted in the formation of two epimers at C-20. Since their separation could not be easily achieved on a desired scale, the two C_{23} monohydroxylated BA, norlithocholic acid (4a) and isonorlithocholic acid (4f), were obtained from lithocholic acid acetate (5b) according to the reaction sequence outlined in Fig. 3.

A recent modification of the Hunsdiecker reaction (27) was utilized for the side chain degradation of 3-acetoxylithocholic acid to the C_{23} bromide 9b. Subsequently, this bromide was submitted to a Kornblum oxidation (dimethyl-sulfoxide, bicarbonate (34)). Although the aldehyde 11b was the expected product of the oxidation, we found that 23-formate 10b was formed as the principal compound. The expected aldehyde 11b was present among the products of the reaction in trace amounts only. The observation of the formate 10b formed in the Kornblum oxidation of 9b adds novel information to the mechanistic aspect of this relatively obscure reaction.[3]

Despite its rather unexpected formation, the formate 10b was easily utilized with only a slight modification of the original synthetic protocol. Gentle hydrolysis with bicarbonate in alcohol–water solution resulted in the 23-alcohol 12b which was oxidized (for preparative purposes without separation) to the 3-acetate 4b and subsequently fully hydrolyzed to the desired acid 4a.

The second 3-epimeric C_{23} acid 4f was obtained by the Mitsunobu type inversion as discussed before.

[3] Structures of other products formed in this oxidation and mechanistic implications are beyond the scope of this paper and will be dealt with separately; compare ref. 35.

Spectral and chromatographic characterization of 3-monohydroxylated BA

The entire set of products and intermediates obtained in the course of the side chain degradation of lithocholic acid acetate (5b, Fig. 3) was characterized by ^{13}C NMR (36). The multiplicity of carbon signals was assigned employing INEPT spectra (17, 18). This technique is illustrated for the 3-acetate of 3α,23-dihydroxy-24-nor-5β-cholane (12b). The INEPT spectrum obtained with a delay time of 5.5 msec permitted the differentiation of signals of CH and CH_3 carbon atoms (positive singlets) from those of CH_2 (negative singlets). A delay time of 3.5 msec was occasionally utilized for the differentiation of CH and CH_2 signals. In most cases, however, it was sufficient to measure only the "5.5 INEPT Spectrum," since, in the case of studied steroidal acids, the ranges of CH and CH_3 carbon atoms do not overlap. The only exception is the OMe signal; it can, however, be easily assigned by its characteristic chemical shift value. The INEPT technique is evidently superior to the traditional single frequency off resonance decoupling technique (compare spectra C and D, **Fig. 4**), especially in the case of complex molecules.

Mass spectral fragmentation of the whole set of methyl esters 1c–5c and 1g–5g follows the pattern typical of saturated steroidal 3-alcohols (37). This is expressed in the relative intensities of fragment ions at m/z 215 and 230. Ions at m/z 233 and 248 are derived from the ABC part (37). They are more abundant in the case of 3β,5β isomers; in this case the relative intensity of M^+ is also considerably greater. A significant difference in fragmentation patterns was observed for C_{21} esters 2c and 2e. In these cases, the simple cleavage of the side chain,

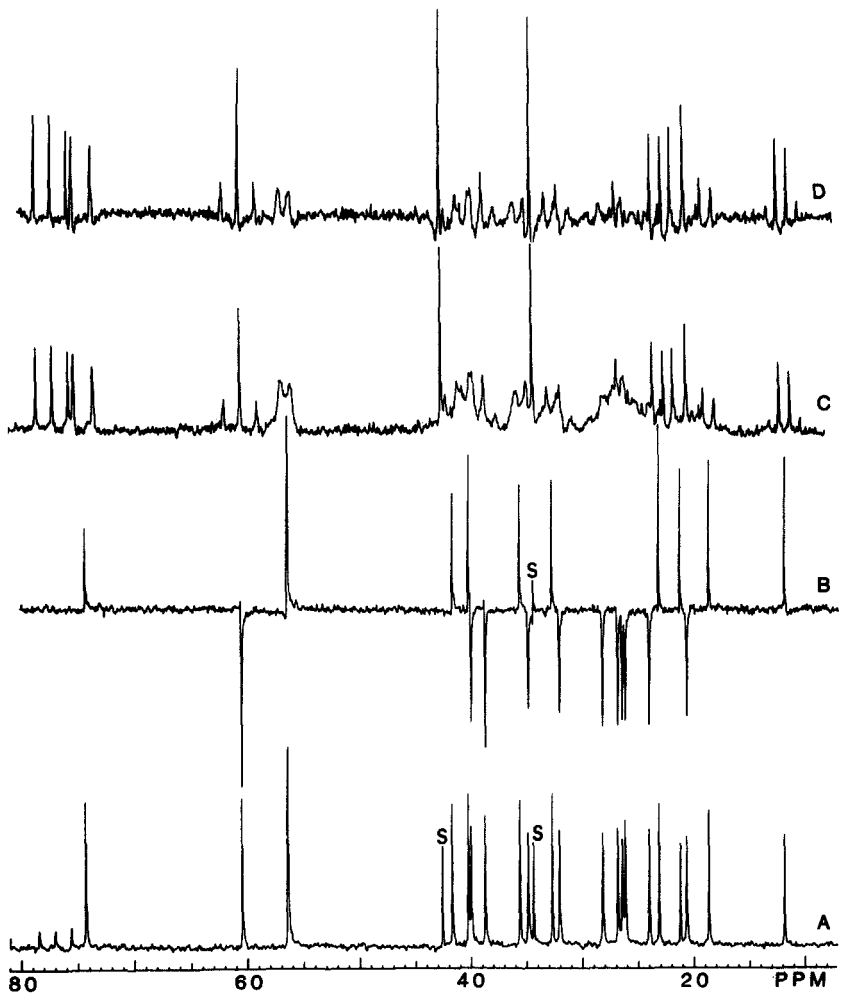


Fig. 4. Ten–80 ppm part of 22.50 MHz ^{13}C FT NMR spectrum of 3α-acetoxy-23-hydroxy-24-nor-5β-cholane (12b, Fig. 3), measured in $CDCl_3$; A: noise decoupled spectrum; B: INEPT spectrum with delay time of 5.5 msec; C: single frequency off-resonance decoupled spectrum; D: resolution enhanced off-resonance spectrum.

observed as a minor but consistent process for other compounds, was replaced by the McLafferty rearrangement (38) leading to the ion M-s.c.-H.

Capillary GLC data of all monohydroxylated BA in the form of methyl ester-acetates are presented in **Fig. 5.** On two nonpolar "bonded" stationary phases DB-1 and DB-5 axial 3β-acetates 1i-5i precede the equatorial counterparts 1d-5d by ~0.2 methylene unit (MU). In a temperature range 230-260°C, satisfactory "base-line" separation can be obtained even on relatively short capillary columns. MU values increase with temperature at a rate of ~0.017 MU/°C with a slight deviation from linear dependence. It is apparent from Fig. 5 that there is no strict uniform increase of MU value when comparing all C_{20}-C_{24} compounds. The very close difference of 1.25 MU is limited only to C_{20} and C_{21} pairs on one side and C_{22}, C_{23}, and C_{24} pairs on the other, since only these acid series can be considered as truly homologous (differing by CH_2). The corresponding methyl esters with the free hydroxyl group are not differentiated under these conditions (8, 9).

The five pairs of free acids, C_{20}-C_{24}, with both epimeric configurations of C-3 are separable by reverse phase HPLC (**Table 2**). The table includes also the relative R_v of several C_{23} acids with the unnatural side chain configuration (20S) as well as of principal C_{24} di- and trihydroxylated BA analyzed under the same conditions. The composition of the solvent system was optimized for monohydroxylated BA with various side chains rather than for more hydroxylated compounds.

The availability of a set of short-chain bile acids of defined configuration at C-3, in both labeled and unlabeled form, opens the possibility of metabolic studies.

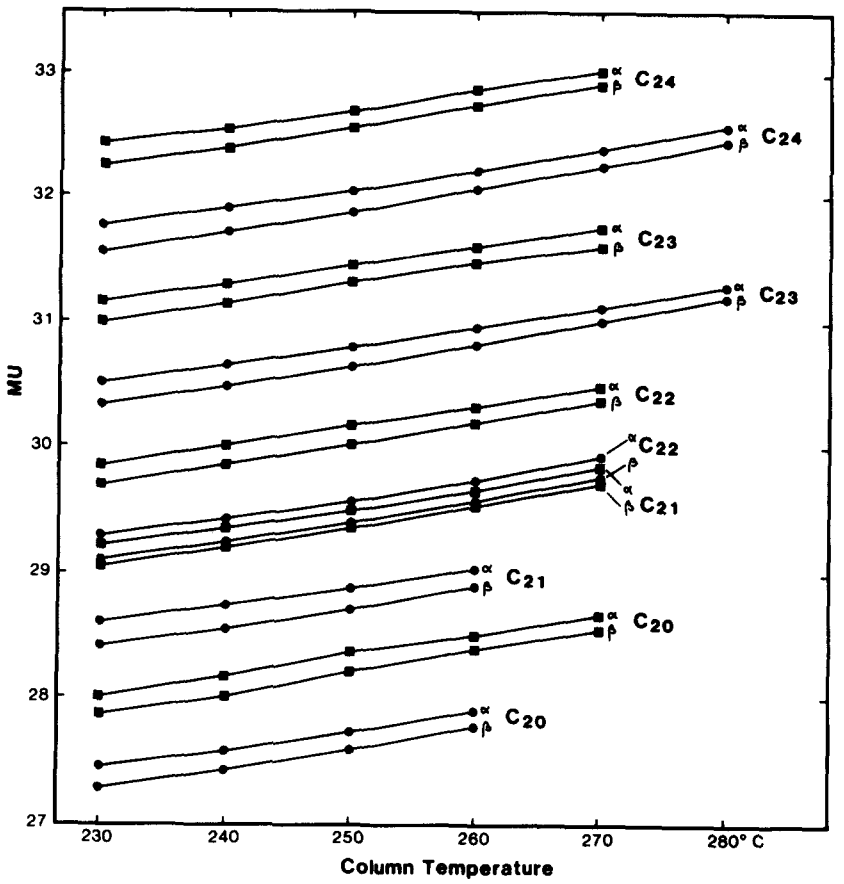


Fig. 5. Capillary GLC separation of 3-monohydroxylated BA in the form of methyl ester-acetates: dependence of methylene unit equivalent (MU) from column temperature. (●) DB-1: 15 m, 0.25 mm ID, 0.1 μ; (■) DB-5: 15 m, 0.25 mm ID, 0.1 μ. Carrier gas N_2, 70 kPa.

TABLE 2. Reverse phase HPLC separation of free bile acids

Number of Carbon Atoms	Hydroxyl/ Ketone	Name of Free Bile Acid	R_v / R_v (Lithocholic Acid)
20	3α	3α-hydroxy-5β-androstane-17β-carboxylic (1a)	0.25
20	3β	3β-hydroxy-5β-androstane-17β-carboxylic (1f)	0.20
20	3=O	3-oxo-5β-androstane-17β-carboxylic (1m)	0.33
20	3=O	3-oxo-5α-androstane-17β-carboxylic	0.23
21	3α	3α-hydroxy-5β-pregnan-21-oic (2a)	0.39
21	3β	3β-hydroxy-5β-pregnan-21-oic (2f)	0.25
22	3α	[20S]-3α-hydroxy-23,24-bisnor-5β-cholan-22-oic (3a)	0.50
22	3β	[20S]-3β-hydroxy-23,24-bisnor-5β-cholan-22-oic (3f)	0.43
23	3α	[20R]-3α-hydroxy-23,24-nor-5β-cholan-23-oic (4a)	0.62
23	3β	[20R]-3β-hydroxy-23,24-nor-5β-cholan-23-oic (4f)	0.59
23	3=O	[20R]-3-oxo-24-nor-5β-cholan-23-oic[a] (4m)	0.89
23	3=O	[20S]-3-oxo-24-nor-5β-cholan-23-oic[a]	0.73
23	3=O	[20R]-3-oxo-24-nor-5α-cholan-23-oic[a]	0.71
23	3=O	[20S]-3-oxo-24-nor-5α-cholan-23-oic[a]	0.78
24	3α	3α-hydroxy-5β-cholan-24-oic (lithocholic) (5a)	1.00
24	3β	3β-hydroxy-5β-cholan-24-oic (isolithocholic) (5f)	0.85
24	3=O	3-oxo-5β-cholan-24-oic acid (5m)	1.13
24	3β	3β-hydroxy-5α-cholan-24-oic	0.77
24	3β	3β-hydroxychol-5-en-24-oic	0.67
24	3α,6α	3α,6α-dihydroxy-5β-cholan-24-oic (hyodeoxycholic)	0.14
24	3α,6β	3α,6β-dihydroxy-5β-cholan-24-oic	0.14
24	3α,7α	3α,7α-dihydroxy-5β-cholan-24-oic (chenodeoxycholic)	0.14
24	3α,7β	3α,7β-dihydroxy-5β-cholan-24-oic (ursodeoxycholic)	0.29
24	3α,12α	3α,12α-dihydroxy-5β-cholan-24-oic (deoxycholic)	0.32
24	3α,7α,12α	3α,7α,12α-trihydroxy-5β-cholan-24-oic (cholic)	0.10
24	3α,6α,7α	3α,6α,7α-trihydroxy-5β-cholan-24-oic (hyocholic)	0.08
24	3α,7=O	3α-hydroxy-7-oxo-5β-cholan-24-oic	0.20
24	3α,7α,12=O	3α,7α-dihydroxy-12-oxo-5β-cholan-24-oic	0.11
24	3,7,12(=O)$_3$	3,7,12-trioxo-5β-cholan-24-oic (dehydrocholic)	0.11

Column: C-18 μ Bondapack; solvent: 0.25 M KH_2PO_4–acetonitrile 6:4 (v/v); detection: refractive index and UV at 190 nm.

[a]These four acids were analyzed as a mixture prepared by a Wittig-Horner condensation of pregnenolone and triethylphosphonoacetate (20), oxidation, and catalytical reduction (as described for the synthesis of 2o), followed by alkaline hydrolysis.

An example, the hepatic glucuronidation of these compounds, is described in an accompanying paper (14).

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