papers and notes on methodology

Synthesis of 24-nor-5β-cholan-23-oic acid derivatives: a convenient and efficient one-carbon degradation of the side chain of natural bile acids

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Abstract An efficient procedure for obtaining nor-bile acids from natural (C_{24}) bile acids is described. Treatment of formylated bile acids with sodium nitrite in a mixture of trifluoroacetic anhydride with trifluoroacetic acid gives, through a "second order" Beckmann rearrangement, 24-nor-23-nitriles. These compounds, on alkaline hydrolysis, afford the corresponding nor-bile acids in high yields. The sequence was successfully applied to the synthesis of 3α-hydroxy-24-nor-5β-cholan-23-oic (norlithocholic) acid, 3α,6α-(norhyodeoxycholic), 3α,7α- (norchenodeoxycholic), 3α,7β- (norursodeoxycholic), and 3α,12α-dihydroxy-24-nor-5β-cholan-23-oic (nordeoxycholic) acids, as well as 3α,7α,12α-trihydroxy-24-nor-5β-cholan-23-oic (norcholic) acid. ^{13}C-NMR spectra of their methyl esters are reported. The procedure provides a more rapid alternative to the Barbier-Wieland degradation for shortening by one methylene group the side chain of natural (C_{24}) bile acids.—**Schteingart, C. D., and A. F. Hofmann.** Synthesis of 24-nor-5β-cholan-23-oic acid derivatives: a convenient and efficient one-carbon degradation of the side chain of natural bile acids. *J. Lipid Res.* 1988. **29:** 1387–1395.

Supplementary key words nor-bile acids

Nor-bile acids[2] differ from C_{24} bile acids in their metabolism and physiological properties, and their study has provided new insights into hepatic biotransformation of bile acids and mechanisms of bile secretion (1–6). Nor-bile acids have also been found in bile (7) and urine (8) of healthy subjects, in bile and urine of patients suffering from cerebrotendinous xanthomatosis (7, 9), and in urine of patients with cholestasis (8).

Because of their occurrence in only trace proportions in biological fluids, nor-bile acids must be synthesized if they are needed in quantity. The practice in recent studies has been to prepare them by the classic Barbier-Wieland degradation or one of its modifications (7, 9, 10). This is a lengthy procedure that requires anhydrous conditions and gives moderate to low yields of products that require extensive purification. Only a few alternative methods have been applied to bile acids: periodate oxidation of a 23-hydroxy-bile acid (obtained through the 23 bromide) to give the 23-aldehyde (11), and more recently, Kornblum oxidation of a 23-bromonorcholate (obtained by a modified Hunsdiecker reaction) and oxidation of the resultant nor-aldehyde to the nor-bile acid (12).

The degradation of fatty acids by α-oxidation of their enolates with O_2 and oxidation with periodate/chromium trioxide has been reported (13). Although the α-hydroxylation of the methyl esters of tetrahydropyranyl (THP)-protected bile acids with LDA/oxidoperoxymolybdenum complex is known to proceed with good yields (14), the periodate/chromium trioxide degradation does not seem to have been tried.

The degradation of carboxylic acids to the next lower homologue nitrile by an α-nitrosation-fragmentation sequence was reported some years ago by Smushkevich, Usorov, and Suvorov (15). We report here a modification of this method which has resulted in a new and efficient methodology for the one-carbon degradation of bile acids. The procedure gives products of high purity in very good yields and can be scaled up easily.

Abbreviations: GLC, gas-liquid chromatography; TLC, thin-layer chromatography; MS, mass spectra; HPLC, high performance liquid chromatography.

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[2]In the bile acid field, the prefix *nor* has generally been used to denote 24-norcholan-23-oic acids (C_{23} bile acids), and we have followed that practice in the remainder of the paper for convenience.

EXPERIMENTAL

Materials

Lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid, **1a**), deoxycholic acid (3α,12α-dihyroxy-5β-cholan-24-oic acid, **4a**), and cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid, **5a**) were purchased from Aldrich Chemical Co., Milwaukee, WI and recrystallized until the purity was more than 99% as judged by gas–liquid chromatography (GLC). Chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid, **2a**) and ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid, **3a**) were a generous gift from Diamalt AG, Raubling, West Germany. Hyodeoxycholic acid (3α,6α-dihydroxy-5β-cholan-24-oic acid, **6a**) was a generous gift from Canada Packers Ltd., Toronto, Ontario, Canada. (See Fig. 1 for structures and identification of compounds.) Trifluroacetic acid, trifluoroacetic anhydride, and sodium nitrite were purchased from Aldrich Chemical Co. and were used without further purification.

Methods

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Infrared spectra (IR) were obtained in KBr pellets with a Perkin-Elmer 1330 infrared spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian EM-390 spectrometer at 90 MHz and on a Nicolet QE-300 at 300 MHz (only where indicated). ^{13}C-NMR spectra were taken on a Nicolet NT-200 wide-bore spectrometer with an Oxford magnet at 50.31 MHz; multiplicities were determined with the sequence GASPE (16) with τ = 4.5 and 7 msec. The central peak of the signal of $CDCl_3$ was used as reference δ 77.0 ppm. Electron impact high resolution mass spectra (MS) were determined on a Kratos MS-50S mass spectrometer (direct insertion probe, source temperature: 200–250°C, resolution: 10,000) at the Mass Spectrometry Facility of UCSF (Director, A. L. Burlingame). Gas–liquid chromatography was performed on a Hewlett-Packard 5840 gas chromatograph equipped with a flame ionization detector (carrier gas: N_2, 35 ml/min). The methyl esters of the nor-bile acids were analyzed on a 3% SE-30 on 100/120 Chrom Q column (1.8 m) at 240 and 250°C, and the methyl esters of formyl derivatives of bile acids on a 3% SP-2250M on 100/120 Supelcoport column (1.8 m) (Supelco, State College, PA) at 270°C and on the SE-30 column at 250°C (all analyses were isothermal). High performance liquid chromatography (HPLC) was performed on a C_{18} octadecylsilane column eluted with 75:25 methanol–phosphate buffer (pH 5.35) as mobile phase, with detection by ultraviolet spectrophotometry at 205 nm (17). Visualization of bile acids after separation by thin-layer chromatography (TLC) was obtained by first spraying the chromatogram with 30% sulfuric acid in acetic acid, followed by 0.6% anisaldehyde in ethanol, and heating at 130°C for 1–4 min. The bile acids and derivatives appeared as red-purple spots, while the nitriles and derivatives appeared blue.

Syntheses

Formylated bile acids. A solution or suspension of 50 g of bile acid in 200 ml of 90% formic acid containing 0.5 ml of 70% perchloric acid was stirred at 47–50°C for 1.5 hr. The temperature of the heating bath was lowered to 40°C and 160 ml of acetic anhydride was added over 10 min and the mixture was stirred for 10 more minutes. The solution was cooled to room temperature and poured into 2 liter of water with vigorous stirring. The precipitate was filtered, washed with 800 ml of water, dissolved in ethyl ether, and the organic layer was washed with water to neutrality, dried over sodium sulfate, and evaporated (yields: 95–99%). The crude products were used in the following reaction, except for the chenodeoxycholic acid derivative **2b**, which was recrystallized once from hexanes–ethyl acetate (yield: 83%, mp 186–187°C, Lit. 186–187°C (18)).

The following general method is described using ursodeoxycholic acid as an example.

Diformylnornitriles. The reaction was carried out in a two-neck round-bottom flask provided with magnetic stirrer, ice bath, and an air condenser closed by a moisture trap or a loose plastic stopper. Fifty g of 3α,7β-diformyloxy-5β-cholan-24-oic acid, **3b** (0.111 mol), 175 ml of cold trifluoroacetic acid, and 47 ml (0.333 mol) of trifluoroacetic anhydride were stirred at 0–5°C until dissolution. Sodium nitrite (8.4 g, 0.122 mol) was added in small portions, waiting for most of the salt to react between additions (0.5–1.5 hr). After the addition was complete, the reaction mixture, which contained some excess sodium nitrite, was stirred at 0–5°C for 1 hr. The stopper or trap was removed and the mixture was stirred at 38–40°C for 1 hr (CO_2 and N_2O_3 evolution). The yellowish-brownish solution was cooled to room temperature and poured into a mixture of 1700 ml of 2 N NaOH and 1500 g of ice. Enough ethyl ether was added to dissolve the precipitate and the mixture was transferred to a separatory funnel. The organic layer was extracted with 1 N NaOH until no more starting material or its hydrolysis products were observed by TLC, washed with water to neutrality, dried with sodium sulfate, and evaporated. For yields see following procedure.

Nornitriles. One g (2.4 mmol) of crude 3α,7β-diformyloxy-24-nor-5β-cholane-23-nitrile, **3c**, was refluxed in 20 ml of methanol containing 390 mg (7.2 mmol) of sodium methoxide for 30 min. The solution was poured into 80 ml of water and the product was extracted with ethyl acetate. The organic layer was washed with 20% NaCl solution to neutrality, dried, and evaporated. In all cases only one product was obtained (by TLC), which, when purified through a short silica gel column, afforded the pure nornitrile almost quantitatively. The reported yields are from

the formyl bile acids and represent essentially those of the degradation reaction:

3α-Hydroxy-24-nor-5β-cholane-23-nitrile, **1d:** mp 166–167°C (hexanes–benzene); IR 2241 cm^{-1}; ^{1}H-NMR (Cl_3CD) δ: 0.66 (s, 3H, Me-18), 0.92 (s, 3H, Me-19), 1.14 (d, J = 7 Hz, 3H, Me-21), 2.26 (m, 2H, H-22), 3.61 (m, 1H, H-3); MS m/z: 343.2873 (calc. 343.2875, M^+, 43), 325 (M-18(H_2O), 97), 310 (M-18-15(CH_3), 49), 271 (M-18-54 (C_4H_6, retro Diels Alder, ring A), 25), 248 (M-68 (side chain) -27(C_2H_3, part of ring D), 10), 233 (M-68-42 (C_3H_6, part of ring D), 6), 230 (M - 18 - 68 - 27, 35), 215 (M - 18 - 68 - 42, 100); (yield: 96%).

3α,7α-Dihydroxy-24-nor-5β-cholane-23-nitrile, **2d:** mp 179–180°C (hexanes–benzene); IR 2230 cm^{-1}; ^{1}H-NMR (Cl_3CD) δ: 0.66 (s, 3H, Me-18); 0.86 (s, 3H, Me-19), 1.14 (d, J = 6.5 Hz, 3H, Me-21), 3.43 (bm, 1H, H-3), 3.82 (bs, 1H, H-7); MS m/z: 359.2836 (calc. 359.2825, M^+, 10), 344 (M-15, 5), 341 (M-18, 6), 323 (M-2 × 18, 100), 308 (M-2 × 18-15, 74), 269 (M - 2 × 18 - 54, 31), 264 M - 68 - 27, 10), 255 (M - 2 × 18 -68, 14), 246 (M - 18 - 68 - 27, 13), 213 (M - 2 × 18 - 68 - 42, 54); (yield: 83%).

3α,7β-Dihydroxy-24-nor-5β-cholane-23-nitrile, **3d:** mp 232–233.5°C (EtOAc–MeOH); IR 2235 cm^{-1} ^{1}H-NMR (Cl_3CD) δ: 0.69 (s, 3H, Me-18); 0.93 (s, 3H, Me-19), 1.16 (d, J = 6.6 Hz, 3H, Me-21), 2.28 (m, 2H, H-22), 3.56 (m, 2H, H-3 and 7); MS m/z: 359.2826 (calc. 359.2825, M^+, 24), 344 (M - 15, 15), 341 (M - 18, 32), 323 (M - 2 × 18, 52), 308 (M - 2 × 18 - 15, 100), 269 (M - 2 × 18 - 54, 29), 264 (M - 68 - 27, 22), 257 (17), 256, (10), 255 (M - 2 × 18 - 68, 16), 254 (11), 246 (M - 18 - 68 - 27, 56), 231 (M - 18 - 68 - 42, 36), 228 (M - 2 × 18 - 68 - 27, 55), 213 (M - 2 × 18 - 68 - 42, 41); (yield: 95%).

3α,12α-Dihydroxy-24-nor-5β-cholane-23-nitrile, **4d:** mp 156–157°C (hexanes–acetone); IR 2239 cm^{-1}; ^{1}H-NMR (Cl_3CD) δ: 0.69 (s, 3H, Me-18), 0.91 (s, 3H, Me-19), 1.20 (d, J = 4.5 Hz, 3H, Me-21), 2.28 (m, 2H, H-22), 3.58 (m, 1H, H-3), 3.94 (bs, 1H, H-12); MS m/z: 359.2833 (calc. 359.2825, M^+, 5), 341 (M - 18, 43), 323 (M - 2 × 18, 100), 312 (M - $C_1H_3O_2$, 53), 308 (M - 2 × 18 - 15, 68), 273 (M - 18 - 68, 11), 269 (M - 2 × 18 - 54, 34), 255 (M - 2 × 18 - 68, 78), 246 (M - 18 - 68 - 27, 5), 231 (M - 18 - 68 - 42, 34), 228 (M - 2 × 18 - 68 - 27, 30), 213 (M - 2 × 18 - 68 - 42, 24); (yield: 92%).

3α,7α,12α-Trihydroxy-24-nor-5β-cholane-23-nitrile, **5d:** mp 168–170°C (acetone); IR 2239 cm^{-1}; ^{1}H-NMR (Cl_3CD) δ: 0.68 (s, 3H, Me-18), 0.87 (s, 3H, Me-19), 1.21 (d, J = 5.0 Hz, 3H, Me-21), 3.42 (m, 1H, H-3), 3.81 (bs, 1H, H-7), 3.91 (bs, 1H, H-12); MS m/z: 375.2767 (calc. 375.2774, M^+, 2), 357 (M - 18, 5), 339 (M - 2 × 18, 80), 324 (M - 2 × 18 - 15, 67), 321 (M - 3 × 18, 53), 310 (M-CH_5O_3, 100), 306 (M - 3 × 18 - 15, 59), 280 (M - 68 - 27, 17), 271 (M - 2 × 18 - 68, 53), 267 (M - 3 × 18 - 54, 39), 253 (M - 3 × 18 - 68, 89), 244 (M - 2 × 18 - 68 - 27, 15), 229 (M - 2 × 18 - 68 - 42, 22), 226 (M - 3 × 18 - 68 - 27, 58), 211 (M - 3 × 18 - 68 - 42, 23); (yield: 86%).

Nor-bile acids. The reactions were carried out in a polypropylene bottle with a suitable condenser attached (glass vessels produced soluble silicates that precipitated during the isolation steps). Twenty g of crude 3α,7β-diformyloxy-24-nor-5β-cholane-23-nitrile, **3c,** was refluxed with 10% KOH in ca. 700 ml of ethanol–water 1:1 for 96 hr. The ethanol was evaporated; the solution was saturated with sodium chloride and extracted with ethyl ether until TLC examination showed that no more basic compounds were present (3 to 5 times). The aqueous layer was acidified slowly with 6 N HCl and the nor-bile acid was extracted with ethyl acetate (× 3). The combined organic layers were washed with 20% NaCl to neutrality, dried, and evaporated. The potassium salt of norlithocholic acid, **1e,** is not soluble in water, but the hydrolysis was nonetheless complete in this case. After ethanol evaporation, the solution was acidified and the nor-bile acid was extracted with ether and worked up as above. Occasionally, this treatment gave products (especially in the case of **3e**) slightly contaminated with basic compounds or the amide. In this case the following procedure was applied. The nor-bile acid was dissolved in tetrahydrofuran, and passed through a Dowex 50W-X4 (H^+ form, 0.2–0.4 eq) ion exchange column equilibrated with THF. All nor-bile acids could be decolorized successfully by treatment with activated charcoal and purified by recrystallization.

3α-Hydroxy-24-nor-5β-cholan-23-oic acid, **1e:** mp 183.5–184.5°C (acetone), Lit. 181–182°C (19); ^{1}H-NMR (Cl_3CD + 10% d_6-DMSO) δ: 0.71 (s, 3H, Me-18), 0.93 (s, 3H, Me-19), 0.99 (d, J = 6.0 Hz, Me-21), 3.53 (m, 1H, H-3); (crude yield: 96%; each recrystallization from acetone: 85%).

3α,7α-Dihydroxy-24-nor-5β-cholan-23-oic acid, **2e:** mp 204–205°C (acetone), crystallizes with one molecule of acetone which is lost at 135–160°C, Lit. 197–198°C (MeOH) (19); ^{1}H-NMR (Cl_3CD + 10% d_6-DMSO) δ: 0.69 (s, 3H, Me-18), 0.88 (s, 3H, Me-19), 0.98 (d, J = 6.0 Hz, 3H, Me-21), 3.32 (m, 1H, H-3), 3.73 (bs, 1H, H-7); (crude yield: 90%; each recrystallization from acetone: 90%).

3α,7β-Dihydroxy-24-nor-5β-cholan-23-oic acid, **3e:** mp 248–250°C (acetone–MeOH), Lit. 235–237°C (9), a sample prepared following (10) in our laboratory gave mp 248–250°C; ^{1}H-NMR (Cl_3CD + 10% d_6-DMSO) δ: 0.70 (s, 3H, Me-18), 0.93 (s, 3H, Me-19), 0.98 (d, J = 5.5 Hz, 3H, Me-21), 3.41 (m, 2H, H-3 and 7); (crude yield: 87%; each recrystallization from acetone–MeOH: 91%).

3α,12α-Dihydroxy-24-nor-5β-cholan-23-oic acid, **4e:** mp 212–213.5°C (acetone), Lit. 213.5–214.5°C (acetone) (10); ^{1}H-NMR (Cl_3CD + 10% d_6-DMSO) δ: 0.69 (s, 3H, Me-18), 0.89 (s, 3H, Me-19), 1.03 (d, J = 3.0 Hz, 3H, Me-21), 3.50 (m, 1H, H-3), 3.90 (bs, 1H, H-12); (crude yield: 98%; each recrystallization from acetone: 83%).

3α,7α,12α-Trihydroxy-24-nor-5β-cholan-23-oic acid, **5e:** mp 188–190°C (softens at 165°C), (acetone, crystallizes with one molecule of solvent), Lit. 188–192°C (acetone) (20); ^{1}H-NMR (Cl_3CD + 10% d_6-DMSO) δ: 0.70 (s, 3H, Me-

18), 0.87 (s, 3H, Me-19), 1.06 (bs, $w_{1/2}$ 6 Hz, 3H, Me-21), 3.32 (m, 1H, H-3), 3.75 (bs, 1H, H-7), 3.87 (bs, 1H, H-12); (yield with one recrystallization from acetone-methanol: 82%; each additional recrystallization from the same solvent, 85%).

3α,6α-Dihydroxy-24-nor-5β-cholan-23-oic acid, **6e:** The combined procedures described above were applied (without characterization of the nornitrile) to **6a** to give **6e**, mp 218-219.5°C (ethyl acetate), Lit. 217.5-219°C (ethyl acetate) (19); ^{1}H-NMR (Cl_3CD + 10% d_6-DMSO) 300 MHz δ: 0.67 (s, 3H, Me-18), 0.89 (s, 3H, Me-19), 1.00 (d, J = 5.8 Hz, 3H, Me-21), 3.51 (m, 1H, H-3), 3.97 (m, $w_{1/2}$ 24 Hz, 1H, H-6); (crude yield: 83%; each recrystallization from ethyl acetate: 80%). ^{13}C-NMR of methyl ester, **6f:** see Table 1.

Methyl esters of nor-bile acids. These were prepared with diazomethane and purified by column chromatography and/or recrystallization. ^{13}C-NMR spectra are given in **Table 1**.

Methyl-3α-hydroxy-24-nor-5β-cholan-23-oate, **1f:** mp 107-108°C (MeOH), Lit. 100°C (19); ^{1}H-NMR (Cl_3CD) δ: 0.68 (s, 3H, Me-18), 0.92 (s, 3H, Me-19), 0.97 (d, J = 6.0 Hz, 3H, Me-21), 3.60 (m, 1H, H-3), 3.66 (s, 3H, $-COOCH_3$).

Methyl-3α,7α-dihydroxy-24-nor-5β-cholan-23-oate, **2f:** mp 83.5-85°C (hexanes-acetone), Lit. 86-87°C (hexanes-ethyl ether) (19); ^{1}H-NMR (Cl_3CD) 300 MHz δ: 0.72 (s, 3H, Me-18), 0.92 (s, 3H, Me-19), 0.98 (d, J = 5.7 Hz, 3H, Me-21), 2.22 (dt, J = 11.7, 13.0 Hz, 1H, H-4α), 2.45 (d, J = 13 Hz, 1H, H-22), 3.46 (m, 1H, H-3), 3.68 (s, 3H, $-COOCH_3$), 3.86 (bs, 1H, H-7).

Methyl-3α,7β-dihydroxy-24-nor-5β-cholan-23-oate, **3f:** mp 178-180°C (MeOH-water); ^{1}H-NMR (Cl_3CD) 300 MHz δ: 0.72 (s, 3H, Me-18), 0.96 (s, 3H, Me-19), 0.99 (d, J = 6.5 Hz, 3H, Me-21), 2.45 (dd, J = 14.1 and 2.9 Hz, 1H, H-22), 3.59 (m, 2H, H-3 and 7), 3.67 (s, 3H, $-COOCH_3$); MS m/z: 392.2925 (calc. 392.2926, M^+, 3), 374 (M - 18, 25), 359 (M - 18 - 15, 16), 356 (M - 2 × 18, 55), 341 (M - 2 × 18 - 15, 50), 302 (M - 2 × 18 - 54, 20), 283 (M - 2 × 18 - CH_2COOCH_3, 56), 273 (M - 101 (side chain) - 18, 19), 264 (M - 101 - 27, 27), 255 (M - 2 × 18 - 101, 53), 246 (M - 18 - 101 - 27, 56), 231 (M - 18 - 101 - 42, 38), 228 (M - 2 × 18 - 101 - 27, 52), 213 (M - 2 × 18 - 101 - 42, 100).

Methyl-3α,12α-dihydroxy-24-nor-5β-cholan-23-oate, **4f:** mp 166.5-167.5°C (hexanes-benzene), Lit. 164-165.5°C (benzene) (19); ^{1}H-NMR (Cl_3CD) δ: 0.72 (s, 3H, Me-18), 0.90 (s, 3H, Me-19), 1.02 (d, J = 3.0 Hz, 3H, Me-21), 3.50 (m, 1H, H-3), 3.66 (s, 3H, $-COOCH_3$), 3.96 (bs, 1H, H-12).

Methyl-3α,7α,12α-trihydroxy-24-nor-5β-cholan-23-oate, **5f:** mp 157.5-158°C (hexanes-ethyl acetate), Lit. 155°C (MeOH)

TABLE 1. ^{13}C-NMR chemical shifts for the methyl esters of nor-bile acids[a]

Carbon	1f 3α	2f 3α,7α	3f 3α,7β	4f 3α,12α	5f 3α,7α,12α	6f 3α,6α
1	35.2	35.2	34.7	35.1	35.2	35.6[b]
2	30.4	30.5	30.1	30.3	30.0	29.9[c]
3	71.6	71.8	71.0	71.5	71.6	71.3
4	36.3	39.4[b]	37.1[b]	36.3	39.3	29.2[c]
5	42.0	41.3	42.3	41.9	41.4[b]	48.4
6	27.1	34.6	36.9[b]	27.0	34.6	67.8
7	26.3	68.3	71.0	26.0	68.2	34.6[b]
8	35.7	39.3	43.5	35.9	39.3	34.7
9	40.3	32.7	39.0	33.5	26.1	39.8
10	34.5	34.9	33.9	34.0	34.5	35.8
11	20.7	20.4	21.0	28.5[c]	27.9[c]	20.7
12	40.0	39.7[b]	39.9	72.8	72.8	39.8
13	42.7	42.6	43.6	46.4	46.3	42.8
14	56.4[b]	50.3	55.6[c]	48.1[b]	41.3[b]	56.2[d]
15	24.1	23.5	26.7	23.5	23.1	24.1
16	28.2	28.1	28.6	27.5[c]	27.5[c]	28.2
17	56.0[b]	55.8	54.9[c]	47.2[b]	46.9	56.0[d]
18	12.0	11.7	12.0	12.6	12.3	12.0
19	23.3	22.7	23.2	23.0	22.3	23.5
20	33.7	33.7	33.5	33.5	33.6	33.7
21	19.4	19.4	19.5	18.4	18.4	19.4
22	41.4	41.3	41.3	41.2	41.2	41.3
23	174.0	174.0	173.8	173.9	174.0	173.9
Me	51.3	51.2	51.2	51.3	51.3	51.3

[a]In Cl_3CD, 50.31 MHz, ppm downfield from TMS.
[b,c,d]Assignments can be interchanged in each column.

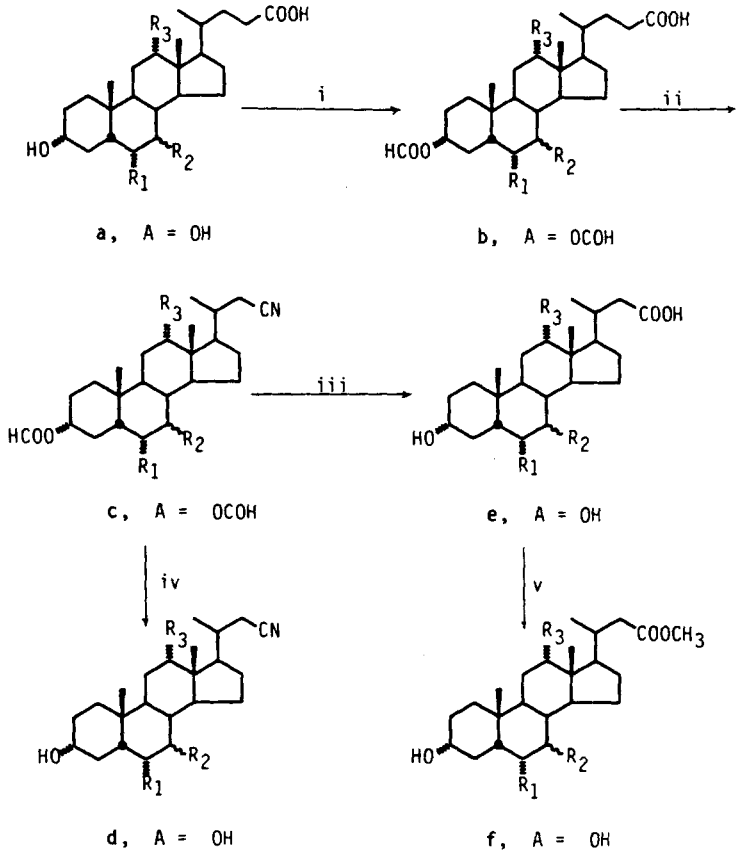
(21), 160–161°C (MeOH) (20); ^{1}H-NMR (Cl_3CD) δ: 0.70 (s, 3H, Me-18), 0.87 (s, 3H, Me-19), 1.06 (bs, $w_{1/2}$ 6 Hz, 3H, Me-21), 3.40 (m, 1H, H-3), 3.66 (s, 3H, -$COOCH_3$), 3.81 (bs, 1H, H-7), 3.94 (bs, 1H, H-12).

RESULTS

In 1975, Smushkevich, Usorov, and Suvorov (15) reported the conversion of a carboxylic acid into its nor-nitrile. Though the model compounds described were very simple and the yields only moderate, we decided to try to apply the procedure to the synthesis of nor-bile acids, because its eventual success would result in a method far simpler than the Barbier-Wieland degradation. In the method reported here, a suitable protected bile acid (**Fig. 1**) is transformed in one step into the nitrile of the lower homologue, which in turn is deprotected and hydrolyzed to the nor-bile acid in another simple operation.

Hydroxyl group protection

The formation of an intermediate mixed anhydride with trifluoroacetic acid (**Fig. 2**) makes it necessary to protect the hydroxyl groups of the bile acid to prevent polyesterification (22). The formyl ester proved to be a most suitable protective group since it can be introduced by a well-known method in quantitative yield without the formation of mixed anhydrides; it is stable under the acidic conditions



	R_1	R_2	R_3	Trivial Name
1	H	H	H	Lithocholic acid
2	H	αA	H	Chenodeoxycholic acid
3	H	βA	H	Ursodeoxycholic acid
4	H	H	A	Deoxycholic acid
5	H	αA	A	Cholic acid
6	A	H	H	Hyodeoxycholic acid

Fig. 1. Synthesis of nor-bile acids and derivatives. i: HCOOH, $(Ac)_2O$, H^+; ii: $NaNO_2$, F_3CCOOH, $(F_3CCO)_2O$; iii: KOH, EtOH-H_2O; iv: NaMeO, MeOH; v: CH_2N_2, MeOH.

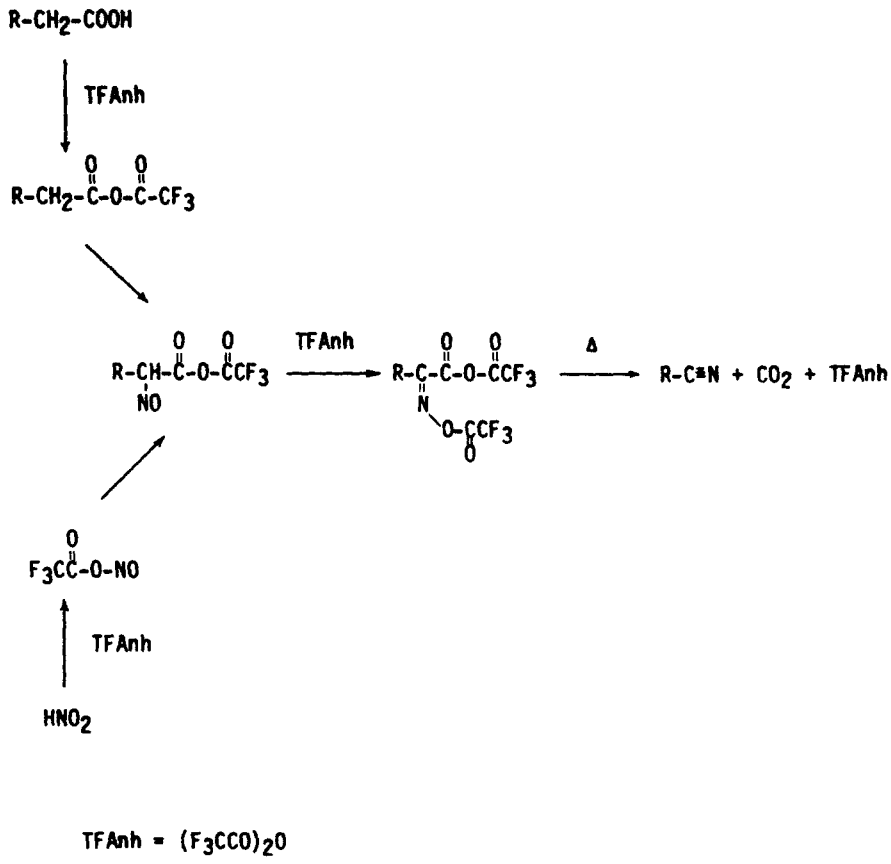


Fig. 2. Proposed mechanism of the degradation.

of the reaction, and it can be eliminated simultaneously during the hydrolysis of the nitrile.

The formylation method of Tserng and Klein (18) was found satisfactory for most cases and only the isolation step was changed to produce dry products (see experimental). However, when the degradation scheme was applied to chenodeoxycholic acid, the nor-bile acid obtained was found to be contaminated with an impurity of higher R_f on TLC. The problem could be traced back to the formylation step. Though the diformylchenodeoxycholic acid obtained had the correct melting point, GLC and TLC showed ca. 15% impurities—probably products of elimination of the 7α-hydroxyl group. Lowering the reaction temperature from 55°C to 48°C cut down substantially the amount of impurities as analyzed by GLC. Recrystallization from ethanol failed to further purify the compound, but one recrystallization from a mixture of ethyl acetate–hexanes afforded a pure product with 83% yield and the same melting point as reported. Subsequently, we applied the lower temperature reaction conditions to the other bile acids and found it successful; no recrystallizations were needed. It was also found that the formylation of chenodeoxycholic acid can be carried out at room temperature if the reaction time after the addition of acetic anhydride is extended to several hours; but no gain in purity was observed.

Side chain degradation

The degradation reaction (Fig. 2) (a "second order Beckmann rearrangement") can be thought to involve activation of the α position of the bile acid by formation of the mixed anhydride with trifluoroacetic acid followed by nitrosation with trifluoroacetyl nitrite (23) (a NO^+ carrier (24)) to form an α nitroso anhydride (15). This tautomerizes to the oxime which is acylated, thus providing a better nucleofugal group for the subsequent fragmentation; trifluoroacetic anhydride has already been shown to promote fragmentation of α-hydroxyketoximes (25). The actual fragmentation can be described as elimination of trifluroacetate from the oxime moiety, shift of an electron pair to form the carbon–nitrogen triple bond (26), liberation of carbon dioxide, and formation of trifluoroacetic anhydride by attack of trifluoroacetate or an equivalent mechanism (27). It was observed by TLC analysis that in the course of the reaction during the cold period, there was no alteration of the starting material; this was transformed into the mixed anhydride (which would revert to the starting material on

TLC), whereas the sodium nitrite was slowly converted into trifluoroacetyl nitrite.

On heating, carbon dioxide evolution was observed, but no intermediate (oxime, derivative, or hydrolysis product) could be detected. We speculate that the nitrosation happens only at moderately high temperature, as can be expected for a not very reactive substrate (28), and is followed by a much faster rearrangement step, thus preventing trapping. Also, we have found that there is no need for stirring the reaction at room temperature after the addition of sodium nitrite is complete, as stated in (15).

The rearrangement could not be carried out at the boiling point of the mixture of trifluoroacetic acid and its anhydride as in (15) because in the case of cholic acid a second less polar product was observed by TLC. The evidence suggested that some of the formyl groups were cleaved under these conditions causing elimination of the hydroxyl groups. Lowering the temperature to the minimum compatible with appreciable reaction rate, 38–40°C, eliminated the problem. The small amount of the remaining original bile acid could be easily separated from the neutral nor-nitrile by basic washings of an ether solution of the reaction mixture. This, however, caused extensive deprotection of the product which was obtained as the mixture of all possible formylated products. The nor-nitriles were therefore characterized as the completely deprotected products. The yields of "formylated" nor-nitriles are above 90% except in the case of cholic acid (85–88%) and chenodeoxycholic acid (80–85%) where some deformylation and subsequent polymerization was observed. The polymeric products are easily separated since they are either hydrolyzed during the washings and the bile acid extracted, or they remain insoluble and are filtered out. The isolated product, as examined by TLC, was composed only of the mixture of partially protected nor-nitriles and had a yellowish color. The intensity of the color depended on the quality of the starting formyl derivative.

Hydrolysis of the nornitriles

Because of the known sensitivity of the 7α-hydroxyl group to acids (29), we chose the classic basic hydrolysis of the nitrile (30) which, at the same time, eliminated the formate groups. Although it was necessary to carry out the reaction for 96 hr for completion, the nor-bile acid was the only product obtained (except in the case of norcholic acid). The remaining nitrile was extracted from the basic hydrolysis solution, but some intermediate amide and other minor basic impurities were difficult to extract completely and accompanied the nor-bile acid when this was extracted from the acidified solution. Saturation of the basic solution with sodium chloride allowed complete extraction of this basic byproduct; alternatively, after the extraction of the neutral compounds, the crude acid could be purified by percolation through a strongly acidic exchange resin in H^+ form using tetrahydrofuran as solvent. The crude products were obtained in ca. 90–98% yields and were pure as judged by TLC and GLC. Nor-cholic acid was obtained accompanied by a small amount of a more polar impurity that could not be isolated, but that could be eliminated by one recrystallization from acetone. The products were of yellowish color, the color being carried over from the degradation step; products could be purified in all cases by recrystallization from acetone. The yield of each recrystallization was ca. 80–90%, depending on the number of crops obtained.

For further chemical work the crude product (one recrystallization in the case of norcholic acid) was satisfactory. Three recrystallizations gave a product of high purity that did not show impurities when analyzed by HPLC with UV detection at 205 nm.

Spectral properties of nor-bile acids

The mass spectra of methyl norursodeoxycholate, **3f**, exhibited the same fragmentations as methyl ursodeoxycholate (31), but showed the base peak at m/z 213 (loss of 2 × H_2O + side chain + C_{15}–C_{17} of ring D) rather than at m/z 255 (loss of 2 × H_2O + side chain). An additional intense peak (56%) was found at m/z 283 ($C_{21}H_{31}^+$), presumably formed by the loss of 2 × H_2O and fragmentation of the C_{20}–C_{22} bond (a branching point) with loss of the relatively stable $^{\bullet}CH_2COOCH_3$ radical. The mass spectra of the nornitriles always showed the molecular ion and fragmentations similar to those of the methyl ester of the corresponding C_{24} bile acids, but with different intensities. The spectrum of nordeoxycholonitrile, **4d**, showed an important peak at m/z 312 ($C_{22}H_{34}N^+$, 53%), whereas the spectrum of norcholonitrile, **5d**, showed the corresponding m/z 310 ($C_{22}H_{32}N^+$, 100%). This fragmentatoin pattern, which is not found in the spectrum of the methyl esters of the C_{24} bile acids or in the other nor-nitriles, could be attributed to loss of one (or two) molecules of water followed by the loss of the $^{\bullet}HCO$ radical, presumably originated from elimination of the C_{12}–O_{12} system accompanied by H transfer. This illustrates the known strong directive effect of the 12α hydroxyl group on the fragmentation patterns of bile acids (32).

The ^{13}C-NMR spectra of the methyl esters of the nor-bile acids (Table 1) were assigned by comparison with that reported for C_{24} bile acid methyl esters (33). As expected, the only modifications were found on the side chain carbon atoms: C-22 was shifted by ca. 10.4 ppm whereas C-20 was shifted by −1.4 ppm. The values paralleled those found when comparing the spectra of 4-methylpentanoic acid and 3-methylbutanoic acid, 11.0 and −2.2 ppm, respectively. The chemical shifts of C-16 and C-17 did not change with respect to the C_{24} methyl esters, thus indicating that the conformations of the side chains are similar (34).

The simplicity and the high yields and purity obtained by this procedure make it possible to prepare large amounts of the common nor-bile acids, thus providing starting materials for the synthesis of more complex C_{23} bile acid derivatives.

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