ELSEVIER

Steroids 64 (1999) 780–784

Steroids

A convenient synthesis of dinorbile acids: Oxidative hydrolysis of norbile acid nitriles☆

Ashok K. Batta[a,*], Subhash C. Datta[a], G. Stephen Tint[a,b], David S. Alberts[c], David L. Earnest[c], Gerald Salen[a,b]

[a]*Department of Medicine, University of Medicine and Dentistry-New Jersey Medical School, Newark, NJ 07103, USA*
[b]*Department of Medicine, GI Section (15A), Veterans Administration Medical Center, 185 Tremont Avenue, East Orange, NJ 07019, USA*
[c]*Department of Medicine, University of Arizona, Tuscon, AZ 85727, USA*

Received 5 April 1999; accepted 5 May 1999

Abstract

We report a convenient method for the synthesis of dinorbile acids (23,24-dinor-5β-cholan-22-oic acids, pregnane-20-carboxylic acids) in fair to good yields from norbile acid nitriles in one step by oxidative hydrolysis with oxygen in the presence of potassium-*t*-butoxide. The method results in stepwise overall removal of two carbon atoms in bile acid side chains in two steps. Dinorbile acids corresponding to several common bile acids have been prepared and their structures confirmed by spectroscopic methods. This simple method for synthesis of dinorbile acids may facilitate their study metabolically.

Keywords: Dinorbile acids; Norbile acids; Bile acids; Synthesis; Nuclear magnetic resonance; Mass spectrometry

1. Introduction

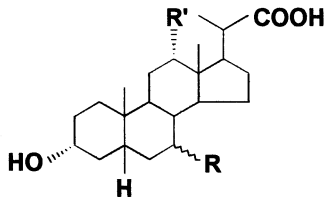
Bile acids (hydroxy derivatives of 5β-cholan-24-oic acid [1]) are the end products of cholesterol biosynthesis in the liver and play a major role in cholesterol and fat absorption. Although the naturally occurring C_{24} bile acids are conjugated with glycine and taurine by hepatic enzymes and are well absorbed from the intestine, the short-chain C_{23} and C_{22} bile acids are choleretic in animals and are poorly conjugated and largely excreted into the urine [1–3]. Because hepatocyte bile acid concentrations are abnormally high in cholestatic liver disease, bile acids that cause choleresis may be potentially useful in patients with cholestasis. Norcholic acid and norchenodeoxycholic acid have been shown to be highly choleretic, but they do not accumulate in the bile. On the other hand, dinorchenodeoxycholic acid has been shown to be secreted into bile in the unconjugated form. Dinorbile acids have not been detected in bile from healthy humans, although dinorcholic acid has been shown to be present in unconjugated form in bile from patients with cerebrotendinous xanthomatosis [4] and dinotlithocholic acid was reported in human meconium [5] and serum [6]. Dinorcholic acid, and its 7- and 12-oxo and 1β- and 2β-hydroxy derivatives, were all detected in small amounts in the urine of patients with cerebrotendinous xanthomatosis [7]. Dinorbile acids are also formed by microbial degradation of bile acids [8,9]. Yeh et al. [3] have shown that dinorchenodeoxycholic acid is metabolized in bile fistula rat or hamster similarly to norchenodeoxycholic acid (a greater proportion of unconjugated dinorbile acid was secreted into the bile than the norbile acid), but quite differently from chenodeoxycholic acid. In the view of renewed interest in dinorbile acids and to learn more about their metabolism, we needed to have a convenient method for the synthesis of these short-chain bile acid derivatives.

Dinorbile acids have been synthesized by the Barbier–Wieland degradation [10] or oxidation [11] of 24-nor-5β-cholan-22-enes obtainable by lead tetraacetate oxidation of the C_{24} bile acids [12,13] or of the noraldehydes [14]. However, the methods involve several steps and the inter-

* Corresponding author. Tel.: +1-201-676-1000/2289; fax: +1-201-676-2991.

☆ This work was supported by U.S. Public Health Service Grant HL-17818, a grant from the Veterans Affairs Research Service, Washington, D.C., and National Cancer Institute Grant PO1-CA-41108. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

0039-128X/99/$ – see front matter
PII: S0039-128X(99)00064-1



1a R = H, R' = H; Dinorlithocholic acid
1b R = H, R' = OH; Dinordeoxycholic acid
1c R = α-OH, R' = OH; Dinorcholic acid
1d R = β-OH, R' = H; Dinorursodeoxycholic acid
1e R = β-OH, R' = OH; Dinorursocholic acid

Fig. 1. Structures of dinorbile acids.

mediate 24-nor-5β-cholan-22-enes are obtained in relatively low yields. Schteingart and Hofmann have reported the synthesis of 24-nor-23-nitriles of bile acids in >90% yields [15] via α-nitrosation-fragmentation with sodium nitrite in trifluoroacetic acid/trifluoroacetic anhydride [16]. We report a convenient synthesis of dinorbile acids (Fig. 1, *1a–e*) by oxidative hydrolysis of the 24-nor-23-nitriles of bile acids, and thus, the synthesis of C_{22} bile acids in two steps from C_{24} bile acids.

$RCH_2CH_2COOH \rightarrow RCH_2CN \rightarrow RCOOH.$

2. Experimental

Ursodeoxycholic acid was a gift from Tokyo Tanabe, Tokyo, Japan. All other bile acids were purchased from Aldrich (Milwaukee, WI, USA). Sil-Prep (hexamethyldisilazane:trimethylchlorosilane:pyridine, 3:1:9) used for making trimethylsilyl ether derivatives was purchased from Alltech Associates, Inc. (Deerfield, IL, USA). All reagents and solvents used were reagent grade and were purchased from Aldrich.

2.1. Methods

The elemental analysis of the synthesized compounds was performed at the Spang Microanalytical Laboratory (Eagle Harbor, MI, USA). Melting points were determined on a Thermolyne 12 000 apparatus (Dubuque, Iowa, USA) and are uncorrected.

2.2. TLC

TLC of the dinorbile acids was performed on precoated silica-gel plates (0.25 mm thickness; Analabs, New Haven, CT, USA). Plates were developed in a solvent system of chloroform/methanol (90:10 v/v). After development, spots were visualized by spraying the plates with phosphomolybdic acid (3.5% in isopropanol) followed by a spray with 10% sulfuric acid and subsequent heating at 110°C for 2 min.

2.3. NMR spectroscopy

The proton NMR spectra of the dinorbile acids were obtained at 200 MHz in deutereochloroform on a Varian Associates XL-200 spectrometer (Palo Alto, CA, USA) and tetramethylsilane was used as the internal standard.

2.4. GLC

A Hewlett–Packard model 5890A gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector and an injector with a split/splitless device for capillary columns was used for GLC. The chromatographic column consisted of a chemically bonded fused silica CP-Sil-5 CB (stationary phase, 100% dimethylsiloxane) capillary column (20 m × 0.22 mm inside diameter; Chrompack, Inc., Raritan, NJ, USA) and helium was used as the carrier gas at a flow rate of 1 ml/min. The GLC operating conditions were as follows. Injector and detector temperatures were 260°C and 290°C, respectively. After injection, oven temperature was kept at 100°C for 2 min, then programmed at a rate of 20°C/min to a final temperature of 260°C. Aliquots of dinorbile acids (10–20 μg) were treated with 3% anhydrous methanolic hydrochloric acid to obtain the methyl esters, which were then reacted with 100 μl of Sil-Prep for 20 min at 55°C. Solvents were evaporated at 55°C under N_2 and the TMS ether derivative formed was taken in 100 μl of hexane. One to 2 μl was injected into the GLC column simultaneously with nordeoxycholic acid used as the internal standard.

2.5. GC-MS

Mass spectra of the dinorbile acids were obtained on a Hewlett–Packard model 5988 gas chromatograph–mass spectrometer (Palo Alto, CA, USA) by using a 25-m CP-Sil-5 CB capillary column [17].

*2.6. Synthesis of dinorursodeoxycholic acid (*1d*)*

The nornitrile (Fig. 2, 3; 2 g) synthesized from ursodeoxycholic acid [2], according to the method of Schteingart and Hofmann [15], was dissolved in anhydrous tetrahydrofuran (20 ml) and cooled at 0°C. Potassium *t*-butoxide (1 g) was added and the resulting solution was stirred for 30 min at 0°C. 18-Crown-6 ether (1 g) was then added to the reaction mixture and a slow stream of oxygen was passed while the temperature was allowed to rise to room temperature [18]. After stirring for 18 h in oxygen atmosphere, ethyl acetate (100 ml) was added and the contents were extracted with water (4 × 20 ml). The combined water extract was acidified to pH 1 and the liberated dinorursodeoxycholic acid was extracted with ethyl acetate (4 × 50 ml),

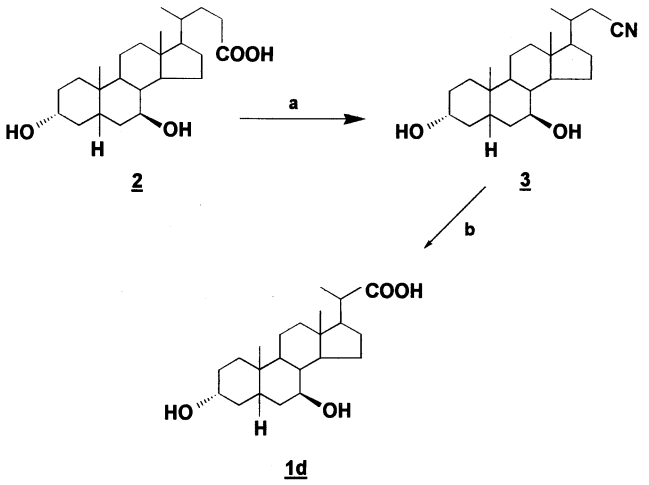


Fig. 2. Synthesis of dinorursodeoxycholic acid. *a*, Trifluoroacetic acid/trifluoroacetic anhydride/sodium nitrite on diformate. *b*, Potassium *t*-butoxide/18-crown-6 ether/O_2.

the ethyl acetate extract was washed with water to neutrality, dried over anhydrous sodium sulfate, and concentrated to a small volume. On keeping overnight in a refrigerator, colorless microscopic crystals of dinorursodeoxycholic acid (*1d*) were separated (yield, 1.05 gm); crystals from acetone, melting point, 177–180°C; TLC, R_f, 0.45 (Table 1); GLC retention time of the methyl ester-TMS ether derivative, 19.333 min (Table 1). Found: C, 72.47; H, 9.92%; calculated for $C_{22}H_{36}O_4$: C, 72.53; H, 9.89. The proton signals in the ^{1}H-NMR spectrum of the methyl ester and the major mass ion fragments in the mass spectrum of the methyl ester-TMS ether derivatives are given in Tables 2 and 3. Silica gel column chromatography of the mother liquor after filtration of *1d* yielded another 310 mg of the compound (total yield, 68% from *2*).

3. Results and discussion

The recently reported synthesis of norbile acids [15] involves the formation of 24-nor-23-nitriles from the C_{24} bile acids via α-nitrosation fragmentation with sodium nitrite in trifluoroacetic acid/trifluoroacetic anhydride [16]. Alkaline hydrolysis of the nornitrile then produces the norbile acid in very good yields and the method may be a preferred alternative to Barbier–Wieland degradation for synthesis of dinorbile acids. However, nitriles are highly resistant to alkaline hydrolysis and 24-nor-23-nitriles of bile acids are hydrolyzed with 10% sodium hydroxide at reflux temperature for 96 h—conditions that result in partial dissolution of silicates from the glass reaction vessel [15]. We considered the penultimate oxidative hydrolysis of nornitriles as a means of converting the nitrile into carboxyl group under milder conditions [18] and thereby providing us with a method to synthesize a C_{22} bile acid in two steps from a C_{24} bile acid. The reaction would involve creation of a carbanion α- to the nitrile, followed by oxidative hydrolysis with O_2 in presence of a strong base. Treatment of the 24-nor-23-nitrile with a slight excess of lithium diisopropylamide at low temperatures (−20°C) in oxygen atmosphere and in the presence of 18-crown-6 ether for 4 h produced the required dinorbile acid, albeit in only 10–15% yield, and the starting nor-nitrile was largely recovered. Prolonged reaction time (up to 48 h) did not significantly improve the yield. In an earlier publication, DiBiase et al. [18] have reported that treatment of nitriles from several long-chain aliphatic acids with potassium-*t*-butoxide in the presence of 18-crown-6 ether at 65°C in oxygen atmosphere resulted in 60–90% yields of the lower homologs. We used this method to make dinorbile acids from bile acid nornitriles and obtained 33–75% yield of the products. The isolation procedure was simple; the unreacted nornitrile could be removed by simple solvent extraction of the basic aqueous reaction product and the dinor bile acid was then isolated after acidification of the aqueous layer, followed by direct crystallization or a simple column chromatography. A typical procedure for the oxidative hydrolysis is described in the experimental section for the synthesis of dinorursodeoxycholic acid. Apparently, the addition of oxygen to the generated anion or the hydrolysis of the acyl nitrile (*A*, below) requires more rigorous conditions [18]; thus, lithium

Table 1
Physical characteristics of dinorbile acids

Dinorbile acid	m.p.	TLC R_f	GLC rt (min)	C,H analysis	
				Found	Calculated
Dinorlithocholic	207–209°C (Lit. 210°C [21])	0.79	16.342	C, 75.80% H, 10.31%	C, 75.86% H, 10.34%
Dinordeoxycholic	236–239°C (Lit. 238°C [22])	0.42	17.483	C, 72.59% H, 9.82%	C, 72.53% H, 9.89%
Dinorcholic	285–288°C (Lit. 285–286°C [23])	0.19	18.206	C, 69.56% H, 9.43%	C, 69.47% H, 9.47%
Dinorursodeoxycholic	177–180°C	0.45	19.333	C, 72.47% H, 9.92%	C, 72.53% H, 9.89%
Dinorursocholic	217–218°C	0.20	19.806	C, 69.41% H, 9.41%	C, 69.47% H, 9.47%

Table 2
Proton signals in the ^{1}H-NMR spectra of the methyl esters of dinorbile acids

Dinorbile acid	^{1}H signal						
	CH_3			CHOH			$COOCH_3$
	C-18	C-19	C-21	C-3	C-7	C-12	
Dinorlithocholic	0.674 (s)	0.925 (s)	1.231 (d, J = 6.8 Hz)	3.630 (m)			3.647 (s)
Dinordeoxycholic	0.687 (s)	0.910 (s)	1.228 (d, J = 6.8 Hz)	3.630 (m)	3.920 (bs)		3.650 (s)
Dinorcholic	0.677 (s)	0.878 (s)	1.232 (d, J = 6.8 Hz)	3.480 (m)	3.820 (bs)	3.920 (bs)	3.643 (s)
Dinorursodeoxycholic	0.686 (s)	0.949 (s)	1.190 (d, J = 6.8 Hz)	3.620 (m)	3.620 (m)		3.648 (s)
Dinorursocholic	0.717 (s)	0.941 (s)	1.244 (d, J = 6.8 Hz)	3.620 (m)	3.620 (m)	3.920 (bs)	3.658 (s)

diisopropylamide at the low temperatures yielded poor yields of the dinorbile acids.

$$RCH_2CN \rightarrow RCH^-CN \rightarrow$$

$$RCH(OO^-)CN \rightarrow RCOCN \rightarrow RCOOH(A)$$

The dinorbile acids, *1a* and *1d* (Fig. 1), were isolated in pure form after crystallization from acetone, whereas the compounds *1b*, *1c*, and *1e* needed initial purification by column chromatography. Care was taken in the isolation of the more water-soluble dinorcholic acid (*1c*) and, in particular, dinorursocholic acid (*1e*) to exhaustively extract these compounds from the aqueous solutions after acidification. Further, whereas the 23-nornitriles of ursodeoxycholic acid and lithocholic acid yielded 68% and 73% of the corresponding dinorbile acids, the nornitriles from cholic acid, deoxycholic acid, and ursocholic acid, the 12α-hydroxylated bile acids, produced the dinor acids in 33%, 40%, and 50% yields, respectively, even when the reaction was performed at 65°C [18], suggesting steric hindrance caused by the 12α-hydroxyl group. Elevated temperatures resulted in undesirable side products. Thus, during the preparation of dinorcholic acid, 20–25% of norcholic acid amide was formed in the neutral fraction in addition to the unreacted nornitrile, and the acidic fraction contained 20–30% of norcholic acid in addition to dinorcholic acid, thus suggesting competing hydrolysis of the nornitrile at higher temperatures.

The structures of the dinorbile acids were confirmed by their ^{1}H-NMR and MS analyses. The proton signals in the ^{1}H-NMR spectra of the methyl esters of compounds *1a–e* are given in Table 2. As expected, both C-18 and C-19 proton signals were not influenced by the length of the side chain and appeared at approximately the same positions, as in case of the corresponding C_{23} and C_{24} bile acids [3,19]. Thus, the C-18 proton signals appeared as a singlet at δ0.674–0.717 ppm in all spectra, and the C-19 protons appeared as singlet at δ0.878–0.949 ppm. However, the shortened side chain showed a strong deshielding effect of approximately δ0.2 ppm on the C-21 proton signals, which appeared as a doublet (J = 6.8 Hz) at δ1.190–1.244 ppm in the spectra of these compounds [8]. The mass spectral fragmentation pattern of the TMS ether methyl esters of the dinorbile acids [4,6,8] was essentially identical to that reported for the corresponding C_{24} bile acids [20] and norbile acids [15] with the ion fragments with intact side chains being 28 and 14 mass units less, respectively. The relative intensities of the ion fragments varied sometimes; e.g. in the mass spectrum of dinordeoxycholic acid derivative, the ion fragment at m/z 180

Table 3
Major ion fragments in the mass spectra of methyl ester-trimethylsilyl ester derivatives of dinorbile acids[a]

Dinorbile acid	Ion fragment (m/z)
Dinorlithocholic	434 (0.1), 419 (8), 344 (35), 329 (20), 257 (12), 215 (34), 75 (100)
Dinordeoxycholic	522 (0.1), 507 (38), 432 (1), 342 (10), 327 (6), 255 (60), 180 (63), 73 (100)
Dinorcholic	595 (7), 505 (1), 430 (13), 415 (3), 340 (13), 325 (5), 281 (6), 253 (37), 73 (100)
Dinorursodeoxycholic	522 (0.1), 507 (4), 432 (32), 342 (12), 327 (11), 283 (4), 255 (10), 73 (100)
Dinorursocholic	595 (9), 520 (9), 433 (5), 430 (7), 343 (10), 340 (12), 281 (3), 253 (45), 73 (100)

[a] Major on fragments above m/z 200 are reported.

(cleavage of rings B and C) was slightly more abundant than m/z 255 (due to loss of side chain + TMS groups), compared with the C_{24} derivative, where the fragment at m/z 255 appeared as the base-ion fragment.

In summary, we have described a method for the synthesis of dinorbile acids from bile acids via penultimate oxidative hydrolysis of norbile acid nitriles. The reaction involves mild hydrolysis conditions and good yields are obtained for dinorbile acids without a 12α-hydroxyl group. The 12α-hydroxyl group interferes with the oxidative hydrolysis with competing hydrolysis of the nornitrile. It is hoped that this facile synthesis of dinorbile acids will generate a renewed interest in their metabolism and physiological properties.

References

[1] Nakatomi F, Kihira K, Kuramoto T, Hoshita T. Intestinal absorption and metabolism of norcholic acid in rats. J Pharmacobiosyn 1985;8: 557–63.

[2] Yoon YB, Hagey LR, Hofmann AF, Gurantz D, Michelotti EL, Steinbach JH. Effect of side chain shortening on the physiological properties of bile acids: hepatic transport and effect on biliary secretion of 23-norursodeoxycholate in rodents. Gastroenterology 1986; 90:837–52.

[3] Yeh H-Z, Schteingart CD, Hagey LR, Ton–Nu H-T, Bolder U, Gavrilkina MA, Steinbach JH, Hofmann AF. Effect of side chain length on biotransformation, hepatic transport, and choleretic properties of chenodeoxycholyl homologues in the rodent: studies with dinorchenodeoxycholic acid, norchenodeoxycholic acid, and chenodeoxycholic acid. Hepatology 1997;26:374–85.

[4] Matoba N, Une M, Hoshita T. Identification of unconjugated bile acids in human bile. J Lipid Res 1986;27:1154–62.

[5] St Pyrek J, Sterzycki R, Lester R, Adcock E. Constituents of human meconium. II. Identification of steroidal acids with 21 and 22 carbon atoms. Lipids 1982;17:241–9.

[6] St. Pyrek J, Little JM, Lester R. Detection of 3-hydroxy-etianic and 3-hydroxy-bisnorcholanic acids in human serum. J Lipid Res 1984; 25:1324–9.

[7] Kuromoto T, Furukawa Y, Nishina T, Sugimoto T, Mahara R, Tohma M, Kihira K, Hoshita T. Identification of short side chain bile acids in urine of patients with cerebrotendinous xanthomatosis. J Lipid Res 1990;31:1895–902.

[8] Leppik RA. Deoxycholic acid degradation by a *Pseudomonas* species: acidic intermediates from the initial part of the catabolic pathway. Biochem J 1982;202:747–51.

[9] Hayakawa S. Microbial transformation of bile acids: a unified scheme for bile acid degradation, and hydroxylation of bile acids. Z Allg Mikrobiol 1982;22:309–26.

[10] Riegel B, Moffett R, McIntosh AV. Nordeoxycholic acid. In: Horning EC, editor. Organic Synthesis Coll.,Vol. 3. New York: John Wiley & Sons, 1955. p. 234.

[11] Huffman JW, Sobti RB. Synthetic approaches to steroidal alkaloids. II. Preparation and reactions of 12-oxo-dinorcholanic acids. Steroids 1970;16:755–70.

[12] Vaidya AS, Dixit SM, Rao AS. Degradation of bile acid side chain with lead tetraacetate. Tetrahedon Lett 1968:5173–4.

[13] Carlson GL, Belobaba DTE, Hofmann AF, Wedmid Y. 24-Nor-5β-chol-22-enes derived from the major bile acids by oxidative decarboxylation. Steroids 1977;30:787–93.

[14] Radominska–Pyrek A, Huynh T, Lester R, St. Pyrek J. 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;27:102–13.

[15] Schteingart CD, Hofmann AF. 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;29:387–95.

[16] Smushkevich YI, Usorov MI, Suvorov NN. Conversion of carboxylic acids into nitriles with shortening of the aliphatic chain by a methylene group. J Org Chem USSR 1975;11:653–5.

[17] Batta, AK, Arora R, Salen G, Tint GS, Eskreis D, Katz S. Characterization of serum and urinary bile acids in patients with primary biliary cirrhosis by gas-liquid chromatography-mass spectrometry: effect of ursodeoxycholic acid treatment. J Lipid Res 1989;30:1953–62.

[18] DiBiase SA, Wolak RP Jr, Dishong DM, Gokel GW. Crown cation complex effects: 10. Potassium *tert*-butoxide mediated penultimate oxidative hydrolysis of nitriles. J Org Chem 1980;45:3630–4.

[19] Waterhaus DV, Barnes S, Muccio DD. Nuclear magnetic resonance spectroscopy of bile acids: development of two-dimensional NMR methods for the elucidation of proton resonance assignments for five common hydroxylated bile acids, and their parent bile acid, 5β-cholanoic acid. J Lipid Res 1985;26:1068–78.

[20] Iida T, Chang FC, Matsumoto T, Tamura T. High resolution mass spectra of mono-, di-, and trihydroxy stereoisomers of bile acids. Biomed Mass Spectrom 1982;9:473–6.

[21] Reindel F, Niederlander K. Nor- und bisnorlithocholsaure. Ber Dtsch Chem Ges 1935;68:1969–73.

[22] Hoehn WM, Mason HL. The degradation of desoxycholic acid to etio-desoxycholic acid through etio-desoxycholyl methyl ketone. J Am Chem Soc 1938;60:1493–7.

[23] Shimizu T, Kazuno K. Uber die Konstiturion der Trioxybufostercholensaure und den Systematischen abbau det Cholsaure. Z Physiol Chem 1936;244:167–72.