



Chemical Synthesis, Structural Analysis, and Decomposition of *N*-Nitroso Bile Acid Conjugates[†]

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Abstract—*N*-nitrosoamides of 7 β -hydroxylated bile acid conjugates, particularly of the ursodeoxycholic acid family have been synthesized. The products and synthetic intermediates were fully characterized by the results of high-resolution ¹H NMR, FT-IR, FAB/MS and ESI-MS studies. The compounds, *N*-nitrosoglycoursodeoxycholic acid (NOGUDCA), *N*-nitrosoglycoursodeoxycholic acid (NOGUCA) and *N*-nitrosoglycodeoxycholic acid (NOGDCA) decomposed between pH 6 and 9 in aqueous buffer solutions, indicating a *t*_{1/2} of 5–7 h while *N*-nitrosotauroursodeoxycholic acid (NOTUDCA) indicated a much longer *t*_{1/2} of 15–17 h. These results suggest that the compounds are relatively stable and may enter the enterohepatic circulation. Their decomposition is similar to that of other *N*-nitrosamides, which generate alkylating agents and thereby act as DNA mutagens. Copyright © 1996 Elsevier Science Ltd

Introduction

N-nitroso compounds have been found to produce tumors in 40 animal species.¹ These compounds may be introduced into humans by exogenous sources such as food substances and environmental pollution. It has been suggested that *N*-nitroso compounds may also exist endogenously, forming carcinogens associated with cancers of the gastrointestinal tract.² There is a growing realization that normal cells contain DNA adducts derived from electrophiles generated endogenously, and that the potential of endogenous adducts to initiate carcinogenesis must be compared with that of exogenous compounds.³ Considerable research has been directed towards the formation and biological activation of the *N*-nitrosamines, while less effort has been focused on the *N*-nitrosamides which require no activation and are known to be potent direct-acting mutagens and carcinogens.^{4–6} The major sources of nitrosable amides in the human body are the bile acids,⁷ which are synthesized from cholesterol and conjugated in the liver with the amino acids glycine or taurine.^{8,9} The conjugated bile acids are stored in the gall bladder and secreted in bile into the upper intestinal tract during and after meals.¹⁰ Bile acids aggregate into micelles,^{11,12} which have a well-documented catalytic effect on *N*-nitrosation at the concentrations normally found in the bile.^{13,14} Having performed their

function of assisting in the absorption of fat, they are reabsorbed and recirculated in the enterohepatic circulation (EHC).¹⁰ This pool of bile acids circulates 2 or 3 times during each meal or 5–10 times daily. Since the body maintains a constant amount of 2–4 g of bile acids, an estimated 10–40 g of conjugated bile acids travel through the small intestine daily. At the terminal ileum, there are special receptor sites, which initiate the absorption of most of the conjugated bile acids and return them to the liver in portal blood. The remaining bile acids enter the large intestine, where they are degraded by intestinal bacteria, and free bile acids are passively absorbed in the colon.^{8,9,15} However, a portion of the bile acids (ca. 50–250 mg/day) are not reabsorbed by the terminal ileum or colon. Some are consequently excreted in the feces while a few may bind to the tissues in the region of the ileum and colon.¹⁵ The most likely human exposure to direct acting *N*-nitroso bile acid conjugates would result from their *in vivo* formation. A number of investigators have proposed that nitrosamides are produced in the stomach from amides and nitrite derived from food and act in that organ to induce cancer.^{16–21} The acidic environment of the stomach after meals is ideal because amide nitrosation is catalysed by acid.²² Nitrite enters the stomach (ca. 4.3 mg/day) from two sources: 20% arises from nitrite in the diet and 80% from the reduction of salivary nitrate by oral and gastric bacteria, especially under mild acidic conditions.²² Cured meats, baked goods, cereals, and vegetables can contribute to a dietary nitrite load that increases gastric nitrite levels at the same time that the conjugated bile acids are circulating. The nitrite is converted by gastric acid to nitrous acid (HNO₂), which upon decomposi-

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tion to NO can react with nitrogen compounds such as the nitrosamides to yield *N*-nitroso derivatives.²³ In fact, nitrous acid was the reagent used in the chemical synthesis of *N*-nitroso bile acid conjugates in the present study. Nitrosation can also be mediated by bacteria and activated macrophages in infected and inflamed organs.²⁴ Endogenously occurring *N*-nitroso compounds may induce DNA modification by the formation of DNA adducts. In addition, time-dose relationships observed for tumor formation in animals indicate that lifetime or chronic exposure to even low levels of certain carcinogenic *N*-nitroso compounds may represent a greater cancer risk to man because of his long-life span.²⁵

Thus far, studies of *N*-nitrosated bile acid conjugates have been limited to derivatives of cholic acid, namely *N*-nitrosotaurocholic acid (NOTC) and *N*-nitrosoglycocholic acid (NOGC).²⁶ Both compounds were found to be mutagenic, in the absence of activation, in both forward and reversion bacterial mutation assays, while their precursors taurocholic acid and glycocholic acid were not. In a human lymphoblast assay NOGC was approximated to be 9000 times more potent than NOTC in causing a mutant fraction.²⁶ Investigation of the carcinogenic activities of NOTC and NOGC in rats showed that both induced significant levels of hepatocellular carcinoma and malignant stomach tumors.²⁷

It is essential to note that cholic acid (3 α ,7 α ,12 α -tri-hydroxy-5 β -cholan-24-oic acid, CA) and chenodeoxycholic acid (3 α ,7 α -dihydroxy-5 β -cholan-24-oic acid, CDCA) are two primary bile acids in the human gastrointestinal tract, and many other stereoisomeric and ketonic products are formed by bacterial action. Once in the hepatocyte, primary bile acids are conjugated with glycine or taurine to form the corresponding *N*-acyl conjugates. As conjugates they are secreted from the hepatocyte into the bile and with the bile they enter the intestine. In the distal small intestine and in the colon, these conjugates are converted by bacterial enzymes into the corresponding secondary bile salts by initial deconjugation, followed by 7 α -dehydroxylation. This process in the case of cholic acid leads to the formation of deoxycholic acid (3 α ,12 α -dihydroxy-5 β -cholan-24-oic acid, DCA). DCA often becomes a major bile acid in the EHC of human adults because 7-rehydroxylation does not occur.¹⁰ This bile acid has been implicated in the promotion of adenoma growth and polyp formation, although the mechanism by which this occurs is not clear.^{28,29} In addition, epimers of the major bile acids having 3 β , 7 β , and 12 β stereochemistry may also be produced by the bacterial action in the distal ileum or colon.³⁰ Bile acids of the urso family, with a 7 β -epimeric configuration, include ursodeoxycholic acid (3 α ,7 β ,12 α -trihydroxy-5 β -cholan-24-oic acid, UCA) and ursodeoxycholic acid (3 α ,7 β -dihydroxy-5 β -cholan-24-oic acid, UDCA), respectively. UDCA, present in bear's bile, is of special interest³¹ because it has been approved by the FDA as an efficient gallstone solubilizer (Actigall, Ursodiol) and is being consumed daily in large amounts by those suffering from gallstone disease³²⁻³⁴ and primary biliary cirrhosis.³⁵ Although the

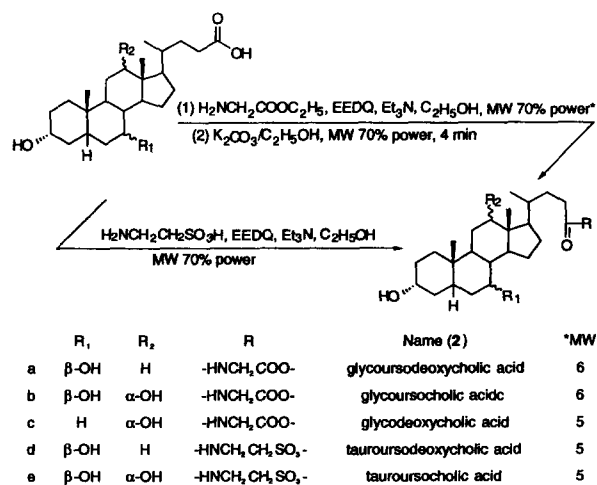
toxicity of the drug has been observed to be minimal thus far, the long-term consequences of therapy with ursodeoxycholic acid remain to be discovered.

N-Nitroso bile acid conjugates may form endogenously, whose end products upon decomposition may be very potent alkylating agents. The general class of *N*-nitroso compounds occur in normal gastric juice, however, in subjects who were prone to develop gastric cancer and had undergone Billroth I or II operations these compounds are at elevated levels.³⁶ Analytical methods cannot identify the subgroup or the individual compounds until the principal compounds have been purified, identified, and biologically assayed.²² In order to investigate further the role of the *N*-nitrosated derivatives of bile acid conjugates, particularly those of 7 β configuration in the development of cancer, sufficient quantities of the compounds were required for experimentation. In this paper, we report a relatively simple, rapid, and highly efficacious methodology for the chemical synthesis of these molecules as well as their pH dependent activities and stabilities.

Chemistry

The bile acid conjugates were prepared using a new, innovative technique which employs a domestic microwave oven,³⁷⁻⁴¹ as shown in Scheme 1. Glyco-bile acid conjugates (**2a–2c**) were synthesized by reacting the corresponding free bile acid (**1**) with the ethyl ester of glycine in the microwave, followed by hydrolysis of the ester with potassium carbonate and ethanol, also in the microwave oven.⁴¹ Tauro-bile acid conjugates (**2d** and **2e**) were synthesized by the reaction of **1** with taurine in the microwave oven. After usual work up, the desired products were isolated in good to excellent yields.

Treatment of the bile acid conjugates (**2a–2e**) with nitrous acid, according to Scheme 2, resulted in the formation of the *N*-nitrosated derivatives (**3a–3e**) of each molecule. The reagent, nitrosyl chloride gas



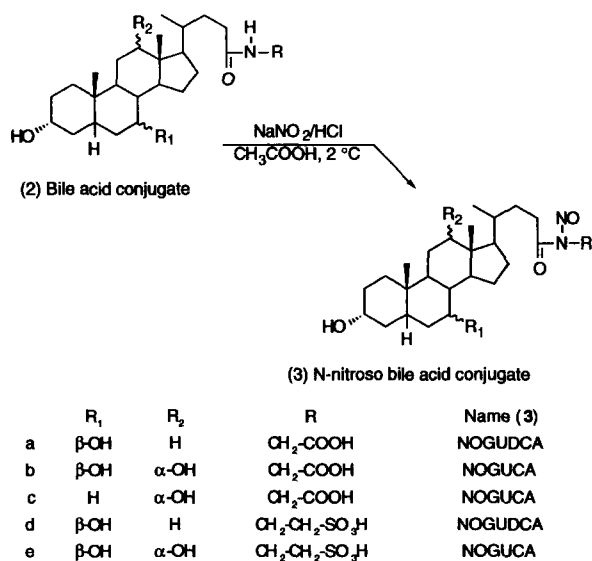
Scheme 1. Conjugation of bile acids with glycine and taurine.

(NOCl), used previously for the synthesis of NOGC and NOTC,⁷ is very expensive and labor intensive. On the other hand, we found that the expense in preparing nitrous acid in situ from sodium nitrite and hydrochloric acid is much lower and the efficiency of reaction is also higher. The various *N*-nitroso derivatives synthesized in this study were characterized by high-resolution ¹H NMR and FAB/MS or ESI-MS. It is important to note that we did not observe *E* and *Z* configurational isomers (rotomers) in the ¹H NMR spectrum for the *N*-nitrosated bile acid conjugates while recent studies described from our laboratory⁴⁰ and also by Schmassmann et al.⁴² have indicated a general rotameric population in the ¹H NMR spectrum of *N*-alkyl amides of bile acid conjugates.

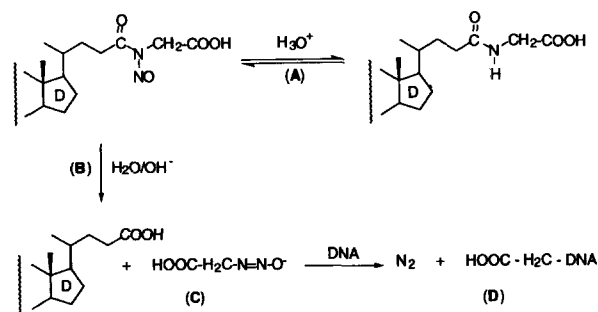
The chemical half-lives of the *N*-nitroso bile acid conjugates were determined by following the change in the maximum UV absorbance of the compound at room temperature. Above pH 8, the reactions were carried out in borate buffer, which is known not to catalyse *N*-nitrosamide decomposition. Phosphate buffer, which does catalyse the reaction,⁷ was used below pH 8. The half-life of the *N*-nitroso glyco-derivatives (3a–3c) was found to be 5–7 h on average at zero buffer concentration and neutral pH. The half-life of *N*-nitrosotauroursodeoxycholic acid was observed to be much longer (15–17 h) under the same conditions. The kinetics of the decomposition reaction were first-order over 3 half-lives. The decomposition that we have observed is significantly slower than the half-life of 2–4 h reported for NOTC and NOGC.⁷

Results and Discussion

N-Nitroso bile acid conjugates may form in the stomach as described previously, and then flow into the intestine with other semiliquid mass from the stomach because their half-lives are relatively long (15–17 h for NOTUDCA and 5–7 h for NOGUDCA, NOGUCA,



Scheme 2. *N*-nitrosation of bile acid conjugates.



Scheme 3. Decomposition of *N*-nitroso glyco-bile acid conjugates.

and NOGDCA) under the conditions that might be encountered in the gastrointestinal tract. Consequently, the effect of these *N*-nitroso bile acid conjugates upon the enterohepatic circulation must be considered. Decomposition was apparent especially in strong acid or alkali (Scheme 3). In very strong acid, denitrosation (A) was predominant. Above pH 1, however, only deamination (B) is important, and this pathway⁶ is catalysed by OH[−]. As the *N*-nitroso bile acid conjugates travel through the intestine, they would slowly decompose, and the end products of simple alkaline hydrolysis (C) are very reactive with DNA (Scheme 3).

Alkyldiazoates are postulated to be reactive intermediates in the DNA-alkylating activity of a large number of compounds that contain an *N*-nitroso-*N*-alkyl functionality, evolving nitrogen gas during the decomposition.^{6,7} For example, the glyco-side chain (from NOGC) gives rise to a carboxymethyldiazoate (C), which has been observed by the formation of carboxymethyl adducts (D) to the nitrogenous bases guanine and adenine in calf-thymus DNA in vitro.⁴³ A similar experiment has not been performed with an *N*-nitrosated-tauro derivative, although strong evidence of the alkylating capacity of both the *N*-nitrosated tauro- and glyco-side chains is shown in the FAB and ES-MS data for the compounds under present study. All of the *N*-nitrosated glyco derivatives produced a carboxymethyl fragment (*m/z* 59) and both of the *N*-nitrosated tauro derivatives gave rise to a 2-sulfoethyl fragment (*m/z* 108). If such electrophiles are generated in the cells of the gastrointestinal tract, *N*-nitrosated bile acid conjugates may be promoters of liver, colon, or stomach carcinogenesis. The consequences of the formation of *N*-nitroso derivatives of the bile acid conjugates, because of their enhanced stability in comparison to other nitrosamides, may have a wider scope than acting only on the stomach, the organ in which they were formed.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were determined with a Thomas-Hoover unimelt capillary melting point apparatus and are uncorrected. Ursodeoxycholic acid, ursocholic acid, and deoxycholic acid of pharmaceutical grade were

donations of Prodotti Chimici e Alimentari S.p.A., Alessandria, Italy. The reactions were monitored by TLC using 0.25 mm E. Merck precoated silica gel-60 plates (F-254). Solvent systems: 26:8:4:2, CHCl_3 :MeOH: CH_3COOH : H_2O (a), 40:6:4, CHCl_3 :MeOH: CH_3COOH (b), 70:50:5, CHCl_3 :(Me) $_2$ CO:MeOH (c), and 70:50:10, CHCl_3 :(Me) $_2$ CO:MeOH (d) were used as appropriate.^{40,41,44,45} The spots were made visible with 3.5% phosphomolybdic acid in isopropanol and 10% (wt/vol) sulfuric acid. A commercial microwave oven (Whirlpool, 2450 MHz, total cooking power 650 watts) was used in irradiation experiments. The reaction mixture was held in an Erlenmeyer flask, covered with an inverted funnel, and placed in a beaker containing water (ca. 150 mL). The beaker served as a heat sink to dissipate excess microwave energy.³⁷⁻⁴¹ The ^1H NMR spectra were recorded on a Varian XL-400 (400 MHz) spectrometer in DMSO solution. Proton chemical shifts are expressed as parts per million downfield from internal tetramethylsilane (TMS). FABMS studies were conducted in the positive ion mode using 3-nitrobenzyl alcohol, (NBA)/DMSO/NaCl as a matrix on a JEOL HX-110 sector mass spectrometer as described previously.⁴⁵ ESI-MS was performed in the negative ion mode with MeOH as the solvent on a Sciex API tandem mass spectrometer.^{40,41} IR spectral data were obtained on a Perkin-Elmer 1600 spectrophotometer. All material required for the preparation of phosphate and borate buffers were obtained from Sigma Chemical Company. A Beckman DU-600 spectrophotometer was used to measure the rate of decomposition of the *N*-nitrosobile acid conjugates.

Glycoursodeoxycholic acid (GUDCA) (2a). Ursodeoxycholic acid (392 mg, 1 mmol), glycine ethyl ester hydrochloride (396 mg, 2.82 mmol), *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 403 mg, 1.63 mmol), triethylamine (Et_3N , 2.3 mL), and absolute EtOH (20 mL) were mixed in an Erlenmeyer flask, stirred manually and the clear solution heated in a domestic microwave oven for a period of 5–7 min at 70% power (total microwave output 650 watts).⁴¹ In most cases, it was found to be appropriate to irradiate in intervals. After cooling, the residual ethanol was evaporated and the reaction mixture dissolved in ethyl acetate and washed with water, 0.5 N NaOH, and 0.5 N HCl. The organic phase was washed with water again, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to yield the ethyl ester of ursodeoxycholic acid, which was subsequently hydrolysed to free acid by dissolving in EtOH (10 mL), treatment with potassium carbonate (10% aqueous solution, 7 mL) and irradiation for a total of 4 min in the microwave oven. The final solution was then poured into ice water (100 mL) with vigorous stirring, and acidified with 0.5 N HCl. The white solid that precipitated was collected by filtration and dried to yield **2a** (391 mg, 87%). Physical characteristics: TLC (solvent system d) $R_f = 0.21$, and (solvent system b) $R_f = 0.54$; ^1H NMR: δ 7.03 (1H, tr, $J = 4$ Hz, NH), 3.26 (2H, d, $J = 4$ Hz, $\text{NH}-\text{CH}_2$).

Compounds **2b** and **2c** were synthesized in an identical manner, and their physical properties are given below.

Glycoursodeoxycholic acid (GUCA) (2b). GUCA yield 85%; TLC: $R_f = 0.24$, solvent system b; ^1H NMR: δ 8.09 (1H, tr, $J = 6$ Hz, NH), 3.70 (2H, d, $J = 6$ Hz, $\text{NH}-\text{CH}_2$), 12.4 (1H, s, COOH).

Glycodeoxycholic acid (GDCA) (2c). GDCA, 90% yield; TLC: $R_f = 0.22$, solvent system d; ^1H NMR: δ 8.10 (1H, tr, $J = 6$ Hz, NH), 3.70 (2H, d, $J = 6$ Hz, $\text{NH}-\text{CH}_2$), 12.45 (1H, s, COOH).

Tauroursodeoxycholic acid (TUDCA) (2d). A mixture of ursodeoxycholic acid (73.0 mg, 0.2 mmol), *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 62.0 mg, 0.25 mmol), taurine (50 mg, 0.4 mmol), *N*-methylmorpholine (NMO) or triethylamine [$(\text{C}_2\text{H}_5)_3\text{N}$, 0.35 mL], and absolute EtOH (6.0 mL) were irradiated for 3.0 min in an Erlenmeyer flask in a commercial microwave oven. After cooling, the reaction mixture was poured slowly into chilled anhydrous ether (25 mL). The suspension was stirred at 5 °C for 30 min and then filtered. The solid was dissolved in chloroform (10 mL) and filtered to remove unreacted taurine. The filtrate was poured into chilled anhydrous ether (25 mL). TUDCA was filtered and dried under vacuum (66.9 mg, 67%). TLC: $R_f = 0.80$, solvent system a; ^1H NMR: δ 7.67 (1H, tr, NH), 3.28 (2H, q, $\text{NH}-\text{CH}_2$), 2.50 (2H, tr, CH_2-SO_3); ESI-MS (in the negative ion mode) $[\text{M}-\text{H}]^- = 498$ and other polymeric ions, $[2\text{M}-\text{H}]^- = 997$, $[2\text{M}-2\text{H} + \text{Na}]^- = 1019$ and $[3\text{M}-3\text{H} + 2\text{Na}]^- = 1540$ were also observed.

Tauroursodeoxycholic acid (TUCA) (2e). TUCA was prepared from ursodeoxycholic acid in 65% yield following the same procedure that was used for the preparation of TUDCA. TLC: $R_f = 0.60$, solvent system a; ESI-MS (in the negative ion mode) $[\text{M}-\text{H}]^- = 514$ and similar clusters of polymeric ions as in TUDCA were observed: $[2\text{M}-\text{H}]^- = 1029$, $[2\text{M}-2\text{H} + \text{Na}]^- = 1051$ and $[3\text{M}-3\text{H} + 2\text{Na}]^- = 1588$.

***N*-Nitroso glycoursodeoxycholic acid (NOGUDCA) (3a).** Caution! In view of the hazardous nature of *N*-nitroso compounds, all work was carried out in an efficient fume hood. GUDCA (100 mg, 0.22 mmol) was dissolved in concentrated glacial acetic acid (2 mL) in a three-necked round bottom flask. The flask was kept at 2–4 °C in an ice bath and the contents stirred by magnetic stirrer. In a separate round bottom flask, sodium nitrite (10 g) was allowed to react with concentrated hydrochloric acid (15 mL), and added dropwise through a separatory funnel. The liberated nitrous acid gas HNO_2 (g) was passed through a rubber tube and was bubbled into the reaction flask containing the bile acid conjugate dissolved in glacial acetic acid. After all the nitrous acid gas had passed (ca. 15 min) and a green color persisted, TLC was taken while the mixture was kept frozen. The reaction was terminated if the TLC showed complete disappearance of the GUDCA ($R_f = 0.21$, solvent system d) and therefore maximal

formation of the *N*-nitroso derivative ($R_f = 0.44$, solvent system d). If a trace of the starting material remained, additional sodium nitrite (5 g) and concd hydrochloric acid (8 mL) were reacted to prepare more nitrous acid. After terminating the reaction, excess acetic acid was evaporated by passing N_2 (g), and methanol (5 mL) was added to the reaction mixture. Purging with nitrogen afforded only a single product as confirmed by TLC ($R_f = 0.44$, solvent system d). The resulting concentrated clear yellow solution was filtered through Celite to remove precipitated salts, all excess solvents evaporated, and cold distilled water added to the concentrated solution to precipitate a pale yellow solid. This material was filtered and redissolved in ethanol (2 mL), excess solvent evaporated under N_2 (g), and precipitated again with cold distilled water. The final compound, NOGUDCA, was separated by filtration and dried under vacuum (106 mg, 95%); mp 116–119 °C. IR (film): 1725 ($-C=O$), 1507 ($-N=O$) cm^{-1} ; 1H NMR: δ 4.42 [2H, s, $N(NO)-CH_2$], 13.25 (1H, s, $COOH$). The FABMS (positive-ion mode) showed the molecular ion at a m/z of 501.4 $[M+Na]^+$ (the calcd molecular weight for $C_{26}H_{41}O_6N_2$ was 478). The m/z peaks at 472.4 and 413.4 were attributed to the loss of N_2 and a proton, and $-CH_2COOH$, respectively.

***N*-Nitroso glycoursocholic acid (NOGUCA) (3b).** NOGUCA was prepared using the procedure outlined for NOGUDCA, with 93% yield; TLC: $R_f = 0.44$, solvent system b, mp 123–126 °C. IR (film): 1725 ($-C=O$), 1507 ($-N=O$) cm^{-1} ; 1H NMR: δ 4.42 [2H, s, $N(NO)-CH_2$]. The FABMS (positive-ion mode) showed the molecular ion at a m/z of 517.4 $[M+Na]^+$ (the calcd molecular weight for $C_{26}H_{41}O_7N_2$ was 494). The m/z peaks at 488.4 and 429.4 were attributed to the loss of N_2 and a proton, and CH_2COOH , respectively.

***N*-Nitroso glycodeoxycholic acid (NOGDCA) (3c).** NOGDCA was synthesized using the procedure described for NOGUDCA, in 96% yield; TLC: $R_f = 0.54$, solvent system d, mp 109–111 °C. IR (film): 1725 ($-C=O$), 1507 ($-N=O$) cm^{-1} . 1H NMR δ 4.41 (2H, s, $N(NO)-CH_2$), 12.0 (1H, s, $COOH$). The FABMS (positive-ion mode) showed the molecular ion at a m/z of 501.3 $[M+Na]^+$ (the calculated molecular weight for $C_{26}H_{41}O_6N_2$ was 478). The m/z peaks at 472.4 and 413.4 were attributed to the loss of N_2 and a proton, and CH_2COOH , respectively.

***N*-Nitroso tauroursodeoxycholic acid (NOTUDCA) (3d).** NOTUDCA was synthesized according to the procedure described for NOGUDCA, although chilled anhydrous ether was used instead of water for precipitation. The final compound (NOTUDCA) was separated by filtration and dried under vacuum, with 75% yield; TLC: $R_f = 0.89$, solvent system a, mp 190–193 °C. IR (film): 1725 ($-C=O$), 1507 ($-N=O$) cm^{-1} ; 1H NMR: δ 3.93 [2H, tr, $J = 8$ Hz, $N(NO)-CH_2$], 2.36 (2H, tr, CH_2-SO_3). ESI-MS (negative ion mode) showed the molecular ion at a m/z

of 527.1 $[M-H]^-$ (the calcd molecular weight for $C_{26}H_{43}O_7N_2S$ was 528). The peaks at m/z 499.1 and 391.1 were as a result of the loss of N_2 and $-CH_2CH_2SO_3$, respectively.

***N*-Nitroso tauroursocholic acid (NOTUCA) (3e).** NOTUCA was prepared from the TUCA in 78% yield following the same procedure that was used for preparation of NOTUDCA; TLC: $R_f = 0.75$, solvent system a; IR (film): 1725 ($-C=O$), 1510 ($-N=O$) cm^{-1} . ESI-MS (negative ion mode) m/z 542.8 $(M-H)^-$, and other peaks corresponding to loss of N_2 and $CH_2CH_2SO_3$, were observed at m/z 515 and 487, respectively.

Decomposition of the *N*-nitroso bile acid conjugates

NOGUDCA, NOGUCA, NOGDCA, and NOTUDCA were dissolved in buffer solutions (50 $\mu g/mL$; borate buffer, above pH 8 and phosphate buffer, below pH 8) of varying concentrations ranging from pH 6.2 to 9.5. Their half-lives were determined by monitoring the disappearance of the UV absorbance at 246 nm for the glyco-compounds and at 239 nm for the tauro-compound over a 2–3 day period. Rates of decomposition at zero buffer concentration were obtained by extrapolation of values obtained at the higher buffer concentrations.^{5–7}

Solutions of NOGUDCA and NOTUDCA in borate buffer (0.2 M, pH 8) were stirred until UV spectrophotometry showed that all the *N*-nitrosamide functionality had disappeared. TLC and capillary GLC showed that the free bile acid, UDCA,⁴¹ was the remaining species. Other products of decomposition such as 2-hydroxyethanesulfonic acid (isoethionic acid) from NOTUDCA and 2-hydroxyacetic acid (glycolic acid) from NOGUDCA were identified as described previously.⁷

Conclusion

The compounds synthesised in this study would be very useful for studying the role of bile acids in carcinogenesis in the intestinal tract, as well as in extraintestinal organs. This area of research has gained increased attention due to strong evidence linking bile acids to the promotion of colon cancer. In addition, this field of investigation has come increasingly in to focus because of the expanding use of ursodeoxycholic acid in the treatment of both cholesterol gallstones and chronic liver diseases.

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