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MICROWAVE-INDUCED RAPID SYNTHESIS OF SARCOSINE CONJUGATED BILE ACIDS

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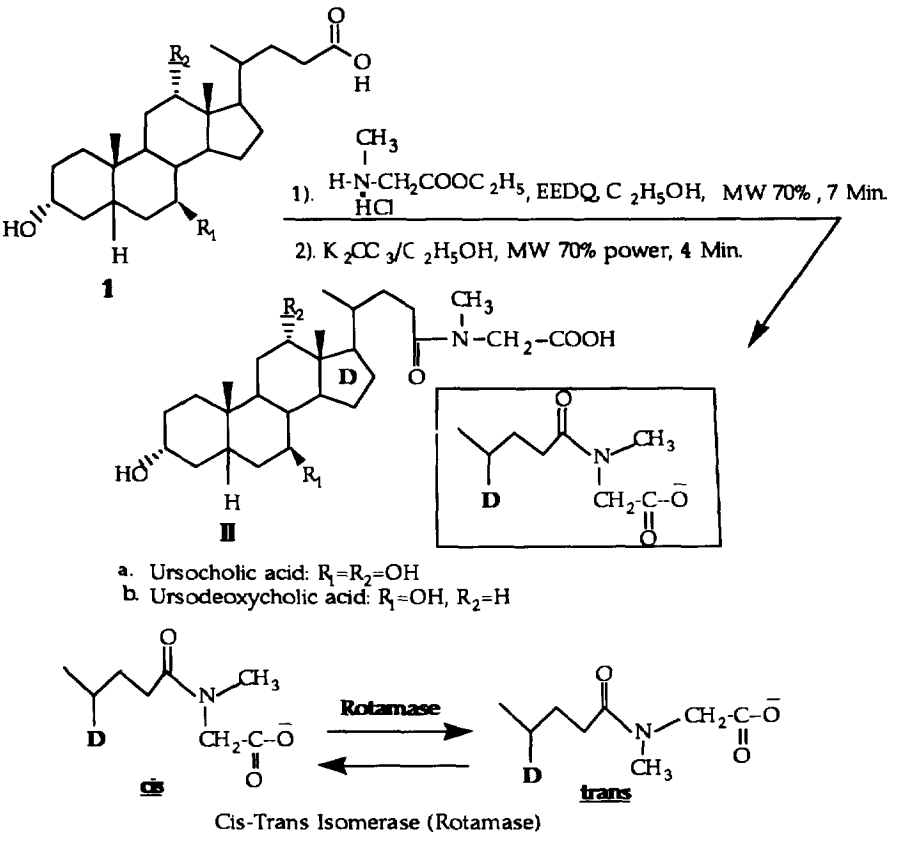
Abstract: Efficient syntheses of a wide variety of sarcosine conjugated bile acids were achieved in absolute ethanol in a domestic microwave oven. The title compounds were characterized by proton nuclear magnetic resonance and fast atom-bombardment mass spectrometry.

Microwave irradiation has become an invaluable tool in performing a great number of common chemical reactions.[1-3] Recently, many laboratories, including our own, have suggested that microwave irradiation can be used to increase the rate of many chemical reactions.[4-16] Rapid esterification, formylation, deformylation, hydrolysis and conjugate formation reactions of bile acids under microwave irradiation were recently reported from our laboratory.[13-15] In the present studies, we describe highly efficient syntheses of sarcosine (N-methylglycine) conjugated bile acids employing a domestic microwave oven as shown in Scheme 1. The use of a microwave oven drastically expedited the procedure for conjugation of bile acids and the desired compounds were achieved within minutes in high yields and at low level of microwave irradiation.



Scheme 1

Experimental: In a typical experiment, ursodeoxycholic acid (3α, 7β-dihydroxy-5β-cholan-24-oic acid, 395 mg, 1 mmol), sarcosine ethyl ester hydrochloride (CH_3N-CH_2-$COOC_2H_5$.HCl,396 mg, 2.57 mmol), N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 403 mg, 1.63 mmol), 2.3 ml of triethylamine, and 20 ml of absolute ethanol were mixed in an Erlenmeyer flask (Scheme1). The reaction mixture in the flask was covered with an inverted funnel, which was then placed in a tall beaker containing 300 ml of water and was heated in a domestic microwave oven (2450 MHz, total cooking power=650 watts) for a period of 7 minutes. [In some cases, it was found to be more

convenient to irradiate in intervals]. After cooling, the reaction mixture was dissolved in ethyl acetate and was washed with water, 0.5 N NaOH, and 0.5 N HCl. The organic solvent was dried with sodium sulfate and was evaporated to yield ethyl ester of ursodeoxycholylsarcosine, which was subsequently converted to free acid with potassium carbonate (10% aqueous solution, 7ml) and irradiating the reaction mixture for a total of 4 minutes at 70% power in the microwave oven (Scheme 1). It was observed that heating done in 1 minute intervals at 70% power (= 455 watts) gave maximum yield.

The solution was acidified with HCl (0.5 N solution) in an ice bath. The precipitate thus formed was filtered and dried (382 mg, 97% yield, mp.198-200 ^{0}C). TLC, R_f=0.72, solvent system: $CHCl_3$:$(CH_3)_2CO$:CH_3OH 70:50:5 (v/v/v). FAB-MS (Nitrobenzyl alcohol/DMSO) of ursodeoxycholylsarcosine provided protonated molecular ions at m/z 464, and 927 corresponding to $[M+H]^+$, and $[2M+H]^+$, respectively. ^{1}H-NMR (DMSO, 400 MHz), sarcosine of UDCA, corresponding to both E and Z configurational isomers were (the chemical shifts (δ) of the signals due to the minor isomer are given in parentheses when they are resolved): (δ) 0.63 (0.61) (s, 3H, CH_3-18), 0.85 (0.84) (s, 3H, CH_3-19), 0.91 (0.90) (d, 3H, CH_3-21), 3.0 (2.75) (s, 3H, N-CH_3), 3.25 (m, 1H, H-3), 3.35 (m, 1H, H-7), 3.95 (4.10) (s, 2H, N-CH_2).

Using a similar protocol as described above, ursocholylsarcosine, and 7-keto lithocholylsarcosine were also synthesized in 92% to 95% yields respectively. For ursocholylsarcosine, TLC, R_f=0.61, solvent system: $CHCl_3$:$(CH_3)_2CO$:ACOH 40:6:4 (v/v/v). FAB-MS (NBA/DMSO) under identical conditions provided significant protonated molecular ions which were observed at m/z 480 and 959 corresponding to $[M+H]^+$ and $[2M+H]^+$, respectively. FAB-MS (NBA/DMSO/NaCl) provided significant sodiated molecular ions at m/z 502=$[M+Na]^+$, and 524=$[M+2Na]^+$ respectively. ^{1}H-NMR (corresponding to both E and Z configurational isomers (3:1) were: respectively δ 0.60 (0.59) (s, 3H, CH_3-18), 0.85 (s, 3H, CH_3-19), 0.96 (0.90) (d, 3H, CH_3-21), 3.0 (2.8) (s, 3H, N-CH_3), 3.28 (m, 1H, H-3), 3.3 (m, 1H, H-7), 3.75 (m, 1H, H-12), 3.95

(4.1) (s, 2H, N-CH_2). For 7-keto lithocholylsarcosine, TLC, R_f=0.42, solvent system: $CHCl_3$:$(CH_3)_2CO$:CH_3OH 70:50:3 (v/v/v). ^1H NMR($CDCl_3$+CD_3OD) corresponding to both E and Z configurational isomers were: (δ) 0.67 (0.65) (s, 3H, CH_3-18), 1.2 (s, 3H, CH_3-19), 0.95 (0.91) (d, 3H, CH_3-21, 6Hz), 3.1 (2.96) (s, 3H, N-CH_3).

Discussion: The classical method for synthesizing sarcosine conjugated bile acids [17-19] which does not employ the convenience and speed of a microwave oven, requires 16-30 hours of reflux. In our present report, microwave-induced conjugate formation was extremely facile and required 6 to 9 minutes. Also, the reaction conditions were simplified and the yields were increased. It was our observation that the use of microwave oven was convenient and cleaner as compared to other means of heating, (conventional reflux, oil baths or heating mantles). This protocol is being used in our laboratory to produce sarcoursodeoxycholic acid and sarco-ursocholic acid conjugates in a multigram scale without any noticeable reduction in yields.

The stereochemical results of the E and Z rotomeric population of the sarcosine conjugated bile acids revealed interesting features in the ^1H-NMR spectrum. For, ursodeoxycholylsarcosine, ursocholylsarcosine and 7-keto lithocholylsarcosine E and Z configurational isomers (rotomers) were observed in a ratio of 3:1 while lithocholylsarcosine showed 2:1 ratio of E and Z rotomers . The fast atom-bombardment mass spectroscopy (MS) of ursodeoxycholylsarcosine and ursocholylsarcosine exhibited polymeric ions $[2M+H]^+$ at m/z 927 and 959 respectively.

Importance: Ursodeoxycholic acid (UDCA, 3α, 7β-dihydroxy-5β-cholan-24-oic acid) and its tauro-conjugate are increasingly being used for effective and safe medical dissolution of cholesterol gallstones and treatment of cholestatic liver diseases with reduced side effects.[20-22] Recently, it has been found that the synthetic amino acid sarcosine conjugated with UDCA travels freely in the enterohepatic circulation (EHC) while resisting bacterial 7-dehydroxylation and deconjugation.[17,18] Furthermore, very recent reports have suggested that ursodeoxycholylsarcosine inhibits intestinal cholesterol absorption in

rats with intact enterohepatic circulation.[23] Therefore, multigram scale preparation of sarco-conjugated UDCA, and its closely related analogs, which we have described above, will be of immense use in the prevention and treatment of atherosclerosis.

In conclusion, We have demonstrated fast and simple preparation of sarcosine conjugated bile acids under microwave irradiation conditions.

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