Preparation of laurylsarcosyltaurine: a surface active constituent of crab gastric juice

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Abstract The conditions for the preparation of laurylsarcosyltaurine from lauric acid and sarcosyltaurine, a crystalline dipeptide, are described. The preparation of sarcosyltaurine from the reaction of taurine and the mixed anhydride of benzyloxycarbonylsarcosine and isobutyl chloroformate is also described.

Supplementary key words conjugated bile acid synthesis • mixed anhydride • sarcosyltaurine

Research into the surface active constituents of crab gastric juice (1) made necessary the synthesis of a detergent, *N*-(*N*-dodecanoyl-sarcosyl)taurine, representative of a set of detergents synthesized by the crustacean hepatopancreas. Although various fatty acid sarcosyl- and tauryl-amides have been prepared (2), the desired compound has not been synthesized.

Van den Oord, Danielsson, and Ryhage (3) and Vonk (4), in reporting on the preparation of decanoylsarcosyltaurine, via the reaction of taurine with a mixed carboxylic acid anhydride of laurylsarcosine, referred only to Norman's method of synthesis (5) and gave no experimental detail. The reaction of sarcosine and the mixed anhydride of lauric acid and ethyl chloroformate, following the Norman procedure, resulted in an oily product which was difficult to manipulate, purify, and characterize, and failed to produce any laurylsarcosyltaurine on reaction with taurine. In contrast, the procedure described below gave an intermediate product (sarcosyltaurine) which was readily purified to the crystalline dipeptide and easily characterized by amino acid analysis. By this method the final product could also be readily purified and obtained in good yield. Consequently, we wish to report on a new method for the preparation of laurylsarcosyltaurine. The same method has also been applied to the preparation of the compound labeled with [1-^{14}C]lauric acid.[3]

The reaction sequence involved the coupling of taurine with the mixed anhydride of benzyloxycarbonylsarcosine and isobutylchloroformate to give benzyloxycarbonylsarcosyltaurine which was then hydrogenolyzed to remove the benzyloxycarbonyl protecting group. The overall yield was 55%. The sarcosyltaurine was a highly crystalline material having a proper amino acid analysis. The mixed anhydride of lauric acid with isobutylchloroformate was then reacted with sarcosyltaurine to yield laurylsarcosyltaurine. Separation of the fatty acid conjugate from the reaction mixture was achieved by reversed phase chromatography on Sephadex LH-20. A final ion exchange chromatography on a Dowex 50W × 1 column eluted with 50% aqueous ethanol gave sodiolaurylsarcosyltaurine, which upon lyophilization gave a powder in 17% overall yield. The material was characterized by thin-layer chromatography and elemental analysis (Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.). In its hydrogen form, the detergent has been shown to promote efficient cholesterol solubilization in mixed micelles (1).

The details of the synthesis are given in the following section. The ease of handling and of characterizing the intermediates and the final product in this approach seem to recommend this procedure over other routes.

Benzyloxycarbonylsarcosine

The oily protected amino acid, benzyloxycarbonylsarcosine (6) (6.7 g), was purified by reaction with dicyclohexylamine (DCHA) (5.4 g). The resulting salt (10 g) was recrystallized by dissolving it in a minimum volume of hot ethyl acetate and adding 10 volumes of petroleum ether (bp 30–60°C). Collection of the crystals gave 8.7 g, mp 142–144°C. Benzyloxycarbonylsarcosine DCHA salt (7.6 g) was then distributed between ether (50 ml) and 1 *N* citric acid (50 ml). The two phases were separated, and the acid layer was extracted with ether (3 × 30 ml). The ether layers were combined and washed with 1 *N* HCl (1 × 30 ml), with water (2 × 30 ml) to pH 4, and with saturated NaCl (1 × 30 ml). The solution was dried over sodium sulfate, filtered and then evaporated to

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Abbreviations: DCHA, dicyclohexylamine; HOAc, acetic acid; Sar, sarcosine; TEA, triethylamine; THF, tetrahydrofuran TLC, thin-layer chromatography; Z, benzyloxycarbonyl.

yield a clear oil, which was dried in vacuo for 1 hr at 40°C (0.5 mm).

Sarcosyltaurine

Triethylamine (2.76 ml, 20 mmole) was added to a stirred solution of benzyloxycarbonylsarcosine (4.4 g, 20 mmole) dissolved in 50 ml of THF (freshly distilled from $LiAlH_4$) at −15°C, and this was followed by addition of isobutylchloroformate (2.6 ml, 20 mmole). The resulting suspension was stirred for 2 min, and the solution was then added slowly to taurine (2.5 g, 20 mmole) dissolved in 50 ml of water containing TEA (5.52 ml, 40 mmole). The reaction mixture was stirred at 1–10°C for 20 min, and then at room temperature overnight. Evaporation of the solvent at 40°C using a rotatory evaporator gave a clear, viscous solution. The solution was diluted to 200 ml with water and applied to an AG 1-X 2 column (resin bed volume, 125 ml; fraction volume, 10 ml). Taurine was eluted with 500 ml of water and unreacted Z-Sar was eluted with 50 ml of 10% HOAc; elution with 1 *N* HCl gave the product (fractions 96–135). The composition of fractions was monitored by TLC on plates prepared from silica gel G (Merck & Co., Darmstadt, W. Germany) in two solvent systems: I, chloroform–methanol–HOAc–water 65:20:10:5; II, 1-butanol–pyridine–HOAc–water 30:20:6:24 The plates were treated with fluorescamine (0.01% in acetone), followed by chlorine solution, then potassium iodide/starch solution to detect the components. Fractions 95–135 were combined, reduced to 70 ml and then lyophilized. The resulting oil showed Cl_2/KI-positive spots at R_f^{II} 0.62 (intense), 0.40 (weak) and 0.32 (weak); the latter two were also ninhydrin positive. The oil readily dissolved in 35 ml of 20% HOAc. The solution was stirred and hydrogenolyzed over Pd for 22 hr in a Vibro-Mixer apparatus (Chemapec, Inc., Hoboken, N.J.). The solution was filtered through Celite, evaporated to a small volume, and 4 volumes of water added to the residual light oil; further evaporation gave crystalline material, 2.46 g (55%), mp > 240°C. TLC (solvent II) on silica gel showed the presence of spots of R_f^{II} 0.30 and 0.40 (very faint). On MN 300 cellulose plates prepared according to Jones and Heathcote (7), single spots were observed in system 1 [propanol-2–formic acid–water 40.2.10] R_f 0.24, and in system 2 [*t*-butanol–methylethylketone–ammonia–water 50:30:10:10] R_f 0.31. Recrystallization of 120 mg from water–ethanol 1:20 gave 90 mg of crystalline material, R_f^{II} 0.27, with an amino acid ratio in an acid hydrolysate of 1:1 sarcosine-taurine (100% recovery).

Laurylsarcosyltaurine

To a stirred solution of lauric acid (1.02 g, 5.1 mmole) dissolved in 10 ml of dioxane (distilled over sodium) containing TEA (0.7 ml, 5.1 mmole) was added isobutylchloroformate (0.7 ml, 5.1 mmole) at 10°C. The suspension was stirred for 5 min at 10°C ,and a solution of sarcosyltaurine (1 g, 5.1 mmole) in 1.0 *N* NaOH (5.1 ml, 5.1 mmole) was added all at once. The reaction mixture was stirred at room temperature overnight. Evaporation of the solvent gave a viscous liquid containing some solid. The product was divided into two halves and each half was dissolved in a minimum volume of water saturated with butanol. The solution was applied to a Sephadex LH-20 column (112.5 g, 2.5 × 90 cm), prepared according to a modification of Norman's procedure (8) using a reversed phase system of water and butanol. The column was developed with water saturated with butanol, and fractions were identified by TLC. Sarcosyltaurine was eluted first (125–305 ml) followed by the product (335–1000 ml), while lauric acid was strongly retarded. The product-containing fractions were pooled and lyophilized to give a translucent glass. Examination by TLC showed the presence of two Cl_2/KI positive spots (R_f^{I} 0.25 and 0.37, R_f^{II} 0.25 and 0.45). In an effort to remove the slower moving material, the product was dissolved in 50% ethanol and the solution was applied to a Dowex 50W × 1 column (Na form, 2.5 × 38 cm). Elution with 50% ethanol gave peptide-containing material (50–100 ml). These fractions were pooled and lyophilized to yield a white crystalline powder, sodio-laurylsarcosyltaurine, mp 190–192°C, 610 mg (32%), R_f^{I} 0.34, R_f^{II} 0.42.

Analysis: $C_{17}H_{33}N_2O_5SNa$;

calculated: C, 51.00; H, 8.25; N, 7.00; S, 8.00

found: C, 50.87; H, 8.21; N, 7.11; S, 7.81

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