Synthesis of Long Chain Fatty Acid Amides of Amino Acids[1]

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The preparation of long chain fatty acid amides of sphingosine and dihydrosphingosine in a nonaqueous medium was reported in an earlier communication.[2] This procedure has been successfully extended to the synthesis of several long chain fatty acid amides of serine and its methyl ester. *N*-palmitoylserine has been made previously, but no information was given concerning its preparation and physical properties.[3] In the present method, the amino acid was converted to the methyl ester which was allowed to react with the acyl chloride in dimethylformamide in the presence of pyridine as acid acceptor. The resulting *N*-acylamino acid methyl ester yielded the free acid after mild saponification. The difficulties encountered in the long chain acylation of monoamino polycarboxylic acids by Jungermann *et al.*[4] were obviated in this system as illustrated in the preparation of *N*-palmitoylglutamate. Advantages of the present nonaqueous procedure are (1) reasonable yields, (2) avoidance of emulsions, (3) ease of isolation of the product, particularly with increasing chain length of the fatty acid moiety, and (4) elimination of the necessity for *p*H regulation of the reaction mixture.[4]

EXPERIMENTAL

N-Acylamino acid methyl ester. DL-Serine methyl ester hydrochloride, m.p., 133–135°, was prepared according to the method of Fischer and Suzuki.[5] The stoichiometric amount of sodium methoxide in methanol[5] was added to 4.0 g. (25.7 mM) of DL-serine methyl ester hydrochloride and, after the addition of one-sixth volume of ether, the reaction mixture was successively chilled, filtered and concentrated to a sirup. To the resulting free base in 35 ml. of dimethylformamide and 2 ml. of pyridine were added 23.1 mM of the acyl chloride[2] (*trans*-2-hexadecenoyl chloride was prepared from the corresponding acid according to the procedure of Shapiro *et al.*[6]) in 15 ml. of dimethylformamide. After standing at room temperature for 1 hr., the reaction mixture was chilled overnight at 5°. The precipitate was removed by suction filtration and crystallized successively from petroleum ether, b.p., 60–70°, and methanol (Table I).

The L-glutamic acid dimethyl ester hydrochloride was prepared in essentially the same manner as was the serine compound by saturating 70 ml. of anhydrous methanol containing 5.0 g. of L-glutamic acid with dry HCl. After concentrating the reaction mixture below 50°, the residue was reconcentrated upon the addition of 50 ml. of methanol and dried *in vacuo* over phosphorus pentoxide and potassium hydroxide. The free base was liberated from the hydrochloride and reacted with the acyl chloride in exactly the manner as was described for the corresponding serine methyl ester (Table I).

TABLE I

Compound	Yield, %	M.P.	Analysis: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
N-trans-2-hexadecenoyl-DL-serine methyl ester	51	79–80	67.55	67.55	10.50	10.54	3.94	3.82
N-palmitoyl-DL-serine methyl ester	67	86–87	67.17	67.44	11.00	11.08	3.92	3.82
N-stearoyl-DL-serine methyl ester	70	90–91	68.51	68.34	11.25	11.31	3.63	3.66
N-palmitoyl-L-glutamic acid dimethyl ester	47[a]	73–74	66.77	67.03	10.49	10.64	3.39	3.40
N-trans-2-hexadecenoyl-DL-serine	88	122–123	66.81	66.79	10.34	10.38	4.10	4.00
N-palmitoyl-DL-serine	91	108–109	66.42	67.13	10.86	11.03	4.08	3.97
N-stearoyl-DL-serine	94	110–111	67.86	67.91	11.13	11.30	3.77	3.68
N-palmitoyl-L-glutamic acid	87	104–106	65.40	66.09	10.20	10.20	3.63	3.67

[a] Calculated on the basis of the L-glutamic acid employed in the preparation of the dimethyl ester; see text.

N-acylamino acid. One g. of the *N*-acylamino acid methyl ester was dissolved in 100 ml. of methanol followed by the addition of 10 ml. of *N* NaOH. A precipitate formed after several minutes and the reaction mixture was allowed to stand overnight at room temperature. After acidification with 6*N* HCl, the solution was chilled and the precipitate, removed by suction filtration, was crystallized from 95 ml. of 85% ethanol containing 2 ml. of 6*N* HCl and then from 100 ml. of 95% ethanol; *N*-palmitoyl-L-glutamic acid was crystallized from 50% ethanol (Table I.). All of the *N*-acylamino acids gave negative ester[7] and ninhydrin (conducted in 95% ethanol) reactions.

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Preparation of Chenodeoxycholic Acid

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During the course of our work on the isolation of bile acids, it became necessary for us to obtain

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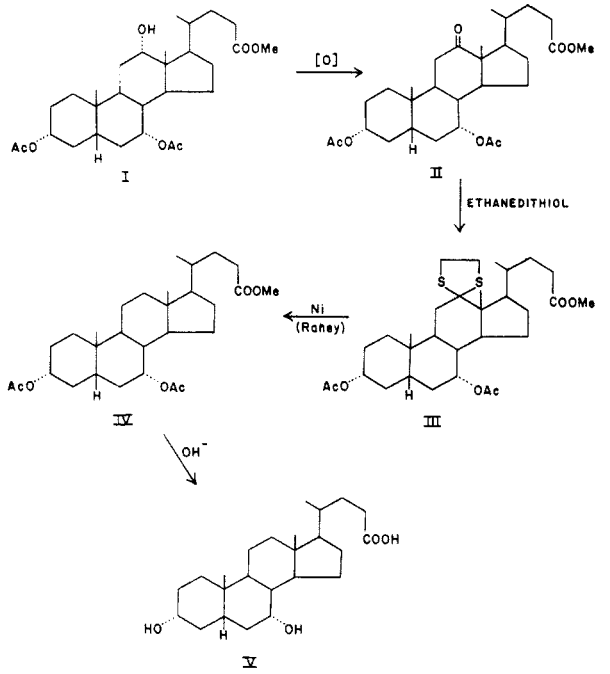
pure specimens of chenodeoxycholic acid. Although the procedure of Fieser and Rajagopalan[2] seemed quite satisfactory for the production of this acid, we wished to explore the possibilities of the Raney nickel desulfurization of the thioketals in connection with another project where somewhat milder conditions were desired.

Accordingly methyl cholate 3,7-diacetate (I)[2] was oxidized with Kiliani's reagent[3] in acetone to the 12-oxo derivative II and after conversion to the corresponding 12-thioketal III with ethanedithiol, it was desulfurized successfully with Raney nickel to the acetate methyl ester of chenodeoxycholic acid (IV). Subsequent hydrolysis of IV



yielded chenodeoxycholic acid (V) of high purity. Product yield at each step was excellent, being no less than 95% in any of the individual operations. Paper chromatograms[4] run on each product revealed no detectable impurity.

Although an extra step[2] is involved in its preparation, it would seem that this is the preferred method for the preparation of chenodeoxycholic acid.

EXPERIMENTAL[5]

Methyl 3α,7α-diacetoxy-12-oxocholanate (II). A solution of 2 g. of methylcholate 3,7-diacetate (I)[2] in 20 ml. of acetone was treated dropwise with 2.5 ml. of Kiliani's reagent[3] until a slight permanent orange color was obtained. After standing for 5 min., several drops of water were added and the resulting greenish oily droplets removed by filtration. The addition of 1 ml. of *N* sodium hydroxide and 80 ml. of water to the clear filtrate resulted in a copious crystallization of needles of m.p. 176–178°; yield 1.984 g. (99%). An analytical sample recrystallized from methanol afforded a product of m.p. 178–179°, $[\alpha]_D^{20}$ +73.5° (dioxane).

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.02; H, 8.79. Found: C, 68.76; H, 8.97.

Methyl 3α,7α-diacetoxy-12-oxocholanate 12-ethylenethioketal (III). To the above 12-oxo derivative II (1.5 g.), dissolved in 2 ml. of ethanedithiol by warming for a short time in the water bath, was added 2 ml. of boron fluoride ethyl ether. After standing for 15 min., 3 ml. of 1*N* sodium hydroxide was added while cooling in an ice bath and the mixture was extracted with ether. The ethereal solution was washed with dilute sodium hydroxide and water and dried over anhydrous sodium sulfate. After removal of the solvent the residual solid crystallized from methanol as plates, m.p. 174–176°; yield 1.64 g. (95%).

A recrystallized analytical specimen melted 175–176°, $[\alpha]_D^{20}$ +65.8° (dioxane).

Anal. Calcd. for $C_{31}H_{48}O_6S_2$: C, 64.15; H, 8.33; S, 11.04. Found: C, 64.45; H, 8.61; S, 11.09.

Methyl chenodeoxycholate diacetate (IV). One gram of 12-ethylenethioketal III was dissolved in 70 ml. of absolute ethanol and refluxed for 8 hr. with Raney nickel (5 teaspoons). The catalyst was removed by filtration and washed repeatedly with alcohol. Upon removal of the solvent (*in vacuo*) from the combined filtrate, the residue was obtained as fine needles, m.p. 124–128°; yield 840 mg. (100%). Recrystallization from methanol yielded 756 mg. (90%) needles which melted at 128–130°; analytical sample, m.p. 129–130.5°,[6,7] $[\alpha]_D^{20}$ +12.5° (dioxane).

Anal. Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 71.11; H, 9.50.

Chenodeoxycholic acid (V). Five hundred mg. of the above ester IV was hydrolyzed with 80 ml. of ethanolic 5% potassium hydroxide for 4 hr. After partial concentration of the volume and addition of water, the reaction product was acidified with hydrochloric acid. The resulting precipitate was collected, dried, and crystallized from ethyl acetate. A quantitative crop (400 mg.) of prisms melting at 143–145° were obtained. Recrystallization from the same solvent yielded a product of m.p. 145–146°, $[\alpha]_D^{20}$ +10.7° (dioxane).

Anal. Calcd. for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found: C, 73.49; H, 10.31.

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(2) L. F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **72**, 5530 (1950).

(3) H. Kiliani, *Ber.*, **46**, 676 (1913). A solution of 53 g. of chromium trioxide and 80 g. of concentrated sulfuric acid in 400 g. of water. We have found this reagent an extremely useful oxidant for certain alcohols [Y. Sato and H. G. Latham, Jr., *J. Org. Chem.*, **22**, 1946 (1957); *J. Am. Chem. Soc.*, **78**, 3146 (1956)]. Yields are generally excellent and the reaction extremely rapid.

(4) We are indebted to Dr. E. Weiss of this institute for the paper chromatograms. The method of Sjövall [*Arkiv Kemi*, **8**, 299 (1955)] was used for the free acid. The procedure for the acetate esters will be published by him elsewhere.

(5) All melting points were taken on the Kofler block and are uncorrected. Microanalyses are from the institute's service analytical laboratory under the direction of Dr. W. C. Alford.

(6) Fieser and Rajagopalan[2] report m.p. 96–98°, $[\alpha]_D^{25}$ +20 ± 2° for this compound.

(7) I. D. P. Wootton and H. S. Wiggins, *Biochem. J.*, **55**, 292 (1953) report m.p. 127–128°, $[\alpha]_D^{20}$ +14° (chlf).