The results showed the 1,4-diazabicyclo[2.2.1]heptane structure proposed by Pettit.[2,3]

Two cycles of isotropic least squares for all atoms reduced the R factor to 18% and one cycle of full-matrix anisotropic least squares gave an R value of 14.6%. Bond distances and angles are given in Figure 1 (a second set of data taken with Ni-filtered Cu radiation gave an R factor of 13.1% after one cycle of anisotropic least squares). A list of atomic coordinates for this structure is given in Table I.

New Compounds

Synthesis of Enantiomeric Chloroacetylcarnitine Chlorides

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Convincing experimental evidence exists for the role of (*R*)-(−)-carnitine in enzyme-mediated transport of activated acyl groups across mitochondrial and possibly other membranes.[1-6] Further, the structural similarity of carnitine (**1**) and acetylcarnitine (**2**) to choline and acetylcholine, respectively, the possible biotransformation of **1** and **2** to β-methylcholine,[7] and the use of **1** in the clinic,[8] point to possible therapeutic potential[9] and/or pharmacologic utility of these types of biological molecules or related derivatives. We report a convenient synthesis of (*R*)-(−)-, (*S*)-(+)-, and racemic chloroacetylcarnitine chlorides (**3**),[10] which have been investigated for cholinergic activity,[12,13] and in tissue culture.[14]

$$(CH_3)_3N^+CH_2CHO{-}X$$
$$\qquad\qquad\quad |$$
$$\qquad\qquad CH_2COO^-$$

1, X = H
2, X = $COCH_3$
3, HCl, X = $COCH_2Cl$

(1) G. Wolf, Ed., "Recent Research on Carnitine," M.I.T. Press, Cambridge, Mass., 1965, Parts II and III, and ref cited therein.

(2) I. B. Fritz and N. R. Marquis, *Proc. Nat. Acad. Sci. U. S.*, **54**, 1226 (1965).

(3) K. R. Norum and J. Brenner, *J. Biol. Chem.*, **242**, 407 (1967).

(4) J. F. A. Chase, *Biochem. J.*, **104**, 510 (1967).

(5) A. M. Snoswell and G. D. Henderson, *ibid.*, **119**, 59 (1970).

(6) B. Wittels and P. Hochstein, *J. Biol. Chem.*, **242**, 126 (1967).

(7) E. A. Khairallah and G. Wolf, *ibid.*, **242**, 32 (1967).

(8) E. Gravina and G. Gravina-Sanvitale, *Clin. Chim. Acta*, **23**, 376 (1969).

(9) E. A. Hosein, S. J. Booth, I. Gasoi, and G. Kato, *J. Pharmacol. Exp. Ther.*, **156**, 565 (1967).

(10) (*R*)-(−)-Bromoacetylcarnitine has been synthesized,[11] but the yield was not reported and purity was ascertained only by chemical assay and by tlc. This derivative in the presence of CoA has been demonstrated to be a reversible inhibitor of acetyl-CoA:L-carnitine *O*-acetyltransferase [E.C. 2.3.1.7]; in the absence of CoA it is an irreversible inhibitor, which is postulated to act by an active-site-directed mechanism.[11]

(11) J. F. A. Chase and P. K. Tubbs, *Biochem. J.*, **116**, 713 (1970).

(12) R. T. Louis-Ferdinand, K. R. Cutroneo, D. C. Kosegarten, R. C. Vasavada, J. G. Turcotte, and D. R. DeFanti, *J. Pharm. Pharmacol.*, **22**, 704 (1970).

(13) K. R. Cutroneo, R. T. Louis-Ferdinand, R. C. Vasavada, J. G. Turcotte, and D. R. DeFanti, *ibid.*, **22**, 940 (1970).

(14) (*RS*)-**3**, (*R*)-(−)-**3**, and (*S*)-(+)-**3** showed modest and comparable inhibition of murine leukemic lymphoblast (L5178Y) growth in culture, indicating that these quaternary ammonium salts may cross the plasma membrane; radioactive **1** has been shown to be progressively taken up by intact Ehrlich ascites tumor cells.[15]

(15) A. A. Spector, *Arch. Biochem. Biophys.*, **122**, 55 (1967).

Experimental Section[16]

(*R*)-(−)-**Chloroacetylcarnitine Chloride (3).**—A mixt of 1.0 g (0.005 mole) of (*R*)-(−)-carnitine chloride, 0.9 g (0.005 mole) of chloroacetaic anhydride, and 0.1 g of *p*-TsOH was stirred at 70–75° for 75 min. The syrupy reaction mixt then was cooled to 25°, washed with Et_2O (3 × 5 ml), and taken up in 3.5 ml of *i*-PrOH. After standing 2–3 hr at 25°, and overnight at 5°, 0.85 g (62%)[18] of a cryst product was obtd. One recrystn from EtOH–*i*-PrOH afforded white crystals: mp 186–188°; $[\alpha]^{22}D$ −27.7° (*c* 8.03, H_2O); tlc (silica) R_f 0.07, CH_3CN–CH_3OH–NH_3 (10:5:2); ir (μ, Nujol) 5.69 (C=O, ester), 5.89 (C=O, acid); pmr (δ, D_2O) 2.91 (2, d), 3.2 (9, s), 3.81 (2, m), 4.32 (2, s), 5.67 (1, m). *Anal.* ($C_9H_{17}Cl_2NO_4$), C, H, N, Cl.

(*S*)-(+)-**Chloroacetylcarnitine chloride** (**3**) was obtained in 62% yield:[18] mp 186–188°; $[\alpha]^{22}D$ +29.8° (*c* 8.89, H_2O). *Anal.* C, H, N, Cl.

(*RS*)-**Chloroacetylcarnitine chloride** (**3**) with *p*-TsOH·H_2O as catalyst was obtained in 66% yield,[18] mp 179°. *Anal.* C, H, N, Cl.

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(16) (*R*)-(−)-Carnitine chloride, $[\alpha]^{22}D$ −21.7° (lit.[17] −23.7°), (*S*)-(+)-carnitine chloride, $[\alpha]^{22}D$ +23.1° (lit.[17] +23.6°), and (*RS*)-carnitine chloride were obtd from Nutritional Biochemicals Co., Cleveland, Ohio 44128. (*R*)-(−)-**3**, (*S*)-(+)-**3**, and (*RS*)-**3** were prepd using the same method and only details of the synthesis of (*R*)-(−)-**3** are given. Optically active precursors and products were found to be extremely hygroscopic, and it was necessary to use *anhyd* *p*-TsOH as catalyst and to scrupulously exclude moisture in order to obtain cryst products—operations requiring moisture-free conditions were carried out in a Labconco controlled atm glove box. The ir and pmr spectra of each compd were consistent with the expected structure and are reported for (*R*)-(−)-**3**. Melting points were detd with a Thomas-Hoover Uni-Melt capillary melting point apparatus and are uncorrected. Where analyses are indicated only by the symbols of the elements, anal. results obtained for those elements were within ±0.4% of the theor values; analyses by Micro-Analysis, Inc., Marshallton, Wilmington, Del.

(17) G. Kato and E. A. Hosein, *Can. J. Chem.*, **47**, 1177 (1969).

(18) Yields by this method approach those reported[19] using three different methods designed to improve yields of O-acylation of (*RS*)-carnitine chloride.

(19) H. J. Ziegler, P. Bruckner, and F. Binon, *J. Org. Chem.*, **32**, 3989 (1967).

3-Amino-4-hydroxy-L(−)-butyramide Hydrochloride[1]

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In the current investigation of compounds related to asparagine for antitumor activity, 3-amino-4-hydroxy-

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L(−)-butyramide·HCl[2] was synthesized for testing as an inhibitor of L-asparagine synthetase.

The conversion of L-asparagine to its *N*-Cbz derivative and subsequent esterification were achieved by adaptations of known procedures. Reduction of the latter blocked L-asparagine methyl ester was effected by $Ca(BH_4)_2$,[3] a less commonly known reagent. Attempts to reduce either L-asparagine, *N*-carbobenzoxy-L-asparagine, or *N*-carbobenzoxy-L-methyl ester with LAH were unsuccessful.

Experimental Section[4]

***N*-Carbobenzoxy-L-asparagine.**[5]—To a mixt of 39.6 g (0.30 mole) of L-Asp and 25.8 g (0.64 mole) of MgO in 300 ml of H_2O was added in 4 portions at 5° 51.0 g (0.30 mole) of carbobenzoxy chloride. After stirring 15 min longer, the thick reaction mixt was stirred for 3 hr at room temp, acidified with 2 *N* HCl, and filtered, and the solid was washed with H_2O, giving 66.2 g of air-dried product, mp 158–161°. The entire amt was recrystd from 700 ml of MeOH to yield 36.7 g, mp 164–165° (lit.[5] mp 165°). Recrystn in the same manner of the residue obtained from concn of the mother liquors to dryness gave 9.4 g, mp 165°. Again concn of the mother liquors and recrystn of the solid thereof led to 8.3 g, mp 164–166°, for a total yield of 54.5 g (74.5%).

***N*-Carbobenzoxy-L-asparagine Methyl Ester.**—A suspension of 30 g (0.113 mole) of *N*-Cbz-L-Asp in 800 ml of 0.1 *N* MeOH–HCl was stirred overnight at room temp. The resulting soln was concd to dryness, the residue was triturated with Et_2O, and the ester was filtered off and air-dried to yield 31.6 g (100%), mp 150° (lit.[5] mp 150°).

3-Carbobenzoxyamino-4-hydroxy-L-butyramide.—A mixt of anhyd $CaCl_2$, 55.5 g (0.5 mole), and $NaBH_4$, 76 g (1.0 mole), in 1000 ml of abs EtOH was stirred for 1 hr at −10° to −20°. *N*-Cbz-L-Asp-OMe, 25 g (0.1 mole), was added and stirring was contd at the same temp for 4 hr after which 100 ml of H_2O was added dropwise at 0–5°. The mixt was stirred for 30 min, acidified with concd HCl to congo red, and concd to dryness *in vacuo*. The residue was stirred vigorously on the steam bath with 1000 ml of H_2O, and the mixt was filtered. From the cooled filtrate 15.6 g (69.3%) of 3-carbobenzoxyamino-4-hydroxy-L-butyramide was obtd, mp 142–144°. A mmp of the product with starting material showed a depression of 50°: nmr (60 MHz, DMSO-d_6), δ 2.33 ppm (d, 2, CH_3CO), 3.41 (t, CCH_2O), 3.92 (sextet, 1, CCNHC), 4.80 (t, 1, OH), 5.07 (s, 2 $PhCH_2O$), ~6.9 (broad s, $CONH_2$), ~7.0 (d, CONHC), 7.40 (s, Ph).

3-Amino-4-hydroxy-L(−)butyramide·HCl.—A soln of 15.6 g (0.0618 mole) of 3-carbobenzoxyamino-4-hydroxy-L-butyramide in 780 ml of MeOH was hydrogenolyzed for 4 hr at room temp using 3.12 g of 10% Pd/C. The filtered soln was concd *in vacuo* to about 250 ml and again filtered to remove trace impurities. The clear filtrate was further concd to an oil which was dissolved in 10 ml of H_2O, acidified with concd HCl, and filtered. An addl 10 ml of H_2O was used for the transfer. Me_2CO (400 ml) was added and the cloudy soln stored at 5° overnight. After decantn of the supernatant phase, the semisolid residue was stirred with 400 ml of Me_2CO for 2 hr at 5°. The filtered crude product was very hygroscopic. It was recrystd from 200 ml of MeOH, stored at 5° overnight, and filtered, and the product was washed with cold MeOH and Et_2O. After drying over P_2O_5 (0.1 mm) (25°, 18 hr), the yield was 2 g. *Anal.* ($C_4H_{11}N_2O_2Cl$) C, H, N, Cl. The residue from the concn of the mother liquors and washes to dryness was recrystd in the same manner from 200 ml of EtOH to give an addl 2.5 g. *Anal.* ($C_4H_{11}N_2O_2Cl$) C, H, N, Cl. The total yield of pure material was 4.5 g (47.1%): mp 134–135°; $[\alpha]^{25}D$ −7.6° (*c* 1.0, H_2O); ir spectrum supported the proposed structure; nmr (60 MHz, D_2O) δ 2.6–2.8 (m, 2, CH_2CO) and 3.5–4.0 ppm (m, 3, OCH_2 and CH), remaining active proton sites of the molecule are deuterated in this medium.

(2) During the prepn of the manuscript, M. R. Harnden and T. O. Yellin reported the synthesis of L(−)-3-amino-4-hydroxybutyramide trifluoroacetate by a different route as one of a series of agents for evaluation as inhibitors of L-asparaginyl-tRNA lygase. The compd was not fully characterized. *J. Med. Chem.*, **13**, 1095 (1970).

(3) J. Kollonitsch, O. Fuchs, and V. Gábor, *Nature (London)*, **175**, 346 (1955).

(4) Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncor. Analyses indicated only by the symbols of the elements were within ±0.3% of the theor values. The ir spectra were obtained using a Perkin-Elmer Model 621 recording spectrophotometer, and a Varian A60 spectrometer was used to obtain the nmr spectra. The authors are grateful to Dr. A. W. Douglas for the ir and nmr spectra and to Mr. R. N. Boos and associates for the elemental anal.

(5) M. Bergman and L. Zervas, *Ber.*, **65**, 1192 (1932).

Solid Phase Synthesis of [4-Proline]oxytocin, [4-Proline]mesotocin, [4-Proline]glumitocin, and [4-Lysine]mesotocin

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In an attempt to establish the identity of a postulated evolutionary intermediate between the 4-serine-containing and 4-glutamine-containing neurohypophysial hormones, the following 4-substituted analogs, [4-proline]oxytocin, [4-proline]mesotocin, [4-proline]glumitocin, and [4-lysine]mesotocin were synthesized. The pharmacological properties of these analogs have been presented elsewhere.[1,2] This communication describes the solid phase synthesis of these analogs *via* their respective protected nonapeptide intermediates. The methods used were essentially the same as those described previously for the synthesis of oxytocin[3] and [4-threonine]oxytocin.[4] The physical characteristics of all of these compds together with the individual yields obtained are presented in Tables I and II.

Experimental Section[5]

***N*-BOC-*S*-benzyl-L-Cys-*O*-benzyl-L-Tyr-L-Ile-L-Pro-L-Asp-*S*-benzyl-L-Cys-L-Pro-L-LeuGly-NH_2 (1).**—BOC-glycyl resin (5.0 g, 0.805 mmole of glycine) was treated in an 8-cycle procedure as described for the synthesis of (4-threonine)oxytocin,[4] except that BOC-L-proline was used in the sixth incorporation step to give the protected nonapeptide resin, weight 6.0 g. Ammonolytic cleavage of the protected nonapeptide resin (3.0 g) was carried out as described for [4-threonine]oxytocin and the protected peptide was extd with DMF and MeOH. Solvents were removed *in vacuo*, and the residue was purified by trituration with 95% EtOH (30 ml) to give **1** as an amorphous white powder, weight 550 mg (Table I). Amino acid analysis gave Asp, 1.00; Leu, 1.00; Gly, 1.08; Bzl-Cys, 2.08; Ile, 0.99; Tyr, 0.82; Pro, 2.00; NH_2, 2.30.

[4-Proline]oxytocin (2).—1 (142 mg) was reduced, reoxidized, and purified by the procedure used in the synthesis of [4-threonine]oxytocin. **2** was obtained as a fluffy white powder, weight 41.5 mg, shown to be homogeneous by tlc and paper electrophoresis at different pH values as described for [4-threonine]-oxytocin (Table II). Amino acid analysis gave: Asp, 1.00; Gly, 1.00; Pro, 2.16; Cys, 1.96; Ile, 0.95; Tyr, 0.94; Leu, 1.05; NH_3, 2.12.

***N*-BOC-*S*-benzyl-L-Cys-*O*-benzyl-L-Tyr-L-Ile-L-Pro-L-Asp-*S*-benzyl-L-Cys-L-Pro-L-IleGly-NH_2 (3).**—BOC-glycyl resin (5.0 g,

(1) W. H. Sawyer, T. C. Wuu, J. W. M. Baxter, and M. Manning, *Endocrinology*, **85**, 385 (1969).

(2) W. H. Sawyer and M. Manning, *J. Endocrinol.*, **49**, 151 (1971).

(3) M. Manning, *J. Amer. Chem. Soc.*, **90**, 1348 (1968).

(4) M. Manning, E. Coy, and W. H. Sawyer, *Biochemistry*, **9**, 3925 (1970).

(5) The abbreviations used for amino acids and protecting groups are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **241**, 2491 (1966); *Biochemistry*, **5**, 1445, 2485 (1966).