of Turner[27] who showed that although the difference in energy between *cis*- and *trans*-decalin is 2.4 kcal. per mole, introducing an angular methyl group reduces the difference to 0.8 kcal., per mole.[28]

(27) R. B. Turner, THIS JOURNAL, **74**, 2118 (1952).

(28) See D. Barton, *Experientia*, **6**, 316 (1950), for a related discussion of the stereoisomerism found in sterols.

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[CONTRIBUTION FROM ABBOTT LABORATORIES]

The Preparation of Some N-Carbethoxyamino Acids

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Fifteen N-carbethoxyamino acids have been synthesized and examined for antiviral activity. Some have been tested as oncolytic agents as well, but none has proved to be of significant value.

In the course of screening compounds for antiviral activity, it was found that N-carbobenzoxy and N-carbethoxyglycylaminomalonic acid diethyl esters seemed to have a slight virus inhibiting effect.[1] It appeared possible that the malonic ester residue was unnecessary and that the N-substituted amino acids were functioning as antimetabolites against essential amino acids. This theory was tested by the synthesis of N-carbethoxy derivatives of some of the essential amino acids, and those from valine and phenylalanine showed some apparent activity.[1] This stimulated the synthesis of a whole series of such derivatives, but the inhibitory effects first observed were not later substantiated and none of the members showed antiviral activity of any significance.[1,2]

The carbethoxyamino acids are urethans and therefore were of interest in their effect on malignancies.[3] Those tested showed no inhibition of mouse sarcoma or leukemia.

Most of the amino acids used were the synthetic racemates and formed solid carbethoxy derivatives. It is of interest that when the *l*-form of the amino acid was used the product was frequently an oil.

Some of these N-carbethoxyamino acids have been prepared before by various methods.[4] The procedure employed here made use of the sodium salt of the amino acid rather than its ester and a second mole of alkali was furnished by sodium carbonate in the mixture from the beginning of the reaction. Equimolar quantities of α-amino acid, ethyl chloroformate, sodium hydroxide and sodium carbonate were used in the preparation of all the derivatives reported except that of lysine (in which case two moles of ethyl chloroformate and sodium carbonate were employed). It is noteworthy that the doubly substituted derivative of tyrosine as well as that of lysine was obtained, but that only monocarbethoxy derivatives were formed from tryptophan and serine. Apparently the hydrogens of the hydroxyl group of serine and the indole function of tryptophan are not sufficiently acidic to react with ethyl chloroformate under these reaction conditions.

The results obtained were fairly good with most of the α-amino acids used except *l*-arginine, *l*-histidine and *l*-cystine.[5] In these cases, the products isolated from the reaction of ethyl chloroformate with the amino acids were insufficiently pure to be reported.

Experimental[6]

N-Carbethoxyglycine.—Thirty-two grams (0.8 mole) of sodium hydroxide and 85 g. (0.8 mole) of sodium carbonate were dissolved in 500 ml. of water, stirred and cooled to 20° in an ice–salt-bath. To this solution was added 60 g. (0.8 mole) of glycine, and stirring and cooling were continued until the solution temperature dropped to 9.5°. Ethyl chloroformate, 87 g. (0.8 mole), was then added dropwise to the stirred solution at such a rate that the temperature did not rise above 10.5°. After stirring another hour in the cold and an additional two hours at room temperature, the reaction mixture was acidified by the careful addition of 100 ml. of concentrated hydrochloric acid solution, bringing the *p*H to about 4.

The resulting solution was concentrated to a sirup *in vacuo* on the steam-bath, and then to a sticky solid in an open evaporating dish. This residue was triturated portionwise with about a liter of ether; the ethereal solution dried by anhydrous magnesium sulfate, filtered and concentrated to about 100 ml. Addition of an equal volume of petroleum ether, b.p. 63–68°, caused the formation of an oil which slowly crystallized. The solid was separated by filtration, washed well with petroleum ether and dried; m.p. 73–74°,[7] yield 78 g. (66%).

By the same procedure as above (*i.e.*, equimolar quantities of amino acid, ethyl chloroformate, sodium hydroxide and sodium carbonate in water) at the temperature indicated, the following carbethoxy derivatives were prepared. The quantity of amino acid used, individual variations in working up the reaction mixture and purifying the product are given.

N-Carbethoxy-*dl*-alanine.—Reaction temperature 9 to 15°; 0.5 mole. The product was isolated by evaporation of the acidified reaction mixture and extraction of the residue with ether. The dried ethereal extract was evaporated to a sirup which slowly crystallized. The product was tritu-

(1) Private communication from C. J. Rickher, the late Dr. H. W. Cromwell and co-workers of Abbott Laboratories.

(2) Private communication from Dr. R. N. Bieter, University of Minnesota.

(3) E. Paterson, I. A. Thomas, A. Haddow and J. M. Watkinson, *Lancet*, [1], 677 (1946).

(4) (a) A. Hantzsch and W. V. Metcalf, *Ber.*, **29B**, 1680 (1896); (b) E. Fischer and E. Otto, *ibid.*, **36B**, 2108 (1903); (c) E. Fischer and W. Axhausen, *Ann.*, **340**, 123 (1905); (d) T. Curtius and W. Sieber, *Ber.*, **55B**, 1543 (1922); (e) L. Havestadt and R. Fricke, *ibid.*, **57B**, 2048 (1924); (f) E. Abderhalden and K. Kautzsch, *Z. physiol. Chem.*, **68**, 487 (1910).

(5) R. A. Gortner and W. F. Hoffman, *J. Biol. Chem.*, **72**, 433 (1927), report tetracarbethoxy-*l*-cystine as a deliquescent brown powder.

(6) All melting points are corrected.

(7) Reference 4a gives m.p. 67–69°; 4b, m.p. 75°.

rated with petroleum ester, filtered and dried; m.p. 80–81.5°,[8] yield 46%.

Anal. Calcd. for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.99; H, 6.81; N, 8.74.

N-Carbethoxy-*dl*-valine.—Reaction temperature 7 to 9°; 0.4 mole. The crude product separated as an oil on acidification of the reaction mixture, and was extracted with ether. The ethereal extract was dried by magnesium sulfate and decolorized by charcoal. Evaporation of the ethereal solution left an oily residue which turned to a semi-solid and was triturated with petroleum ether, filtered and dried; m.p. 56–58°, yield 70%.

Anal. Calcd. for $C_8H_{15}NO_4$: C, 50.78; H, 7.99; N, 7.41. Found: C, 50.84; H, 8.24; N, 7.24.

N-Carbethoxy-*l*-leucine.—Reaction temperature 8 to 15°; 0.15 mole. The product separated as an oil and was worked up in the same way as the *dl*-valine derivative except that the oily residue left after evaporation of the ethereal extract did not solidify, yield 80%.

Anal. Calcd. for $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89. Found: C, 53.26; H, 8.13; N, 6.85.

N-Carbethoxy-*dl*-isoleucine.—Reaction temperature 9 to 18°; 0.15 mole. Acidification of the reaction mixture caused a colorless oil to separate. The mixture was extracted with ether; the extract dried, filtered and concentrated to an oil which slowly crystallized. Trituration of the oil with petroleum ether, followed by filtration and drying gave a 61% yield of product melting at 91–93°. This was further purified by recrystallization from dilute alcohol; m.p. 93–95°.

Anal. Calcd. for $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89. Found: C, 53.19; H, 8.48; N, 7.04.

N-Carbethoxy-*dl*-norleucine.—Reaction temperature 6°; 0.15 mole. A solid formed on acidification of the reaction mixture. This was separated by filtration and added to the ethereal extract of the filtrate. Evaporation of the dried ethereal solution gave a slowly crystallizing oil which was triturated with petroleum ether, filtered and dried; m.p. 56–57°, yield 86%.

Anal. Calcd. for $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89. Found: C, 53.06; H, 8.29; N, 7.00.

N-Carbethoxy-*dl*-phenylalanine.—Reaction temperature 9 to 9.5°; 0.15 mole. The product separated as an oil and was worked up in the same way as the *dl*-isoleucine derivative except that the product was satisfactory after trituration with petroleum ether; m.p. 92–94°, yield 85%.

Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.78; H, 6.37; N, 5.91. Found: C, 60.86; H, 6.28; N, 6.08.

N-Carbethoxy-*dl*-methionine.—Reaction temperature 8 to 10°; 0.25 mole. On acidification of the reaction mixture, the product separated as an oil and was extracted with ether. The dried ether extract was evaporated to an oil which did not solidify, yield 98%.

Anal. Calcd. for $C_8H_{15}NO_4S$: C, 43.42; H, 6.83; N, 6.33. Found: C, 43.61; H, 6.66; N, 6.10.

N-Carbethoxy-*dl*-aspartic Acid.—Reaction temperature 10°; 0.25 mole. The water-soluble product was removed from the acidified reaction mixture by continuous ether extraction for 16 hours. The dried ethereal extract was evaporated to a solid which was purified by solution in ether and evaporation of the solution until a solid again formed; m.p. 139.5–141°, yield 42%.

Anal. Calcd. for $C_7H_{11}NO_6$: C, 40.98; H, 5.40; N, 7.17. Found: C, 41.15; H, 5.36; N, 6.97.

N-Carbethoxy-*l*-glutamic Acid.—Reaction temperature 5 to 10°; 0.25 mole. The water-soluble product was extracted from the acidified reaction mixture with ether. The dried, charcoal treated ethereal solution was evaporated to an oil which did not solidify, yield 70%.

Anal. Calcd. for $C_8H_{13}NO_6$: C, 43.83; H, 5.98; N, 6.39. Found: C, 44.89[9]; H, 5.88; N, 6.26.

N-Carbethoxy-*dl*-tryptophan.—Reaction temperature 5 to 10°; 0.25 mole. The oil which separated from the acidified reaction mixture was extracted with ether, the ethereal extract dried, decolorized with charcoal and evaporated to a heavy oil which slowly crystallized. The solid was triturated with water, filtered and dried; m.p. 106–109°, yield 85%. Recrystallization from ethyl acetate–petroleum ether gave material of m.p. 108–110°.

Anal. Calcd. for $C_{14}H_{16}N_2O_4$: C, 60.42; H, 5.84; N, 10.14. Found: C, 60.69; H, 5.74; N, 9.98.

N-Carbethoxy-*dl*-serine.—Reaction temperature 6°; 0.2 mole. The water-soluble product was extracted from the dried residue of the reaction mixture with ether. The dried, decolorized ethereal solution was evaporated to a very small amount of colorless oil, yield < 10%.

Anal. Calcd. for $C_6H_{11}NO_5$: C, 40.68; H, 6.26; N, 7.91. Found: C, 40.85; H, 6.30; N, 7.87.

N,O-Dicarbethoxy-*l*-tyrosine.—Equimolar quantities of reactants were used as usual; reaction temperature −2 to +2°; 0.15 mole. A solid separated upon acidification and was filtered off. The aqueous filtrate was dried and the two solids combined and triturated with ether. The dried ethereal extract was evaporated to a tan oil which solidified when triturated with petroleum ether. The solid, m.p. 76–86°, was taken up in ether and charcoal treated, then the solution filtered and evaporated to a solid of m.p. 90–91°; yield 48% based on the ethyl chloroformate.

Anal. Calcd. for $C_{15}H_{19}NO_7$: C, 55.38; H, 5.89; N, 4.31. Found: C, 55.55; H, 5.91; N, 4.44.

N$^{\alpha}$,N$^{\epsilon}$-Dicarbethoxy-*l*-lysine.—Two moles of ethyl chloroformate and sodium carbonate were used to one of *l*-lysine and sodium hydroxide. Reaction temperature 0°; 0.15 mole *l*-lysine. The oil which separated on acidification of the reaction mixture was continuously extracted with ether for 16 hours. The dried extract was filtered and evaporated to an oil which did not solidify, yield 96%.

Anal. Calcd. for $C_{12}H_{22}N_2O_6$: C, 49.64; H, 7.64; N, 9.65. Found: C, 49.84; H, 7.54; N, 9.56.

N-Carbethoxy-α-aminoisobutyric Acid.—This material was prepared by the method of Bergmann, *et al.*,[10] for N-carbobenzoxy-α-aminoisobutyric acid, starting with 0.2 mole of ethyl α-aminoisobutyrate hydrochloride. The water-soluble product was obtained by extraction of the dried reaction mixture with ether, and evaporation of the dried ethereal solution to an oil which slowly solidified; m.p. 78–80°, yield 52%, based on the starting ethyl α-aminoisobutyrate hydrochloride.

Anal. Calcd. for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.00. Found: C, 48.21; H, 7.53; N, 8.15.

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(8) Reference 4c gives m.p. 84°.

(9) The analytical value found was beyond the usual margin of error, but could not be improved by repeated charcoal treatment of an ethereal solution of the oil.

(10) M. Bergmann, L. Zervas, J. S. Fruton, F. Schneider and H. Schleich, *J. Biol. Chem.*, **109**, 325 (1935).