water. The filtered solution was extracted with benzene and then acidified with dilute sulfuric acid. The liberated cyclohexanecarboxylic acid was taken up in several portions of benzene, freed of solvent and warmed with 5 ml. of thionyl chloride. Treatment with 4 g. of aniline in benzene solution yielded the anilide, which was recrystallized from aqueous ethanol; yield 1.4 g., m.p. 147–148°.[10]

(10) A. M. Schwartz and J. R. Johnson, THIS JOURNAL, **53**, 1065 (1931), report m.p. 146° for cyclohexanecarboxanilide.

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Preliminary Investigations on the Preparation of Optically Active Peptides Using Mixed Carbonic–Carboxylic Acid Anhydrides

BY JAMES R. VAUGHAN, JR.

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The use of mixed carbonic–carboxylic acid anhydrides for the synthesis of peptides has been reported recently from this Laboratory[1] and independently from two European laboratories.[2] The general over-all equation for the reaction may be given by

$$\text{X-NHCH(R)COOCOOR''} + \text{H}_2\text{NCH(R')COOR''} \longrightarrow \text{X-NHCH(R)CONHCH(R')COOR''} + \text{R'''OH} + \text{CO}_2\uparrow$$

In our latest publication, the general nature of the reaction was demonstrated and it was reported that no racemization was observed in the preparation of simple, optically active dipeptide derivatives. The behavior of larger, optically active peptides, however, in which racemization may occur by mechanisms not operative in the case of dipeptides was not studied.

In the present work, the investigation of the retention of optical activity by this method of synthesis has been extended to a study of the reaction of amino acid esters with the mixed anhydrides of carbobenzoxy dipeptide acids in which the terminal amino acid having the free carboxylic function is optically active.

As model compounds, two dipeptide derivatives, carbobenzoxyglycyl-L-leucine and carbobenzoxyglycycl-L-phenylalanine, were examined. The first of these formed a toluene-soluble triethylamine salt and was caused to react with isobutylchlorocarbonate and then with methyl glycinate, by a modification of the method previously described,[1] to give methyl carbobenzoxyglycyl-L-leucylglycinate in 60% yield after purification. A 7% yield of the DL-isomer was also isolated.

The triethylamine salt of carbobenzoxyglycyl-L-phenylalanine, however, was only slightly soluble in toluene, and it was necessary to add a second solvent to effect solution. The use of toluene as the main solvent was desirable in order to obtain reaction temperatures of about −5° for the anhydride-forming step. When chloroform was used for this purpose and the reaction was carried through using isobutyl chlorocarbonate and ethyl glycinate in the usual manner, almost complete racemization occurred. Ethyl carbobenzoxyglycyl-DL-phenylalanylglycinate was obtained in 64% yield, whereas only 4% of the L-isomer was formed.

On further investigation, it was found that the amount of racemization observed could be greatly reduced by using the minimum amount of chloroform (1:8) necessary to solubilize the salt starting material and by reducing the time allowed for mixed anhydride formation to about 5 minutes. A summary of this work appears in the Experimental section.

When the use of chloroform in the anhydride-forming step was avoided and dioxane or tetrahydrofuran was used in its place, practically no racemization occurred. Thus, using a toluene–dioxane (5:2) solvent system in the above preparation, a 77% yield of ethyl carbobenzoxyglycyl-L-phenylalanylglycinate and only 2% of the DL-isomer was obtained after purification of all fractions. The use of dioxane alone as the solvent necessitated a slightly higher reaction temperature for the anhydride-forming step and resulted in a 64% yield of the L-isomer and 7% of the DL-form. The use of tetrahydrofuran alone, on the other hand, caused no detectable racemization and the pure L-isomer was isolated in 60% yield.[3]

As a check on the optical purity of ethyl carbobenzoxyglycyl-L-phenylalanylglycinate, the tripeptide was also prepared from a mixed isobutylcarbonate–carbobenzoxyglycine anhydride and ethyl L-phenylalanylglycinate. The product obtained was more difficult to purify than the one prepared from the carbobenzoxy dipeptide acid, but its optical rotation and melting point were in good agreement with those previously observed.

In connection with the above work, it was found that the over-all reaction time could be greatly shortened from that previously reported. Optimum time for anhydride formation at −5° is in the neighborhood of 5 to 10 minutes. However, this varies with the individual preparation. Also, after addition of an amino acid or peptide ester to a solution of the preformed mixed anhydride, the amide-forming reaction may be completed rapidly by heating the reaction mixture to reflux and then cooling.

Experimental[4]

Methyl Carbobenzoxyglycyl-L-leucylglycinate.—A solution of 3.22 g. (0.01 mole) of carbobenzoxyglycyl-L-leucine,[5] m.p. 99–100°, $[\alpha]^{25}\text{D}$ −9.5 ± 0.4° (*c* 5, ethanol), and 1.02 g. (0.01 mole) of triethylamine in 100 cc. of toluene was cooled to −5° and 1.37 g. (0.01 mole) of isobutyl chlorocarbonate added with stirring. After 10 minutes at this temperature, an 0.89-g. (0.01 mole) sample of methyl glycinate[6] was added with good stirring and the mixture was then heated rapidly to reflux and immediately cooled. Some of the product separated as a colorless oil. The reaction mixture, therefore, was stirred vigorously with 75 cc. of saturated sodium bicarbonate solution and the resulting heterogeneous mixture allowed to stand overnight at room temperature. The product separated from the toluene phase as colorless crystals, wt. 2.75 g. (70%), m.p. 131.5–132°. The material was recrystallized by dissolving it in

(1) J. R. Vaughan, Jr., THIS JOURNAL, **73**, 3547 (1951); J. R. Vaughan, Jr., and R. L. Osato, *ibid.*, **74**, 676 (1952).

(2) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951).

(3) The yield before recrystallization was 83%, m. p. 112–115°.

(4) All melting points were taken on a Fisher–Johns block and are corrected.

(5) M. A. Stahmann, J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **164**, 759 (1946).

(6) M. Frankel and E. Katchalski, THIS JOURNAL, **64**, 2264 (1942).

TABLE I

PREPARATION OF ETHYL CARBOBENZOXYGLYCYL-L-PHENYLALANYLGLYCINATE UNDER VARYING CONDITIONS

Reaction time for anhydride formation (−5°), min.	Form in which glycine ester was used	Solvent system used	Reaction conditions after addition of the ester	Total yield of pure isomers, %	Racemization, %
5	Base	Tetrahydrofuran	Refluxed	60	0
5 (10°)	Base	Dioxane	Refluxed	71	10
5	Base	Toluene–chloroform (8:1)	Refluxed	65	30
3	Base	Toluene–chloroform (8:1)	Refluxed	68	28
10	Base	Toluene–chloroform (8:1)	Refluxed	70	51
5	HCl	Toluene–chloroform (8:1)	Refluxed	53	17
5	HCl	Toluene–chloroform (2:1)	Refluxed	82	56
5	HCl	Toluene–chloroform (2:1)	Room temp.	69	47
25	HCl	Toluene–chloroform (2:1)	Room temp.	69	93
25	HCl	Chloroform	Room temp.	31	100

The term "refluxed" refers to rapidly heating the reaction mixture to the point of reflux after the addition of the ethyl glycinate and then immediately cooling and working up the mixture. The term "room temperature" refers to allowing the reaction mixture to stand overnight before working it up. The amount of racemization observed in each experiment is expressed as the percentage of the total yield of material which crystallized from a 2% solution of the mixed isomers in alcohol as described above. In all cases, this product had a melting point in the range of 130–133°.

20 cc. of ethyl acetate and diluting this solution with 100 cc. of petroleum ether; wt. 2.35 g. (60%), m.p. 132.5–133°, $[\alpha]^{25}D$ −36.2 ± 0.5° (*c* 2, methanol).[7]

On diluting the original toluene reaction solution with petroleum ether, a small amount of optically inactive material slowly crystallized, wt. 0.25 g. (7%), m.p. 106–108°. The recorded m.p. of methyl carbobenzoxyglycyl-DL-leucylglycinate is 107°.[7]

Ethyl Carbobenzoxyglycyl-L-phenylalanylglycinate. A. By Coupling Carbobenzoxyglycyl-L-phenylalanine with Ethyl Glycinate.—A solution of 1.78 g. (0.005 mole) of carbobenzoxyglycyl-L-phenylalanine,[8] m.p. 125–126°, $[\alpha]^{25}D$ +38.8 ± 0.4° (*c* 5, ethanol), and 0.50 g. (0.005 mole) of triethylamine in a mixture of 40 cc. of toluene and 20 cc. of dioxane was cooled to −5° and a solution of 0.69 g. (0.005 mole) of isobutyl chlorocarbonate in 10 cc. of toluene added. After 5 minutes at this temperature, during which time triethylamine hydrochloride separated, a second solution of 0.52 g. (0.005 mole) of ethyl glycinate in 25 cc. of toluene was added with good stirring. The reaction mixture was then heated rapidly to reflux and immediately cooled and washed with 100 cc. of 1% sodium bicarbonate solution. On standing, the product crystallized from the heterogeneous mixture as colorless needles, wt. 1.60 g. (73%), m.p. 117–119°. Dilution of the organic phase from the filtrate with petroleum ether caused crystallization of a small second crop of material, wt. 0.25 g. (11.5%), m.p. 112–114°. The first crop was dissolved in 80 cc. of alcohol to make a 2% solution and seeded with the DL-isomer of the tripeptide derivative. After 2 hours in the refrigerator only a trace of ethyl carbobenzoxyglycyl-DL-phenylalanylglycinate[9] had separated, wt. 0.05 g. (2%), m.p. 131–132°. The solution was then concentrated to about 25 cc. and diluted hot with water (50 cc.) until cloudy. On cooling, the pure L-isomer crystallized, wt. 1.50 g. (68%), m.p. 118–119°, $[\alpha]^{25}D$ −11.5 ± 0.5° (*c* 2, ethanol). The second crop of product from the original reaction mixture was crystallized separately from ethyl acetate–petroleum ether to give an additional 0.20 g. (9%) of product also melting at 118–119°. The literature records a melting point of 117–118° and $[\alpha]^{24}D$ −12.3° (*c* 2, ethanol) for this compound.[10]

Before these conditions were established for the above reaction a number of experiments were run to determine the effect of changes in the time allowed and the solvent system used for anhydride formation, the effect of heating *versus* room temperature standing after amino acid ester addition, and the results of using pure ethyl glycinate *versus* the base prepared from the hydrochloride plus triethylamine in chloroform solution on the yield and per cent. racemization of the tripeptide derivative. In all cases the anhydride-forming reaction was carried out at −5°, except when dioxane was used as the solvent and a temperature of about 10° was required to prevent freezing. The results of this survey are summarized in Table I.

B. By Coupling Carbobenzoxyglycine with Ethyl L-Phenylalanylglycinate.—A solution of 2.09 g. (0.01 mole) of carbobenzoxyglycine and 1.02 g. (0.01 mole) of triethylamine in a mixture of 50 cc. of toluene and 5 cc. of chloroform was cooled to −5° and a solution of 1.37 g. (0.01 mole) of isobutyl chlorocarbonate in 10 cc. of toluene added with stirring. After 5 minutes at this temperature, a second solution of 3.31 g. (0.01 mole) of ethyl L-phenylalanylglycinate hydrobromide and 1.02 g. (0.01 mole) of triethylamine in 25 cc. of chloroform was added with good stirring and the reaction mixture was then heated rapidly to reflux and immediately cooled. The solution was washed with water and with 3% sodium bicarbonate and concentrated to about 25 cc. on a steam-bath under an air jet. The concentrate was diluted with 150 cc. of petroleum ether to precipitate the product as a colorless oil which slowly solidified. The solvent was decanted and the residue was redissolved in 50 cc. of ethanol and allowed to stand overnight. No crystallization occurred, and therefore, the DL-form of the tripeptide derivative apparently was not formed. The solution was next diluted with 100 cc. of petroleum ether to give a cloudy mixture from which the product slowly crystallized as colorless needles, wt. 3.35 g. (76%), m.p. 114–116°. Recrystallization of this material from alcohol–petroleum ether as above followed by crystallization from 12 cc. of ethanol gave 2.35 g. (54%) of material having a melting point of 116–118° and $[\alpha]^{25}D$ −12.6 ± 0.5° (*c* 2, ethanol). Additional crystallization from ethyl acetate–petroleum ether mixture followed by fractional crystallization from ethanol failed to purify completely this product. Both the melting point and optical rotation remained unchanged.

Ethyl Carbobenzoxy-L-phenylalanylglycinate.—A solution of 5.98 g. (0.02 mole) of carbobenzoxy-L-phenylalanine,[11] m.p. 130–132°, $[\alpha]^{24}D$ + 4.8 ± 0.2° (*c* 2, glacial acetic acid) and 2.04 g. (0.02 mole) of triethylamine in 50 cc. of toluene was cooled to −5° and 2.74 g. (0.02 mole) of isobutylchlorocarbonate added with stirring. After 10 minutes at this temperature, a second solution of 2.06 g. (0.02 mole) of ethyl glycinate in 5 cc. of toluene was added with good stirring and the reaction mixture was heated rapidly to reflux and then immediately cooled. Washing this solution with water caused crystallization of the product. This was filtered off, washed with dilute sodium bicarbonate solution and dried, wt. 5.10 g. (66.5%), m.p. 108–110°. The organic phase was separated from the filtrate, washed as above, dried and diluted with petroleum ether to crystallize a second crop of product, wt. 1.00 g. (13%), m.p. 93–98°. The two crops were combined, dissolved in 40 cc. of hot ethyl acetate and the solution filtered. Dilution of the hot filtrate with 125 cc. of petroleum ether gave a clear solu-

(7) G. W. Anderson and R. W. Young, *ibid.*, **74**, 5307 (1952), give m.p. 132–133° and $[\alpha]^{25}D$ −36.1° (*c* 5, methanol).

(8) K. Hofmann and M. Bergmann, *J. Biol. Chem.*, **134**, 225 (1940).

(9) J. R. Vaughan, Jr., and R. L. Osato, THIS JOURNAL, **73**, 5553 (1951).

(10) G. W. Anderson, J. Blodinger and A. D. Welcher, *ibid.*, **74**, 5309 (1952).

(11) M. Bergmann, *et al.*, *Z. physiol. Chem.*, **224**, 36 (1934), give m.p. 126–128° (cor.) and $[\alpha]^{21}D$ +4.9° (acetic acid).

tion from which the product rapidly crystallized as colorless needles, wt. 5.50 g. (71.5%), m.p. 109–110°, $[\alpha]^{25}D$ −17.3 ± 0.5° (*c* 2, ethanol). The literature melting point is 111°.[12]

When the preparation was repeated using the "standard" conditions previously reported,[1] a 72% yield of recrystallized material was also obtained; m.p. 109–110°.

Ethyl L-Phenylalanylglycinate Hydrobromide.—A 7.69-g. (0.02 mole) sample of ethyl carbobenzoxy-L-phenylalanylglycinate, 300 mg. of palladium black[13] and 1.5 cc. of glacial acetic acid were placed in 150 cc. of absolute alcohol and hydrogen was bubbled through the mixture at room temperature. Carbon dioxide evolution began immediately and was complete within 1 hour. The catalyst was filtered off and the solution concentrated to about 25 cc. and acidified with a slight excess of 1 *N* alcoholic hydrogen bromide. The solution was then reconcentrated to 25 cc. by vacuum distillation and diluted with 50 cc. of ether to precipitate a small amount of "gel-like" material. This impurity was filtered off and the filtrate was diluted with an additional 200 cc. of ether to give a clear solution from which the product crystallized as colorless plates on standing, wt. 4.05 g. (61%), m.p. 135–136°; $[\alpha]^{27}D$ +40.2 ± 0.5° (*c* 2, water). Recrystallization did not change the melting point or rotation.[14]

A second crop of material was obtained from the filtrate by concentration to dryness and recrystallization of the solid residue as above, wt. 0.65 g. (10%), m.p. 131–134°.

(12) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **118**, 414 (1937).

(13) R. Willstätter and E. Waldschmidt-Leitz, *Ber.*, **54**, 128 (1921).

(14) Reference 10 gives m.p. 135–136° and $[\alpha]D$ +40° (*c* 2, water) for this compound.

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Chemistry of Epoxy Compounds. XIV.[1] Reaction of *cis*-9,10-Epoxystearic Acid with Ammonia and Amines[2]

BY DANIEL SWERN AND THOMAS W. FINDLEY

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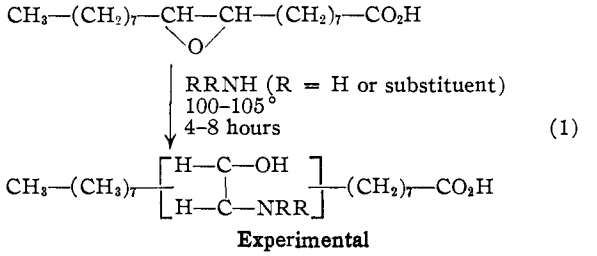
This note reports the opening of the oxirane ring of *cis*-9,10-epoxystearic acid with ammonia and amines (equation 1), the isolation of pure 9,10(10,9)-aminohydroxystearic acid and moderately pure N-substituted aminohydroxystearic acids, and potentiometric titration curves for several of these amino acids in the presence and absence of formaldehyde.



(1)

Experimental

Materials Used.—*cis*-9,10-Epoxystearic acid, m.p. 59.5°, was prepared from oleic acid by epoxidation with perbenzoic acid.[3] A.C.S. reagent grade concentrated aqueous ammonia was used. The Eastman Kodak Co. White Label Grades of 25% aqueous methylamine, 33% aqueous ethylamine, 33% aqueous dimethylamine and diethylamine were used without further purification. Diethylamine was diluted with approximately two parts of water to yield a 33% solution. Aniline was freshly distilled before use.

(1) For paper XIII, see THIS JOURNAL, **74**, 1655 (1952).

(2) Article not copyrighted.

(3) D. Swern, T. W. Findley and J. T. Scanlan, THIS JOURNAL, **66**, 1925 (1944).

Preparation of 9,10(10,9)-Aminohydroxystearic Acid.—A typical experiment is described. Six grams (0.02 mole) of 9,10-epoxystearic acid and 14 ml. of 14.5 *N* aqueous ammonia (0.20 mole) were placed in a Pyrex glass combustion tube sealed at one end and partially constricted at the other. The tube was immersed in Dry Ice–acetone for several minutes and the constricted end of the tube was sealed off. The tube was then placed within a steel pipe which was closed with threaded caps at each end, and the whole assembly was heated and rotated in an oil-bath at 100–105° for four hours. The steel pipe was removed from the bath and allowed to cool to room temperature. The glass tube was again immersed in Dry Ice for several minutes and the tube was opened. The contents were transferred quantitatively to an evaporating dish and 205 ml. of 0.1 *N* aqueous sodium hydroxide was added. The solution was boiled until the odor of ammonia could not be detected and the vapors gave no test for alkalinity with test paper (about one to two hours were required). Sufficient (approximately 205 ml.) 0.1 *N* hydrochloric acid was then added to neutralize the sodium hydroxide and yield a mixture whose *p*H was about 6. A viscous oil precipitated which solidified on standing overnight; weight 6.1 g. The crude 9,10(10,9)-aminohydroxystearic acid was crystallized twice from 95% ethanol (8 ml./g.) at 0°, yielding 3.5 g. (55%) of pure material, m.p. 153–155°. In duplicate experiments, yields ranging from 30–62% were obtained. *Anal.* Calcd. for $C_{18}H_{37}O_3N$: C, 68.6; H, 11.8; N, 4.44; neut. equiv., 315.5. Found: C, 68.9; H, 11.7; N, 4.51; neut. equiv. (formaldehyde present), 316.

Preparation of N-Substituted 9,10(10,9)-Aminohydroxystearic Acids.—The reaction of aqueous methylamine (12.4 g.) with *cis*-9,10-epoxystearic acid (6.0 g.) for eight hours at 100–105° yielded a viscous oil which did not solidify after removal of excess methylamine and acidification, as described in the preceding section under 9,10(10,9)-aminohydroxystearic acid. The reaction product was evaporated to dryness and separated from sodium chloride by solution in 40 ml. of boiling absolute ethanol. The ethanol solution was cooled to −50° for one week, yielding 1 g. of 9(10)-N-methylamino-10(9)-hydroxystearic acid, m.p. 100–103°. *Anal.* Calcd. for $C_{19}H_{39}O_3N$: N, 4.25; neut. equiv., 329.5. Found: N, 4.59; neut. equiv. (formaldehyde present), 331.5. The filtrate was evaporated to dryness yielding 5 g. of viscous oil; N, 4.19; neut. equiv., 348. It was evident, therefore, that the reaction yielded mainly 9(10)-N-methylamino-10(9)-hydroxystearic acid.

Aqueous ethylamine (13.7 g.) and *cis*-9,10-epoxystearic acid (6.0 g.) yielded 7.3 g. of a viscous oil after separation of the sodium chloride as described above. *Anal.* Calcd. for 9(10)-N-ethylamino-10(9)-hydroxystearic acid, $C_{20}H_{41}O_3N$: N, 4.08. Found: N, 4.36. No precipitate was obtained when a solution of this product in absolute ethanol was cooled to −50°.

Aqueous dimethylamine (13.7 g.) and *cis*-9,10-epoxystearic acid (6.0 g.) yielded 7.7 g. of yellow viscous oil from the alcohol solution. *Anal.* Calcd. for 9(10)-N,N-dimethylamino-10(9)-hydroxystearic acid, $C_{20}H_{41}O_3N$: N, 4.08. Found: N, 3.67. This product showed appreciable water solubility; a 5% solution was only slightly turbid.

Aqueous diethylamine (21 g.) and *cis*-9,10-epoxystearic acid (6.0 g.) yielded 7.1 g. of a semi-solid. *Anal.* Calcd. for 9(10)-N,N-diethylamino-10(9)-hydroxystearic acid, $C_{22}H_{45}O_3N$: N, 3.77. Found: N, 2.64.

cis-9,10-Epoxystearic acid (24 g., 0.08 mole) and aniline (72 g., 0.8 mole) were heated on the steam-bath for six hours in a nitrogen atmosphere. The reaction mixture was poured into 1 liter of 1.5 *N* hydrochloric acid in a separatory funnel and the aqueous layer was discarded. The upper oil layer was washed with four 500-ml. portions of 1.5 *N* hydrochloric acid, then with 5% sodium chloride until the wash was neutral, and twice with distilled water. The upper layer was dissolved in ethyl acetate and the solution was dried over anhydrous calcium sulfate. Filtration and evaporation of solvent yielded 16–25 g. of reddish-brown oil. Analysis indicated that it contained about 80% 9(10)-N-phenylamino-10(9)-hydroxystearic acid and about 20% of the anilide of this substance. *Anal.* Calcd. for 9(10)-N-phenylamino-10(9)-hydroxystearic acid, $C_{24}H_{41}O_3N$: N, 3.57; neut. equiv., 391.5; calcd. for the anilide, $C_{30}H_{46}O_2N_2$: N, 6.0. Found: N, 4.05; neut. equiv., 475–481.

Surface Active Properties of N-Substituted 9,10(10,9)-Aminohydroxystearic Acids.—Although 9(10)-N,N-di-