α,α-Dibenzyloxycarbonylamidopropionic Acid Derivatives; Synthesis of 5-Amino-5-methylhydantoin.

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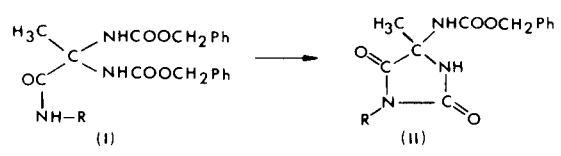
The synthesis and the properties of α-alkoxy-α-phenylacetamidocarboxylic acids and of some oligo peptide derivatives of such acids have been reported previously from this laboratory (1a-c). In the present paper we wish to report some results and observations concerning derivatives of α,α-dibenzyloxycarbonylamidopropionic acid.

It is well known (2) that treatment of benzyloxycarbonyl peptides with alkali can lead to elimination of benzyl alcohol and formation of derivatives of hydantoin-3-acetic acid. This reaction takes place with good yields when alcoholic sodium ethoxide (3) or excess aqueous alkali are used. In the mild conditions usually adopted in peptide synthesis during alkaline hydrolysis of *N*-benzyloxycarbonyl peptide esters (*e.g.* room temperature, 1.5-3 equivalents of 0.1-0.5*N* alkali) hydantoin formation does not generally occur or is very limited.

An exception is represented by *N*-benzyloxycarbonyl-dipeptide esters where glycine is the *C*-terminal residue; in this case two equivalents of alkali at room temperature are reported to give urea derivatives or hydantoin-3-acetic acid derivatives in high yields (2). Attempted saponification of benzyloxycarbonyl-α-methylalanyl-α-methylalanine methyl ester is also reported to give a mixture of the desired acid and of the hydantoin-3-acetic acid derivative (4).

In preparing dipeptide and tripeptide derivatives (I) of α,α-dibenzyloxycarbonylamidopropionic acid we observed the possibility of obtaining hydantoin-3-acetic acid derivatives (II) in high yields in mild conditions; when 3 equivalents of 1*N* sodium hydroxide at room temperature for 45 minutes were used to hydrolyze methyl esters (Ia) and (Ib), formation of 30% and 60% respectively of hydantoin derivatives (IIa) and (IIb) was observed along with the expected hydrolysis products. Using 1.1 equivalents of 1*N* sodium hydroxide, conversion into hydantoin-3-acetic acid derivative (IIa) was complete after three days at room temperature.

Scheme I



(I) (II)

(Ia) R = $-CH(CH_2Ph)-COOCH_3$ (IIa) R = $-CH(CH_2Ph)-COOCH_3$

(Ib) R = $-CH(CH_2Ph)-CO-N$(COOCH$_3$) (IIb) R = $-CH(CH_2Ph)-CO-N$(COOCH$_3$)

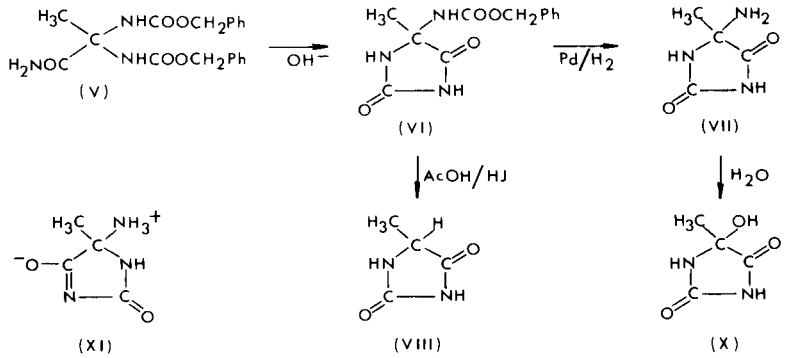
The steric hindrance associated with the presence of an α-α-disubstituted amino acid does not seem the only factor determining the facile cyclization. Benzyloxycarbonyl-α-methylalanyl-L-phenylalanine methyl ester (III) and benzyloxycarbonyl-α-methylalanyl-α-methylalanine methyl ester (IV) were synthesized and treated with sodium hydroxide in both the above cited conditions. Simple hydrolysis of the ester function was observed; only the dipeptide ester (IV) gave 5-10% of the hydantoin-3-acetic acid derivative when treated according to the second procedure (1.1 equivalent of 1*N* sodium hydroxide, two days at room temperature).

The above results prompted us to see this reaction for the synthesis of 5-alkyl-5-amino hydantoins, compounds with potential pharmaceutical interest.

A survey of the literature indicated in fact that attempts to synthesize this class of compounds, based on the route of Gabriel (5) (bromination of 5-phenylhydantoin and treatment with ammonia of the 5-bromo-5-phenylhydantoin), were not successful, because of the formation of unsaturated compounds (6a-b).

Treatment with 2*N* sodium hydroxide of α,α-dibenzyloxycarbonylamidopropionamide (V) gave the expected 5-benzyloxycarbonylamido-5-methylhydantoin (VI) in high yield; this compound could easily be converted into 5-methylhydantoin (VIII) by reaction with hydriodic acid. Hydrogenolysis of (VI) gave 5-amino-5-methylhydantoin (VII) as a crystalline, water soluble compound which decomposed by heating, without melting. By heating (VII) with water, 5-hydroxy-5-methyl hydantoin (X) (7) was

Scheme II



obtained in quantitative yield. Based on the evidence of the ir spectrum (bands at 3000, 2720-2600, 1600 cm^{-1}, typical for primary amine salts) and according to E. Schauenstein and G. M. Perko (8), mesomeric structure (XI) should be given to 5-amino-5-methylhydantoin (VII).

Attempts to obtain this latter compound (VII) starting from α-amino-α-benzyloxycarbonylamidopropionamide (IX) under conditions similar to those in cyclization of (V), resulted in formation of 5-hydroxy-5-methylhydantoin (X) and traces only of the amino derivative (VII). This result is in accordance with the above reported transformation of the 5-amino-5-methyl hydantoin (VII) into the corresponding 5-hydroxy derivative with water and related to the facile substitution of 5-amino group with nucleophiles; investigations of the reactivity of 5-amino-5-alkylhydantoins are now in progress in our laboratory.

EXPERIMENTAL

Melting points were determined in open capillary tubes on a copper block and are not corrected. Anhydrous sodium sulphate was used throughout as drying agent. Evaporations were under reduced pressure.

α,α-Dibenzyloxycarbonylamidopropionyl-L-phenylalanine Methyl Ester (Ia).

A mixture of 5.0 g. of α,α-dibenzyloxycarbonylamidopropionic acid, 2.8 g. of dicyclohexylcarbodiimide and 2.4 g. of L-phenylalanine methyl ester in 60 ml. of tetrahydrofuran was stirred at room temperature overnight; 5.2 g. of Ia was obtained, m.p. 121-122° (from ether), $[\alpha]_D^{20}$ = -10.5° (c 2, methanol).

Anal. Calcd. for $C_{29}H_{31}N_3O_7$: C, 65.28; H, 5.86; N, 7.88. Found: C, 65.29; H, 5.88; N, 7.86.

Alkaline Hydrolysis of Ia.

Procedure A.

Compound Ia (0.900 g.) in 20 ml. of methanol and 5.2 ml. of 1*N* sodium hydroxide was stirred at room temperature for 45 minutes, methanol was removed, 5 ml. of water added and the solution extracted with light petroleum to remove benzyl alcohol; acidification and extraction with ethyl acetate gave 0.72 g. of a mixture; tlc indicated two compounds which were separated by column chromatography on silica gel to give 0.420 g. of α,α-dibenzyloxycarbonylamidopropionyl-L-phenylalanine, m.p. 113-115° (from ethyl acetate), $[\alpha]_D^{20}$ = +21° (c 2.1, methanol).

Anal. Calcd. for $C_{28}H_{29}N_3O_7 \cdot H_2O$: C, 62.56; H, 5.81; N, 7.82. Found: C, 62.22; H, 5.66; N, 7.64.

The second fraction consisted of a viscous oil which failed to crystallize; esterification with diazomethane gave 190 mg. of 5-benzyloxycarbonylamido-5-methylhydantoin-3-*R*-benzylacetic acid methyl ester (IIa); m.p. 153-154° (from ether-light petroleum), $[\alpha]_D^{20}$ = -21° (c 1.2 methanol).

Anal. Calcd. for $C_{22}H_{23}N_3O_6$: C, 62.11; H, 5.45; N, 9.88. Found: C, 62.12; H, 5.50; N, 9.85.

Procedure B.

Compound Ia (0.530 g.) in 30 ml. of methanol was treated at room temperature for three days with 1.1 ml. of 1*N* sodium hydroxide; work up as above gave 98 mg. of benzyl alcohol and 0.380 g. of 5-benzyloxycarbonylamido-5-methyl-hydantoin-3-*R*-benzylacetic acid isolated as methyl ester (IIa).

α,α-Dibenzyloxycarbonylamidopropionyl-L-phenylalanyl-L-proline Methyl Ester (Ib).

A mixture of 0.700 g. of α,α-dibenzyloxycarbonylamidopropionyl-L-phenylalanine, 0.280 g. of dicyclohexylcarbodiimide and 0.175 g. of L-proline methyl ester in 10 ml. of chloroform was stirred at room temperature for 18 hours; solid material was filtered off and the solution fractionated with 1*N* sodium hydroxide and 1*N* hydrochloric acid to give 0.790 g. of a neutral fraction as a colorless oil; preparative tlc (silica gel; 3:1 benzene-ether) gave 0.600 g. of Ib; m.p. 102-103° (from ether-light petroleum), $[\alpha]_D^{20}$ = -35° (c 3, methanol).

Anal. Calcd. for $C_{34}H_{38}N_4O_8$: C, 64.75; H, 6.07; N, 8.88. Found: C, 64.42; H, 6.18; N, 8.57.

Alkaline Hydrolysis of Ib.

α,α-Dibenzyloxycarbonilamidopropionyl-L-phenylalanyl-L-proline methyl ester (Ib) (0.630 g.) in 12 ml. of methanol and 3 ml. of 1*N* sodium hydroxide was stirred at room temperature for 45 minutes; methanol was removed and the residue taken up with water and extracted with light petroleum; the aqueous alkaline solution was extracted with methylene chloride, acidified and extracted with methylene chloride. Evaporation of the dried extracts gave 460 mg. of a neutral fraction and 80 mg. of acidic fraction; 55 mg. of benzyl alcohol were obtained from light petroleum solution. Preparative tlc of neutral fraction (silica gel;

2:1 benzene-ether; six elutions), gave 190 mg. of starting material (Ib) and the two diastereomeric 5-benzyloxycarbonylamido-5-methylhydantoin-3-*S*-benzylacetyl-L-proline methyl esters (IIb); the more polar isomer melted at 197-198° (from ethyl acetate); $[\alpha]_D^{20}$ = -43.4° (c 2, methanol); ν max (potassium bromide) 3350, 3320, 1780, 1740, 1715, 1640, 1530 cm^{-1}.

Anal. Calcd. for $C_{27}H_{30}N_4O_7$: C, 62.06; H, 5.79; N, 10.72. Found: C, 61.98; H, 5.89; N, 10.77.

The less polar isomer melted at 103-105° (from benzene); $[\alpha]_D^{20}$ = -181° (c 2, methanol); ν max (potassium bromide) 3340-3280, 1790, 1735-1715, 1650, 1530 cm^{-1}.

Anal. Calcd. for $C_{27}H_{30}N_4O_7$: C, 62.06; H, 5.79; N, 10.72. Found: C, 61.91; H, 5.76; N, 10.63.

Alkaline Hydrolysis of Benzyloxycarbonyl-α-methylalanyl-L-phenylalanine Methyl Ester (III).

The dipeptide methyl ester (III) was prepared in a yield of 80% with dicycloexylcarbodiimide from benzyloxycarbonyl-α-methylalanine and L-phenylalanine methyl ester, in chloroform; m.p. 92-93° (from ether-light petroleum); $[\alpha]_D^{20}$ = +27.5° (c 2, methanol); lit. (9a-b) m.p. 94.2-94.8°; $[\alpha]_D^{20}$ = +27.2° (c 2.2, dioxane).

Procedure A.

Compound III (400 mg.) in 8 ml. of methanol and 3 ml. of 1*N* sodium hydroxide was kept for 1 hour at room temperature. Methanol was replaced with water and the solution fractionated in ethyl acetate to give 365 mg. of benzyloxycarbonyl-α-methylalanyl-L-phenylalanine, m.p. 60-65° (from ether); $[\alpha]_D^{20}$ = +31° (c 2, dioxane); lit. (9b) m.p. 60-65°, $[\alpha]_D^{20}$ = +34.2°; no benzyl alcohol was detected in the neutral fraction.

Procedure B.

Compound III (400 mg.) in 8 ml. of methanol and 3.3 ml. of 1*N* sodium hydroxide were allowed to stand for three days at room temperature. After working up as above, 355 mg. of benzyloxycarbonyl dipeptide was obtained, but no benzyl alcohol.

Alkaline Hydrolysis of Benzyloxycarbonyl-α-methylalanyl-α-methylalanine Methyl Ester (IV).

The dipeptide methyl ester (IV) was prepared in a yield of 75% from benzyloxycarbonyl-α-methylalanine and α-methylalanine methyl ester by the same procedure used for (III); m.p. 110-111° (from ethyl acetate-light petroleum); lit. (10) m.p. 109-111°.

Procedure A.

Compound IV (670 mg.) in 20 ml. of methanol and 6 ml. of 1*N* sodium hydroxide was kept for 1 hour at room temperature. After working up as in the case of hydrolysis of (III), 435 mg. of benzyloxycarbonyl-α-methylalanyl-α-methylalanine were obtained; m.p. 160-162° (from methanol-water); lit. (10) m.p. 161-162.5°; 215 mg. of starting material was recovered from the neutral fraction.

Procedure B.

Compound IV (200 mg.) in 4 ml. of methanol and 0.66 ml. of 1*N* sodium hydroxide was allowed to stand for three days at room temperature. Work up as in above procedure B, gave 165 mg. of benzyloxycarbonyl dipeptide, 20 mg. of starting material and traces of benzyl alcohol.

α,α-Dibenzyloxycarbonylamidopropionic Acid.

This compound was prepared according to the general procedure of A. E. Martell and R. M. Herbst (11); the reaction time was 5 hours under reduced pressure (20 mm Hg) at a temperature of 70°.

α,α-Dibenzyloxycarbonylamidopropionamide (V).

To a stirred solution of 500 mg. of α,α-dibenzyloxycarbonylamidopropionic acid in 2 ml. of tetrahydrofuran, 136 mg. of triethylamine and 146 mg. of ethylchloroformate, were added at -12°. After 15 minutes at -12° a solution of 23 mg. of ammonia in 3 ml. of tetrahydrofuran was added in drops. The reaction mixture was stirred for 3 hours at 0° and filtered; tetrahydrofuran was removed and the residue fractionated in ethyl acetate with 2*N* hydrochloric acid and 5% sodium bicarbonate to give 410 mg. of V; crystallization from ethyl acetate yielded 270 mg.; m.p. 174-175°; ν max (potassium bromide) 3440, 3395, 3340, 1720, 1670, 1590, 1520, 1500 cm^{-1}.

Anal. Calcd. for $C_{19}H_{21}N_3O_5$: C, 61.45; H, 5.70; N, 11.31. Found: C, 61.35; H, 5.66; N, 11.31.

α-Amino-α-benzyloxycarbonylamidopropionamide (IX).

Methyl-α-amino-α-benzyloxycarbonylamidopropionate (1.19 g.) (12) in 20 ml. of dry methanol was saturated in a pressure bottle with ammonia at -20°. After three days at room temperature the residue from the evaporation was crystallized from ethyl acetate to give 560 mg. of IX, m.p. 123-124°; ν max (potassium bromide) 3460, 3430, 3320, 3240, 1715, 1670, 1530 cm^{-1}.

Anal. Calcd. for $C_{11}H_{15}N_3O_3$: C, 55.69; H, 6.37; N, 17.71. Found: C, 55.59; H, 6.36; N, 17.77.

5-Benzyloxycarbonylamido-5-methylhydantoin (VI).

To a solution of 800 mg. of α,α-dibenzyloxycarbonylamidopropionamide (V) in 80 ml. of methanol was added 3.3 ml. of 2*N* sodium hydroxide. After 2 hours at room temperature the methanol was removed and water added to the residue; the aqueous solution was washed with light petroleum (to extract benzyl alcohol); continuous extraction with ethyl acetate for 12 hours gave 690 mg. of residue; crystallization from ethyl acetate yielded 460 mg. of VI m.p. 174-175°; ν max (potassium bromide) 3300-3200, 1780, 1720, 1685, 1525 cm^{-1}.

Anal. Calcd. for $C_{12}H_{13}N_3O_4$: C, 54.75; H, 4.98; N, 15.96. Found: C, 54.75; H, 4.98; N, 15.96.

5-Methylhydantoin (VIII) from VI.

A solution of 100 mg. of 5-benzyloxycarbonylamido-5-methylhydantoin (VI) in 1.8 ml. of acetic acid containing 1.8 ml. of hydriodic acid (D. 1.7) was heated at 100° for 10 minutes. The mixture was evaporated and the residue washed in ethyl acetate with saturated sodium hydrogen carbonate solution and dilute sodium thiosulphate solution; preparative tlc of the residue from ethyl acetate (silica gel; 60:40 ether-ethyl acetate) gave 25 mg. of VIII; m.p. 149-151° (from ethyl acetate-light petroluem), lit. (13) 150-152°.

Anal. Calcd. for $C_4H_6N_2O_2$: C, 42.11; H, 5.30; N, 24.55. Found: C, 41.91; H, 5.31; N, 24.47.

5-Amino-5-methylhydantoin (VII).

5-Benzyloxycarbonylamido-5-methylhydantoin (1.58 g.) (VI) in 20 ml. of methanol was hydrogenated at room temperature in the presence of 0.69 g. of palladium on aluminium oxide (5% Pd); the mixture was filtered from the catalyst and evaporated to a small volume to give 0.620 g. of VII. This compound decomposed by heating with loss of ammonia, without melting; ν max (potassium bromide) 3340-3300, 3000-2900, 2720-2600, 1750, 1710, 1600 cm^{-1}; mass spectrum [m/e 129 (M^+), peaks at m/e 114,

112, and 101 (base peak)]; nmr [(dimethylsulfoxide-d_6) δ 60 MHz 1.28 (3H, s)].

Anal. Calcd. for $C_4H_7N_3O_2$: C, 37.21; H, 5.46; N, 32.54. Found: C, 37.11; H, 5.48; N, 32.55.

Cyclization of α-Amino-α-benzyloxycarbonylamidopropionamide (IX).

A solution of 480 mg. of amide (IX) in 30 ml. of methanol and 3 ml. of 2*N* sodium hydroxide was stirred for 2 hours at room temperature; methanol was removed and 10 ml. of water was added to the residue; extraction of the aqueous solution with ether gave 260 mg. of a mixture of benzyl alcohol and starting material; the aqueous phase was neutralized with hydrochloric acid and water removed at room temperature. Preparative tlc of the residue (silica gel; 60:40 ethyl acetate-ether) gave 45 mg. of 5-hydroxy-5-methylhydantoin (X) and traces only of 5-amino-5-methylaminohydantoin (VII).

5-Hydroxy-5-methylhydantoin (X).

5-Amino-5-methylhydantoin (130 mg.) in 5 ml. of water was refluxed for 30 minutes. During the reaction ammonia evolution was observed. Water was removed and the residue was purified by preparative tlc (silica gel; ethyl acetate) to give 45 mg. of X, m.p. 173-174°, lit. (7) m.p. 166° (from acetic acid).

Anal. Calcd. for $C_4H_6N_2O_3$: C, 36.93; H, 4.65; N, 21.53. Found: C, 36.83; H, 4.69; N, 21.61.

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