Summary

The electrophoretic mobility and heterogeneity of normal and digested human gamma globulin are increased by cysteine–hydrogen peroxide treatment. It is suggested that such mobility increases result from oxidative changes in the protein. Such treatment likewise causes a marked decrease in the antibody titers of human gamma globulin.

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Esters of α-Aminoisobutyric Acid

BY RALPH A. JACOBSON

Although the methyl and ethyl esters of the more common α-amino acids have been described, in many instances the corresponding esters of the higher alcohols are unknown. The work described in the present paper was undertaken in order to obtain information regarding the properties and, in particular, the stability toward cyclization of the higher esters of α-aminoisobutyric acid.

The preparation of α-aminoisobutyric acid in 30–33% yields by the hydrolysis of α-aminoisobutyronitrile with hydrobromic acid is described in "Organic Syntheses."[1,2] In the present study, 70–75% yields of the acid were obtained by the following two procedures: (a) the hydrolysis of α-aminoisobutyronitrile with 40% sulfuric acid according to the general procedure suggested by Cocker and Lapworth[3] and (b) the hydrolysis of 5,5-dimethylhydantoin with 60% sulfuric acid according to the procedure of Bucherer and Steiner.[4] Procedure (b) is to be recommended since 5,5-dimethylhydantoin is easily prepared in excellent yield.[5]

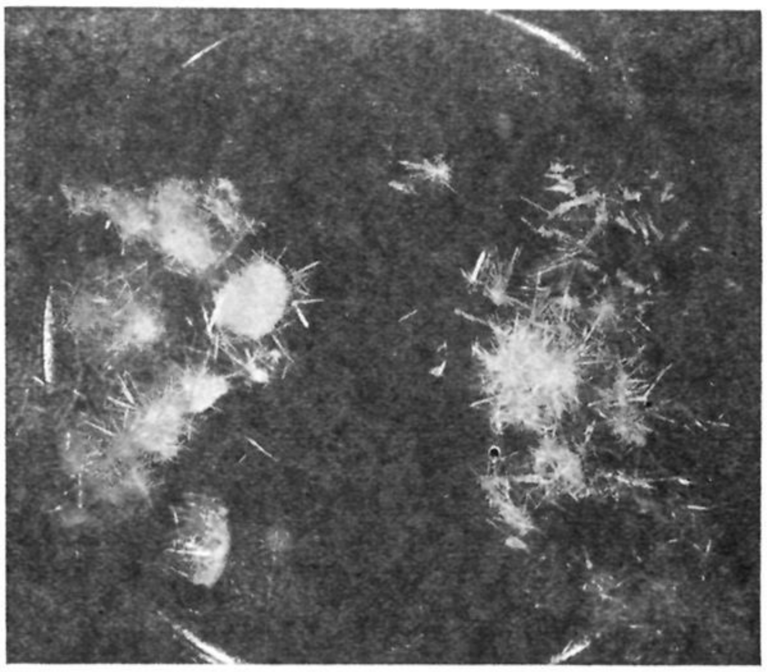

Fig. 1.—A = condensation product from dodecyl ester of α-aminoisobutyric acid; B = tetramethyldiketopiperazine.

The isobutyl, octyl and dodecyl esters of α-aminoisobutyric acid were prepared by esterification in the presence of hydrogen chloride.[6] The hydrochlorides of the esters were first isolated for characterization and then converted to the free esters by treatment with alkali. The hydrochlorides of the esters are crystalline solids and the free esters are colorless liquids of pronounced basicity. The isobutyl ester is slightly soluble in water but the octyl and the dodecyl esters are practically insoluble. The hydrochlorides of the isobutyl and octyl esters are soluble but the dodecyl ester hydrochloride is insoluble in water.

In general, the methyl and ethyl esters of α-amino acids tend to cyclize to diketopiperazine derivatives or to form condensation products even at room temperature. The lower alcohol esters of glycine are particularly unstable and readily form glycine peptide ester chains of considerable length.[7] The methyl ester of α-aminoisobutyric acid, ($NH_2C(CH_3)_2COOCH_3$), appears to be somewhat more stable possibly because of the doubly substituted alpha carbon. Thus Franchimont and Friedmann[6] obtained tetramethyldiketopiperazine in only 34% yield even after prolonged heating of the ester at 230–240°. The present results indicate that the higher esters of α-aminoisobutyric acid possess still greater stability. At room temperature, samples of the isobutyl, octyl and dodecyl esters formed only a trace of solid material over a period of many months. At 210–220° the octyl ester yielded 3.0% of condensation product after twenty-two hours. At its boiling point (177–179°) the isobutyl ester yielded only 0.91% of

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[1] Clarke and Beane, "Organic Syntheses," **XI**, 4 (1931).

[2] Lin and Li, *J. Chinese Chem. Soc.*, **6**, 88 (1938); *C. A.*, **35**, 5096 (1941), prepared the acid by a circuitous route.

[3] Cocker and Lapworth, *J. Chem. Soc.*, 1391 (1931).

[4] Bucherer and Steiner, *J. prakt. Chem.*, **140**, 291 (1934).

[5] (a) Ref. 4; (b) Wagner and Baizer, "Organic Syntheses," **20**, 42 (1940).

[6] Methyl α-aminoisobutyrate has been prepared by Franchimont and Friedmann, *Rec. trav. chim.*, **27**, 201 (1908), and ethyl α-aminoisobutyrate by Zelinski and Kulikow, *Z. physiol. Chem.*, **73** 459 (1911); *Chem. Zentr.*, **82**, 1319 (1911). The bactericidal properties of the hydrochloride of the dodecyl ester have been examined by Baker, Harrison and Miller, *J. Exptl. Med.*, **74**, 611 (1941).

[7] (a) Frankel and Katchalski, THIS JOURNAL, **64**, 2264 (1942); **64**, 2268 (1942). (b) Pacsu, *Nature*, **144**, 551 (1939); Pacsu and Wilson, *J. Org. Chem.*, **7**, 117 (1942); **7**, 126 (1942).

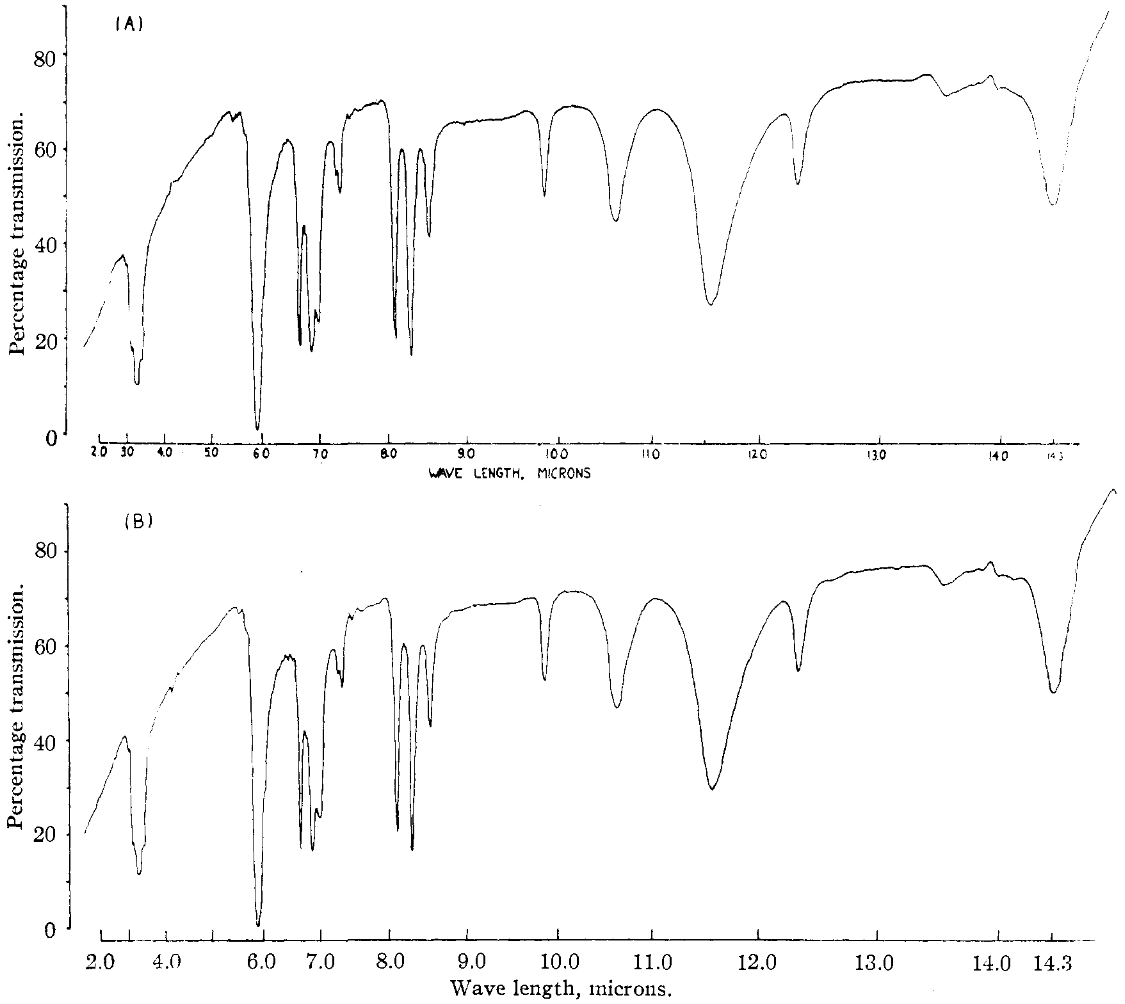

Fig. 2.—Comparison of infra-red spectrograms of (A) reaction product of dodecyl ester of α-aminoisobutyric acid and (B) tetramethyldiketopiperazine. Dry deposits on rock salt plates (about 1 mg./sq. cm.). Recorded by automatic rock salt prism spectrophotometer.

solid after twenty-two hours. A second sample, heated in a sealed tube at 240° for twenty-four hours, gave 3.1% of solid in comparison with 23.1% for the ethyl ester which was heated simultaneously. At 210–220°, the dodecyl ester yielded 0.7% of condensation product after twenty-four hours. The solubility characteristics, sublimation range, crystal structure (Fig. 1), chemical analysis and infrared spectrum (Fig. 2) corresponded to those of tetramethyldiketopiperazine. The octyl ester of α-amino-α-methylbutyric acid yielded only 0.56% of condensation product after twenty-four hours at 220°.

The effect upon stability of replacing one of the hydrogens on the nitrogen of an α-aminoisobutyric ester is also of interest. The introduction of a phenyl group into the molecule would be expected to reduce the tendency toward cyclization or formation of condensation products. This appears to be the case, the isobutyl and octyl esters of N-phenyl-α-aminoisobutyric acid exhibiting unusual stability. Both esters were recovered unchanged (except for darkening) after twenty-four hours at 210–220°.

Experimental

Preparation of 5,5-Dimethylhydantoin.—This compound was prepared by the procedure of Bucherer and Steiner.[4]

α-Aminoisobutyronitrile.—A modification of the procedure of Dubsky and Wensink[4,8] was employed for the preparation of this compound.

A mixture of 2265 g. of acetone cyanhydrin and 7700 g. of liquid ammonia was allowed to stand in a closed container for several days. The ammonia was then distilled out, and solid potassium hydroxide added to the mixture The upper layer was removed, dried with additional potassium hydroxide and distilled. The α-aminoisobutyronitrile was obtained as a colorless liquid boiling at 60° under 21 mm. pressure; yield 1726 g. or 77% of the theoretical.

α-Aminoisobutyronitrile was also prepared in a yield of 57% by passing ammonia gas into acetone cyanhydrin at atmospheric pressure.

Preparation of α-Aminoisobutyric Acid by the Hydrolysis of 5,5-Dimethylhydantoin.—A solution of 142.5 g. of

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(8) Dubsky and Wensink, *Ber.*, **49**, 1134 (1916).

TABLE I

ESTERS OF α-AMINOISOBUTYRIC ACID[a] AND DERIVATIVES

Ester, -butyrate	Formula	Nitrogen %, Ester, Calcd.	Nitrogen %, Ester, Found	Nitrogen %, Hydrochloride, Calcd.	Nitrogen %, Hydrochloride, Found	B. p. °C.	B. p. Mm.	Yield, %	d^{20}_4	n^{20}_D	M. p. hydrochloride, °C.
Isobutyl α-aminoiso-	$C_8H_{17}O_2N$	8.80	9.13	7.16	7.18	60–61	4	65.5	0.9082	1.4210	102–103
Octyl α-aminoiso-	$C_{12}H_{25}O_2N$	6.53	6.69	5.56	5.64	95–96	2	93.4	0.8918	1.4340	84–85
Dodecyl α-aminoiso-	$C_{16}H_{33}O_2N$	5.17	5.98	4.55	4.80	130–132	2	78.3	0.8832	1.4429	95
Octyl N-phenyl-α-aminoiso-	$C_{18}H_{29}O_2N$	4.81	5.16			167–169	1.5	78.8	0.9722	1.4999	
Isobutyl N-phenyl-α-aminoiso-	$C_{14}H_{21}O_2N$	C, 71.49 H, 8.93	C, 71.87 H, 9.07	N, 5.15	N, 5.16	130–135	3–4	87.4	1.0072	1.5082	115–116
Octyl α-amino-α-methyl-	$C_{13}H_{27}O_2N$	6.11	6.43			98	1	85.4	0.8941	1.4380	

[a] Jacobson, U. S. Patent 2,135,641.

5,5-dimethylhydantoin in 587 g. of 60% sulfuric acid was refluxed gently for twenty-four hours. Seven hundred sixty-three grams of barium carbonate was added slowly and a current of steam passed into the mixture on the steam-bath until ammonia was no longer evolved. The mixture was acidified with sulfuric acid until acid to congo paper, filtered and the solid on the filter washed several times by slurrying with hot water. The filtrate and washings were concentrated to a volume of 1 liter. Basic lead carbonate was added until effervescence ceased and the mixture was no longer acid to congo paper but still acid to litmus. The lead sulfate was removed by filtration, washed with hot water, and the filtrate and washings concentrated until crystallization of the α-aminoisobutyric acid began. The yield from this and succeeding crops amounted to 98 g. or 76% of the theoretical amount.

Preparation of α-Aminoisobutyric Acid by the Hydrolysis of α-Aminoisobutyronitrile.—A solution of 84 g. of α-aminoisobutyronitrile in 1225 g. of 40% sulfuric acid was refluxed gently for eight hours. The subsequent procedure was similar to that described above. The yield of α-aminoisobutyric acid amounted to 72 g. or 70% of the theoretical.

Methyl Ethyl Ketone Cyanhydrin.—A mixture of 360 g. (5 moles) of methyl ethyl ketone and 1 g. of sodium cyanide in 2 cc. of water was cooled to 5°, and 141 g. (5 moles) of hydrogen cyanide (98%) slowly added through a separatory funnel. The hydrogen cyanide was added at such a rate (two hours) that the temperature did not rise above 20°. The reaction mixture was cooled to 0° and stabilized by the addition of 1 cc. of 96% sulfuric acid; yield quantitative.

5-Methyl-5-ethyl-hydantoin.—This compound was prepared from 99 g. of methyl ethyl ketone cyanhydrin, 400 cc. of water and 114 g. of ammonium carbonate by the procedure described for 5,5-dimethylhydantoin. The yield was 120.5 g. or 85%. As reported by Bucherer and Steiner,[4] the compound melted at 145–146°.

α-Amino-α-methylbutyric Acid.—This acid was obtained by the hydrolysis of 5-methyl-5-ethyl-hydantoin according to the procedure employed for the preparation of α-aminoisobutyric acid.

Preparation of α-Phenylaminoisobutyronitrile.—A mixture of 7440 g. of freshly distilled aniline and 7000 g. of acetone cyanhydrin was allowed to stand at room temperature in a 5-gallon wide-mouthed bottle. After several days, a crop of crystals weighing 4935 g. had separated. The crystals were filtered off, washed with petroleum ether, and the filtrate (but not the washings) allowed to stand. Additional crops brought the total yield to 11,894 g. or 92.8% of the theoretical. Purification other than washing with petroleum ether was unnecessary. The white crystals melted at 90–92° (lit. 93–94°).[9]

α-Phenylaminoisobutyric Acid.—This acid was prepared by the hydrolysis of the nitrile with hydrochloric acid according to the procedure of Bucherer and Grolee.[9]

Esters of α-Aminoisobutyric Acid

The method described below for the dodecyl ester of α-aminoisobutyric acid illustrates the general preparative procedure employed. The properties of the esters and their hydrochlorides are summarized in Table I.

Dodecyl Ester of α-Aminoisobutyric Acid.—Hydrogen chloride was passed into a suspension of 206 g. of α-aminoisobutyric acid in 2232 g. of dodecyl alcohol until the increase in weight amounted to 278 g. The mixture was warmed gently for eight hours, during which the solid dissolved. The excess alcohol was recovered by vacuum distillation and the ester hydrochloride was obtained as a solid cake. Crystallization of a portion of this product from butyl acetate gave white crystals melting at 95°. The main portion was treated in the cold with a 20% solution of potassium hydroxide in the presence of ether. The ether layer was separated, dried with magnesium sulfate, and distilled. The dodecyl ester was obtained as a colorless liquid boiling at 130–132° under 2 mm.; yield 325 g. or 78.3% of the theoretical.

The ester (70 g.) was heated at 210–220° for twenty-four hours, cooled and filtered. The small amount (0.490 g.) of white solid thus obtained was washed with water, alcohol, and ether. Analysis indicated that it was tetramethyldiketopiperazine. On an electric block, the crystals sublimed slightly at 202°, and more rapidly at 214–220°. An authentic specimen of tetramethyldiketopiperazine behaved similarly (Fig. 1). The infrared absorption spectra of both are shown in Fig. 2.

Anal. Calcd. for $C_8H_{14}O_2N_2$: C, 56.47; H, 8.23; N, 16.47; mol. wt., 170. Found: C, 56.70; H, 8.16; N, 16.42; mol. wt., 165.

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Summary

1. The isobutyl, octyl and dodecyl esters of α-aminoisobutyric acid and the octyl ester of α-amino-α-methylbutyric acid have been prepared.

2. The higher esters of α-aminoisobutyric acid and α-amino-α-methylbutyric acid appear to be relatively resistant to cyclization and intermolecular condensation.

3. The isobutyl and octyl esters of N-phenyl-α-aminoisobutyric acid have been prepared and shown to be unusually resistant to cyclization.

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[9] Bucherer and Grolee, *Ber.*, **39**, 986 (1906).