286. α-*Amino*-β-*keto-acids. Part I. Synthesis and Attempted Isolation of the Free Acids.*

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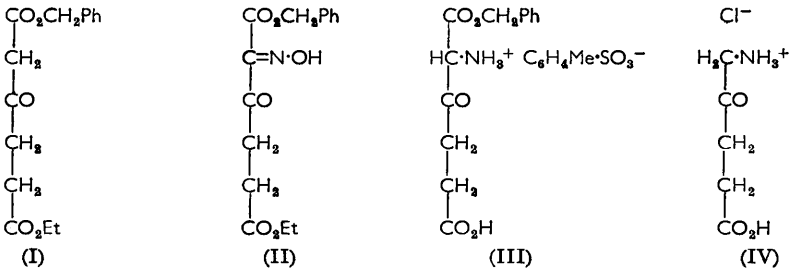
α-Benzyl hydrogen α-amino-β-oxoadipate was prepared by selective transesterification of ethyl β-oxoadipate, nitrosation, reduction with stannous chloride, and hydrolysis of the δ-ethyl ester grouping. Benzyl α-amino-β-oxobutyrate hydrochloride was synthesized in an essentially similar manner. With both compounds catalytic hydrogenolysis occurred readily, but the speed of its decarboxylation rendered impossible the isolation of the α-amino-β-keto-acid formed. Similar instability of various α-acylamino-β-keto-acids was observed. Cleavage of the benzyl esters with hydrogen bromide in glacial acetic acid was slow and accompanied by decarboxylation. Attempts to isolate the desired free acids after reductive splitting of β-keto-α-phenylhydrazono-acids were also unsuccessful.

It is now generally accepted that the first reaction in the biosynthesis of porphyrins consists of the condensation of succinyl-coenzyme A with glycine to give ultimately

δ-aminolævulic acid.[1 2 3] While the reaction can now be carried out *in vitro* with the aid of cell-free enzyme preparations,[4] certain features of the mechanism are still obscure; in particular it is uncertain at what stage the carboxyl group of glycine is lost. It is unlikely that glycine is decarboxylated before its condensation with succinate, since a variety of one-carbon compounds, such as methylamine, cannot be substituted for glycine.[5] However, the possibility that an activated complex of glycine loses carbon dioxide before condensation cannot be excluded. It is also possible that condensation takes place at the same time as the removal of the carboxyl group of glycine. The third possibility is that the primary product is α-amino-β-oxoadipate which is decarboxylated either by a specific enzyme or by spontaneous decomposition and this hypothesis was indeed preferred in the earlier publications.[1 2] In order to decide between some of these possibilities it was proposed to attempt the synthesis of α-amino-β-oxoadipic acid or at least to get information about the stability of this acid under physiological conditions.

No free α-amino-β-keto-acid has so far been prepared and attempts in this laboratory to hydrolyse ethyl and methyl α-amino-β-oxoadipate with acid or alkali without loss of carbon dioxide have been unsuccessful.[6] It seemed clear that α-amino-β-oxoadipic acid, if it existed at all, was not very stable. In the work to be reported in the present paper the α-benzyl ester of this acid was synthesized. It was hoped that hydrogenolysis of the ester would give the acid which could then be isolated under suitable conditions of temperature and pH. Ethyl β-oxoadipate with hot benzyl alcohol gave a benzyl ethyl ester to which structure (I) is ascribed. This is based on the fact that under the conditions used, *i.e.*, heating with the required alcohol without addition of a catalyst, transesterification occurs with esters of β-keto-acids, but not with esters of ordinary aliphatic acids.[7] Moreover, the structure proposed is the only one compatible with the reactions of the compound and the stability of the half-ester (III) derived from it which is described below. Nitrosation of the ester (I) by Adkins and Reeve's method [8] as modified by Albertson *et al.*[9] gave the crystalline α-hydroxyimino-compound (II). Reduction of the latter with stannous chloride in concentrated hydrochloric acid gave the monobenzyl ester (III) of the amino-keto-acid; the ester was isolated in the form of a crystalline toluene-*p*-sulphonate. The removal of tin salts resulting from the reduction of hydroxyimino-compounds by stannous chloride has generally been done by precipitation with hydrogen sulphide after the hydrochloric acid concentration has been lowered by addition of a large volume of water.[10] In the present work it was found that the stannic and stannous chloride can be completely and more conveniently removed by several extractions with ether. The salt of this half ester had m. p. 156·5—158° and did not lose carbon dioxide when heated; it is assumed therefore that the ester group in the β-position to the carbonyl group is still intact. The other ester group must have been split either during the reduction or during the subsequent working up in a strongly acid medium. The preferential hydrolysis of an ester group distal to the charged amino-group has been noted before in this group of compounds [6] and has been ascribed to the operation of electrostatic factors. Hydrogenolysis of the ester (III), with palladium as catalyst, under a variety of conditions yielded only δ-aminolævulic acid (IV). Quantitative aspects of this decarboxylation will be discussed in the following paper.[11]

[1] Shemin and Russell, *J. Amer. Chem. Soc.*, 1953, **75**, 4873.
[2] Neuberger and Scott, *Nature*, 1953, **172**, 1093.
[3] Shemin, Russell, and Abramsky, *J. Biol. Chem.*, 1955, **215**, 613.
[4] Laver, Neuberger, and Udenfriend, *Biochem. J.*, 1958, **70**, 4; Gibson, Laver, and Neuberger, *ibid.*, p. 71; Shemin, Kikuchi, and Bachmann, *Fed. Proc.*, 1958, **17**, 310; Kikuchi, Shemin, and Bachmann, *Biochim. Biophys. Acta*, 1958, **28**, 219; Gibson, *Biochim. Biophys. Acta*, 1958, **28**, 451.
[5] Unpublished experiments quoted by Shemin in "Ciba Foundation Symposium on Porphyrin Biosynthesis," J. and A. Churchill Ltd., London, 1955, p. 9.
[6] Neuberger, Scott, and Shuster, *Biochem. J.*, 1956, **64**, 137.
[7] Bader, Cummings, and Vogel, *J. Amer. Chem. Soc.*, 1951, **73**, 4195; see also Bacon, *Amer. Chem. J.*, 1905, **33**, 68.
[8] Adkins and Reeve, *J. Amer. Chem. Soc.*, 1938, **60**, 1328.
[9] Albertson, Tullar, King, Fishburn, and Archer, *ibid.*, 1948, **70**, 1150.
[10] Gabriel and Posner, *Ber.*, 1894, **27**, 1141.
[11] Laver, Neuberger, and Scott, following paper.



A similar series of reactions was carried out with benzyl acetoacetate. Nitrosation yielded a crystalline α-hydroxyimino-compound which on reduction with stannous chloride gave benzyl α-amino-β-oxobutyrate hydrochloride. After catalytic reduction aminoacetone but no α-amino-β-oxobutyric acid was isolated. In these catalytic reductions a palladium–charcoal catalyst was used in a strongly acid medium; under such conditions the hydroxyimino-group is readily reduced, but the carbonyl group is unaffected, as found by Harington and Randall.[12] However, with a platinum–palladium catalyst,[12] esters of α-amino-β-oxoadipic acid are reduced easily to derivatives of α-amino-β-hydroxyadipic acid which was isolated as a benzyloxycarbonylamino-compound. The material obtained was analytically pure, but probably consisted of a mixture of the two racemic forms as indicated by variations in m. p. of different crystalline fractions.

It seemed not impossible that the metal catalyst used in the catalytic hydrogenation might promote decarboxylation; other methods of preparing α-amino-β-oxoadipic acid were therefore explored. In the war-time penicillin work a crystalline α-hexanoylamino-β-oxopropionic acid was obtained which was unstable on storage.[13] It thus appeared likely that α-acylamino-β-keto-acids might be more stable than the α-amino-β-keto-acids themselves. Various monobenzyl esters of α-acylamino-β-oxoadipic acids and similar compounds in the acetoacetate series were therefore prepared in the hope that the esters might be hydrogenolysed without decarboxylation. The acylamino-linkage might then be split with the aid of enzymes such as amino-acid deacylases or by other methods. The acyl groups used were acetyl, chloroacetyl, benzyloxycarbonyl, and *p*-nitrobenzyloxycarbonyl. This approach was also unsuccessful, as the acylamino-keto-acids, although more stable than the amino-keto-acids, were too labile for isolation.

Similar negative results were obtained when the benzyl α-amino-β-oxo-adipate and -butyrate were treated with hydrogen bromide in glacial acetic acid at room temperature.[14] Liberation of benzyl bromide was slow and was accompanied by evolution of carbon dioxide. The *N*-benzyloxycarbonyl derivatives of the α-amino-β-keto-acids, unlike those of simple amino-acids,[14] were very slowly split by the hydrogen bromide reagent and decarboxylation was always associated with the removal of the acyl group.

Concurrently with these approaches, the reduction of β-oxo-α-phenylhydrazonobutyric acid and of the corresponding derivative of β-oxoadipic acid was explored. But again, apart from aniline, only aminoacetone or aminolævulic acid was obtained and the volume changes [11] occurring during the reduction indicated that decarboxylation under these conditions was even faster than during the hydrogenolysis of the benzyl esters described above. It seemed likely that the aniline formed during the reaction would promote decarboxylation, and the reduction of *N*-acetylphenylhydrazono-acids was therefore examined. But these attempts were also not successful. However, the use of *N*-acetyl derivatives which yield acetanilide on reductive fission [15] permits easy separation by ether-extraction of the two cleavage products of the reduction, as shown by the preparation of

[12] Harington and Randall, *Biochem. J.*, 1931, **25**, 1917.
[13] Cornforth, Cornforth, Abraham, Baker, Chain, and Robinson CPS. 59. Quoted by Cornforth in "The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 805.
[14] Ben-Ishai and Berger, *J. Org. Chem.*, 1952, **17**, 1565.
[15] Bülow and Hailer, *Ber.*, 1902, **35**, 915.

ethyl α-amino-β-oxobutyrate hydrochloride. The method is limited to preparation of the amino-keto-esters, as repeated attempts to isolate free α-*N*-acetylphenylhydrazono-β-oxobutyric acid failed owing to the readiness with which the compound reacted with the solvent to give the phenylhydrazono-acid. It is concluded that α-amino-β-keto-acids were indeed formed in the various reactions studied, but that they were too unstable to be isolated by conventional methods.

Experimental

Almost all evaporations were done under reduced pressure. Ultraviolet absorption data for many of the compounds mentioned here are given in the following paper.[11] Chromatography was done throughout on Whatman No. 1 paper with butan-1-ol–water–acetic acid (63 : 27 : 10, by vol.). Aminolævulic acid was identified by isolation as either the hydrochloride or the toluene-*p*-sulphonate and determination of mixed m. p. or by chromatography (R_F of the hydrochloride = 0·22 [16]); the compound was detected by spraying the paper either with butanolic ninhydrin (yellow colour after 2 min. at 80°) or with ammoniacal silver nitrate (reduction within 2 min., but not immediate as with α-amino-β-keto-esters [6]).

α-Benzyl δ-Ethyl β-Oxoadipate.—Ethyl β-oxoadipate (142 g.) was heated with benzyl alcohol (284 ml.) for 4½ hr. at 140°, a slow stream of nitrogen being passed through the mixture to remove the ethanol liberated. Distillation yielded *α-benzyl δ-ethyl β-oxoadipate* (144 g., 79%), b. p. 164—172°/0·8 mm., 152—160°/0·4 mm. (Found: C, 64·5; H, 6·6. $C_{15}H_{18}O_5$ requires C, 64·6; H, 6·45%).

α-Benzyl δ-Ethyl α-Hydroxyimino-β-oxoadipate.—To a solution of benzyl ethyl β-oxoadipate (144 g.) in glacial acetic acid (300 ml.) was added a solution of sodium nitrite (95% pure; 40·3 g.) in water (90 ml.) during 1 hr., at 25°. Water was then added, just insufficient to cause separation of two phases, and stirring was continued for 1½ hr. The mixture was then extracted with ether; the ether extract was washed free from acid with water and aqueous sodium hydrogen carbonate and dried. Removal of the solvent yielded the *oxime* which on crystallization from benzene–light petroleum (b. p. 40—60°) gave hexagonal plates (142 g., 89%). Recrystallization from chloroform–light petroleum (b. p. 40—60°) gave material of m. p. 72—74° (Found: C, 58·4; H, 5·4; N, 4·3. $C_{15}H_{17}O_6N$ requires C, 58·6; H, 5·6; N, 4·6%).

α-Benzyl Hydrogen α-Amino-β-oxoadipate Toluene-p-sulphonate.—α-Benzyl δ-ethyl α-hydroxyimino-β-oxoadipate (77 g.) was added during 7 hr. to a solution of stannous chloride dihydrate (125 g.) in hydrochloric acid (130 ml.; *d* 1·18). Ethanol (25 ml.) was added at the start of the reaction to facilitate dissolution of the oxime, and the temperature of the reaction mixture was kept below 28°. After all the oxime had dissolved, the solution was left at 20° for 15 hr. and then extracted with ether (4 × 600 ml.). The aqueous residue, on evaporation, gave a viscous oil which solidified to a glass on being dried *in vacuo* over sodium hydroxide but did not crystallize. This material was dissolved in the minimum amount of acetone, and a small amount of insoluble material was removed, consisting of small pyramidal crystals which charred, but did not melt, when heated. Ether was then added and an oil precipitated which again formed a solid glass after removal of the solvent and drying *in vacuo* over sodium hydroxide. Chromatography of this glass on paper showed one major ninhydrin-reacting spot (R_F 0·56) which almost certainly consisted of benzyl hydrogen α-amino-β-oxoadipate hydrochloride, but attempts to crystallize it at this stage failed.

The crude hydrochloride was dissolved in water (600 ml.), and Deacidite G (Permutit Co.) in the basic form (−16 + 50 mesh) was added with stirring until the pH rose to approx. 3·5. At this stage a gelatinous precipitate was formed which was removed together with the resin by rapid filtration. Toluene-*p*-sulphonic acid (43 g.) was added to the filtrate, which on concentration yielded crystalline *benzyl hydrogen α-amino-β-oxoadipate toluene-p-sulphonate* (35 g., 32% based on weight of oxime). Recrystallization from water gave rosettes, m. p. 155—157°, and from dry ethanol–ether needles, m. p. 156·5—158° (Found: C, 54·9; H, 5·5. $C_{13}H_{15}O_5N,C_7H_8O_3S$ requires C, 54·9; H, 5·3%). Paper chromatography gave a single spot of R_F 0·73 after spraying with 1% (w/v) butanolic ninhydrin or ammoniacal silver nitrate solution. The toluene-*p*-sulphonate was converted into the hydrochloride by passing an aqueous solution through a column of ion-exchange resin Deacidite FF (Permutit Co.) in the chloride form. The effluent was taken to dryness *in vacuo* over NaOH, and the residue of

[16] Berlin, Neuberger, and Scott, *Biochem. J.*, 1956, **64**, 80.

α-benzyl hydrogen α-amino-β-oxoadipate hydrochloride crystallized from dry ethanol–ether as needles, m. p. 77—85°. Recrystallization from methanol–ether gave solvated needles, m. p. 82—85° (Found: C, 50·8; H, 6·25; N, 4·4; Cl, 10·6. $C_{13}H_{15}O_5N,HCl,CH_3{\cdot}OH$ requires C, 50·4; H, 6·0; N, 4·2; Cl, 10·6%). Catalytic hydrogenation with palladium–charcoal [12] was done under a great variety of conditions, *i.e.*, by varying the amount of catalyst or solvent or pH, but the only nitrogenous product obtained was aminolævulic acid (these experiments are described in the following paper [11]). The same result was obtained when the charcoal which was used as support for the catalyst was replaced by Celite 545 (Johns-Manville) which had been extensively washed with acid and then water, to remove metallic impurities.

The toluene-*p*-sulphonate of the benzyl ester (2·185 g., 5 mmoles) was dissolved in 50% (w/v) hydrogen bromide in glacial acetic acid (30 ml.) and kept at room temperature. Samples (5 ml.) were withdrawn at intervals, diluted with water (100 ml.), and extracted once with di-*n*-butyl ether (40 ml.). The ether layer was washed twice with water, once with saturated sodium hydrogen carbonate, and again twice with water. Ethanolic 0·1N-silver nitrate (10·0 ml.) was added, and the mixture was set aside for 10 min. Dilute nitric acid (5 ml.) and a few drops of ferric ammonium sulphate solution were then added, and the excess of silver nitrate was estimated by titration with aqueous 0·1N-ammonium thiocyanate. Results were as follows:

Time (min.)	3	25	120	300	1380	Theor.
CH_2PhBr liberated (mmole) ...	0·016	0·048	0·19	0·36	0·57	0·83

There was also evolution of carbon dioxide; this was not accurately measured, but the amount appeared to be smaller than expected on the basis of the benzyl bromide formed.

α-Benzyl Hydrogen α-Acetamido-β-oxoadipate.—To a suspension of the foregoing toluenesulphonate (4·37 g.) in glacial acetic acid (25 ml.) were added successively acetic anhydride (1·5 ml.) and anhydrous sodium acetate (0·82 g.). The mixture was shaken until complete solution was obtained and then left for 20 hr. at 20°. Dry ether (600 ml.) was added and the precipitate of sodium toluene-*p*-sulphonate was filtered off. The filtrate was concentrated to about 100 ml.; adding light petroleum (b. p. 40—60°) precipitated an oil which soon solidified. Recrystallization from hot water gave *benzyl hydrogen α-acetamido-β-oxoadipate* (2·41 g., 78%), m. p. 104—105·5°. Recrystallization from water gave plates (m. p. 105·5—106°), which on drying *in vacuo* over sodium hydroxide formed a powder (Found: C, 58·4; H, 5·6; N, 4·4. $C_{15}H_{17}O_6N$ requires C, 58·6; H, 5·6; N, 4·6%). Further recrystallization from water or from chloroform–light petroleum gave crystals which either softened at 105·5° and melted at 130—131·5° or melted at the higher temperature without preliminary softening. The differences in behaviour could not be correlated with the nature of the solvent used for crystallization. Moreover, it could be shown that the lower-melting form was not a hydrate.

Benzyl Hydrogen α-Chloroacetamido-β-oxoadipate.—To a suspension of benzyl hydrogen α-amino-β-oxoadipate toluene-*p*-sulphonate (6·22 g.) in glacial acetic acid (30 ml.) were added successively chloroacetic anhydride (2·55 g.) and anhydrous sodium acetate (1·168 g.). After 15 hr. ether was added and sodium toluenesulphonate removed. The filtrate was concentrated to about 100 ml. and light petroleum (b. p. 40—60°) was added. The resulting oil, which partially crystallized, was taken up in a large volume of hot water. On cooling, needles separated (1·33 g., 27·5%). Recrystallization from ethanol–water gave needles, m. p. 114—115° (Found: N, 4·1; Cl, 10·3. $C_{15}H_{16}O_6NCl$ requires N, 4·1; Cl, 10·4%).

Ethyl α-Hydroxyimino-β-oxoadipate.—Nitrosation of the ethyl β-oxoadipate was carried out as described above for the corresponding ethyl benzyl ester. After evaporation of the ether, the residue was heated for 2 hr. *in vacuo* on a boiling-water bath to remove traces of water. The product, a light brown oil, was left at −10° for a few weeks until crystallization occurred. The crude *ethyl α-hydroxyimino-β-oxoadipate*, recrystallized from cold (0°) toluene–light petroleum (b. p. 40—60°), gave rectangular needles, m. p. 53—55° (Found: C, 48·9; H, 6·2; N, 5·5. $C_{10}H_{15}O_6N$ requires C, 49·0; H, 6·2; N, 5·7%).

α-Benzyloxycarbonylamino-β-hydroxyadipic Acid.—Ethyl α-amino-β-oxoadipate hydrochloride (10 g.), dissolved in water (75 ml.), was reduced at atmospheric pressure in the presence of platinum–palladium–charcoal prepared as follows.[12] Palladium–charcoal (10 g.) was added to a mixture of platinum chloride (0·5 g.) and ferric chloride (0·025 g.) dissolved in water (10 ml.) containing two drops of concentrated hydrochloric acid; the suspension was then equilibrated with hydrogen. After addition of the ester, hydrogen uptake during 1½ hr. amounted to the

theoretical. The catalyst was filtered off and the filtrate was concentrated to dryness. Ethyl α-amino-β-hydroxyadipate hydrochloride was obtained as a colourless syrup which partly crystallized after some time, but was not purified further. The hydroxy-ester was boiled in 2N-hydrochloric acid (200 ml.) under reflux for 2 hr. The product on evaporation yielded α-amino-β-hydroxyadipic acid as a glass which slowly crystallized but was not further purified. It was dissolved in N-sodium hydroxide (60 ml.) and left at 20° for 15 hr. The solution was then cooled to 0° and benzyl chloroformate (8 g.) was added. The mixture was shaken and N-sodium hydroxide (45 ml.) was added during 30 min., the pH being kept at about 9. The mixture was left for 1 hr. at 0° and then extracted three times with ether. The aqueous layer was acidified with hydrochloric acid until acid to Congo Red and then extracted several times with ethyl acetate. The ethyl acetate extracts were washed twice with water, dried (Na_2SO_4), and filtered. Concentration of the filtrate yielded *α-benzyloxycarbonylamino-β-hydroxyadipic acid* (4·5 g., 40%), m. p. 133—134° (Found: C, 53·8; H, 5·8; N, 4·1. $C_{14}H_{17}O_7N$ requires C, 54·0; H, 5·5; N, 4·5%). On recrystallization from acetone–light petroleum (b. p. 40—60°) the m. p. was 134—135°.

Addition of light petroleum (b. p. 40—60°) to the ethyl acetate liquor yielded crystalline material (m. p. 123—127°) which on recrystallization from ethyl acetate gave material of m. p. 137—138°.

Ethyl α-p-*Nitrobenzyloxycarbonylamino*-β-*oxoadipate*.—A mixture of ethyl α-amino-β-oxoadipate hydrochloride (6 g.) in water (60 ml.) with *p*-nitrobenzyl chloroformate [17] (6 g.) in ether (60 ml.) was cooled to 0°, and magnesium oxide (2·64 g.) was added in portions with intermittent shaking during 15 min. After a further 15 min. at 0°, the mixture was filtered and the ether layer was separated. The aqueous layer was acidified and extracted with ether. The ether extracts were pooled, washed with water and aqueous sodium hydrogen carbonate, dried, and evaporated. The residue on crystallization from cold (0—10°) ethanol–water yielded needles (7·6 g., 82%). On recrystallization from ethanol–water *ethyl* p-*nitrobenzyloxycarbonylamino*-β-*oxoadipate* had m. p. 52—55° (Found: C, 52·7; H, 5·4; N, 6·7. $C_{18}H_{22}O_9N_2$ requires C, 52·7; H, 5·4; N, 6·8%).

Benzyl α-p-*Nitrobenzyloxycarbonylamino*-β-*oxoadipate*.—Ethyl α-*p*-nitrobenzyloxycarbonylamino-β-oxoadipate (2·05 g.) was refluxed under a fractionating column with benzyl alcohol (6·6 g.), toluene-*p*-sulphonic acid (400 mg.), and benzene (60 ml.) for 12 hr. More toluenesulphonic acid (400 mg.), benzyl alcohol (6 g.), and benzene were then added and the mixture was refluxed for a further 1½ hr. Ethanol (270 mg.) was evolved during this period and collected in the distillate. The solution was then washed with water and dried and light petroleum (b. p. 80—100°) was added. An oil was precipitated which partly crystallized after some time at −10°. Filtration yielded a pasty residue which was dissolved in hot ether under pressure. On cooling, crystals formed. These were filtered off and recrystallized from ether (yield 520 mg., 20%). The *benzyl ester* had m. p. 82—84° (Found: C, 62·6; H, 5·1; N, 4·8. $C_{28}H_{26}O_9N_2$ requires C, 62·9; H, 4·9; N, 5·2%). Catalytic hydrogenation (palladium–charcoal [12]) of the ester gave aminolævulic acid. On treatment of the ester with a saturated solution of hydrogen bromide in glacial acetic acid there was a very slow evolution of carbon dioxide during several days. Working up these solutions under a variety of conditions yielded aminolævulic acid as the only identifiable aliphatic compound.

β-*Oxo*-α-*phenylhydrazonobutyric Acid*.—The ethyl ester [18] (2·34 g., 0·01 mole) was dissolved in ethanol (10 ml.), and 3N-sodium hydroxide (0·01 mole) was added (Bülow [19] used 3 equiv. of sodium hydroxide). The free acid was isolated essentially by Bülow's procedure. After recrystallization from hot chloroform–*cyclo*hexane, it had m. p. 161°, in agreement with Bülow. The yield was 96%. The benzyl ester (see below), treated similarly, gave needles, having m. p. 159°, raised to 161° after recrystallization.

Benzyl β-*Oxo*-α-*phenylhydrazonobutyrate*.—Aniline hydrochloride (51·8 g.; 0·4 mole) was dissolved in 3N-hydrochloric acid in a 2 l. flask. Throughout the subsequent diazotization the solution was slowly stirred mechanically and kept at 0—5° by external cooling. A solution of sodium nitrite (30 g.; 95% pure, in 80 ml. of water) was added during about 2 hr., until free nitrous acid persisted in the solution for 5 min. Benzyl acetoacetate (76·9 g., 0·4 mole) mixed with ethanol (500 ml.) was then added, followed at once by powdered sodium acetate (122·5 g.,

[17] Carpenter and Gish, *J. Amer. Chem. Soc.*, 1952, **74**, 3818.
[18] Kjellin, *Ber.*, 1897, **30**, 1965.
[19] Bülow, *Ber.*, 1899, **32**, 200.

0·9 mole of the trihydrate). Cooling was stopped but stirring was continued at a greatly increased rate. After 15 min. most of the sodium acetate had dissolved; the colour of the solution was lemon-yellow. Shortly after the addition of water (100 ml.) spontaneous crystallization of the product yielded a thick paste. Rapid stirring was continued for 30 min.; the crystals were collected and washed with water (1 l.). After drying *in vacuo*, the crude material (m. p. 109—112°) weighed 115 g.; a further crop (2·5 g.) was obtained from the filtrate and washings after refiltration the next day (combined yield close to 100%). *Benzyl β-oxo-α-phenylhydrazonobutyrate* after recrystallization from hot aqueous ethanol had m. p. 98—100° (112 g.). A further crystallization from methanol gave material of m. p. 111·5—113·5° (Found: C, 68·7; H, 5·3. $C_{17}H_{16}O_3N_2$ requires C, 68·9; H, 5·5%). After further recrystallizations from *cyclo*hexane the m. p. were 102—106°, 76—78°, and 76—78° (Found: C, 69·1; H, 5·3%). The analytical samples of low and high m. p. had mixed m. p. 65—69°. When the low m. p. form was recrystallized from hot aqueous ethanol it had m. p. 109—111°. Both forms appeared as golden needles; both were convertible in 98% yield into 3-methyl-1-phenyl-4-phenylhydrazono-5-pyrazolone, whose m. p. (157—158°) was not depressed on admixture with a sample (m. p. 158—159°) prepared similarly from the ethyl ester, by the method of Japp and Klingemann;[20] Eibner[21] found m. p. 158°. Hydrogenation in a freshly prepared solution of hydrogen chloride (2 equiv.) in acetic acid gave aniline hydrochloride and aminoacetone hydrochloride (identified chromatographically).

Benzyl α-(N-*Acetylphenylhydrazono*)-β-*oxobutyrate*.—In contrast to the ethyl ester,[15] preparation of this compound was unsuccessful if an excess of acetic anhydride or an increased proportion of zinc chloride was present, or if heating was prolonged. After short periods of heating with an excess of reagents all the starting material was recovered unchanged; with longer periods of heating a dark brown tar was formed. Anhydrous zinc chloride (100 mg.) was dissolved in acetic acid (50 ml., dried by distillation over $CaSO_4$). Benzyl β-oxo-α-phenylhydrazonobutyrate (2·96 g.; m. p. 76—78°) was added, followed by acetic anhydride (1·02 ml.). The mixture was heated on a boiling-water bath for 1 hr., then rapidly cooled; water (10 ml.) was added slowly and crystallization was induced by scratching. The mixture was filtered, leaving nearly colourless crystals (2·0 g.; m. p. 126—130°); more water (150 ml.) was added to the filtrate, producing a further crop (1·27 g.; m. p. 120—125°). The crops were combined and recrystallized after dissolution in *cyclo*hexane (200 ml.) under reflux, yielding colourless needles (2·4 g., 71%) of the desired N-*acetyl ester*, m. p. 137° (Found: C, 67·5; H, 5·5; N, 8·0. $C_{19}H_{18}O_4N_2$ requires C, 67·4; H, 5·4; N, 8·3%). Yields were consistently 10—15% lower when the high-melting benzyl ester was used. The *N*-acetyl compound was hydrolysed rapidly by hot water, as judged by appearance of a yellow colour.

On catalytic reduction (palladium–charcoal [12]) in acetic acid not containing hydrogen chloride the benzyl group only was removed, yielding a colourless solution which rapidly became yellow in air during removal of the solvent, to give β-oxo-α-phenylhydrazonobutyric acid in quantitative yield. Hydrogenation in acetic acid containing 2 equivs. of hydrogen chloride yielded acetanilide, aniline hydrochloride, and aminoacetone hydrochloride.

Ethyl α-Amino-β-oxobutyrate Hydrochloride.—A suspension of palladized charcoal (3 g.) in a freshly prepared solution of dry hydrogen chloride (1·0 g.) in acetic acid (50 ml.) was equilibrated with hydrogen. A solution of ethyl α-(*N*-acetylphenylhydrazono)-β-oxobutyrate (2·76 g., 0·01 mole) in acetic acid (50 ml.) was then added and worked in with a further 50 ml. of acetic acid. Uptake of hydrogen (0·04 g.-atom) proceeded at a rate compatible with a single first-order reaction ($t_{\frac{1}{2}}$ 5·6 min.). After 1 hr. the catalyst was separated on a centrifuge, and the solvent by distillation, with exclusion of moisture. On extraction of the residue with warm dry ether (3 × 25 ml.) acetanilide (1·27 g., 94%) was removed and identified by m. p. and mixed m. p. (114—115°) after recrystallization from water. The material insoluble in ether crystallized after drying *in vacuo*; recrystallized from dry ethanol–ether it had m. p. 113·5—114·5° (decomp.), raised to 114—116° when mixed with the hydrochloride (m. p. 115—117°; decomp.) prepared by hydrogenation of the α-hydroxyimino-ester. Gabriel and Posner[10] gave m. p. 95° (decomp.). Potentiometric titration showed a $pK' = 5·2$; the equiv. wt. was 181 (calc. for $C_6H_{11}O_3N,HCl$: 181·6). The yield was 1·5 g. (83%).

Ethyl α-(N-*Acetylphenylhydrazono*)-β-*oxoadipate*.—Ethyl β-oxoadipate (20·4 g., 0·1 mole) in ethanol (125 ml.) was brought into reaction with benzenediazonium chloride (prepared on

[20] Japp and Klingemann, *Annalen*, 1888, **247**, 190.
[21] Eibner, *Ber.*, 1903, **36**, 2687.

one-quarter the scale described above) by addition of sodium acetate (47·6 g. of the trihydrate). Since the product was an oil, the mixture was transferred to a separatory funnel and extracted with benzene (3 × 150 ml.). The extracts were washed with water (5 × 100 ml.) and dried; benzene was removed by distillation. The yield of the crude phenylhydrazono-compound was theoretical. To this material was added a suspension of crushed anhydrous zinc chloride (1 g.) in acetic acid (100 ml.), followed by acetic anhydride (9·45 ml.). After 1 hour's heating on a boiling-water bath the product was crystallized and separated as described for the corresponding derivative of benzyl acetoacetate. Recrystallization was first from ethanol and then from *cyclo*hexane, giving material of m. p. 98—99° and 98·5—99·5° respectively. The pure N-*acetyl compound* (7·6 g., 23·7%) formed colourless octagonal plates (Found: C, 59·7; H, 6·1. $C_{16}H_{20}O_5N_2$ requires C, 60·0; H, 6·3%).

3-2′-*Ethoxycarbonylethyl*-1-*phenyl*-4-*phenylhydrazono*-5-*pyrazolone*.—By the procedure described [20] for preparation of the 3-methyl analogue of the foregoing pyrazolone, crude ethyl β-oxo-α-phenylhydrazonoadipate was cyclized with phenylhydrazine to the corresponding pyrazolone in 82% yield. The product recrystallized from hot benzene–ethanol. During subsequent cooling the orange-yellow needles (m. p. 115—117°) underwent a reversible allomorphic change, below about 40°, to orange rectangular plates. When these were heated in a Kofler apparatus, they lost their specific form between 40° and 60° (depending on the rate of heating) before melting at 115—117°. A similar change attended a further crystallization from *cyclo*hexane–ethanol, giving hexagonal plates of m. p. 117—118° after transition below 60° (Found: C, 65·9; H, 5·3; N, 15·3. $C_{20}H_{20}O_3N_4$ requires C, 65·9; H, 5·5; N, 15·4%).

Benzyl α-Hydroxyimino-β-oxobutyrate.—By using the proportions of reagents described below but otherwise following the procedure of Adkins and Reeve [8] for preparation of the corresponding ethyl ester, the desired product was obtained on the first occasion as a pale yellow oil which crystallized after keeping for a day at −10° (yield 90%). Subsequent preparations were simplified and the yield improved by seeding the reaction mixture. This modification is of no use in preparation of the ethyl ester, because crystals of the latter compound form an oil in the presence of water.

Benzyl acetoacetate (192 g., 1·0 mole) was mixed with acetic acid (200 ml.) in a cooled 2 l. flask. A solution of sodium nitrite (77·6 g., 95% pure) in water (160 ml.) was added slowly (about 1 drop/2 sec. during 1·5 hr.) with mechanical stirring so that the temperature was between 23° and 25° and the flask contents remained colourless. When most of the nitrite solution (150 ml.) had been added further addition caused development of a brown colour. A further portion (5 ml.) was added dropwise 15 min. later, followed by water until there was a persistent faint cloudiness. The mixture was then seeded and the stirring rate increased; as more water (total 1 l.) was added during 1 hr., colourless crystals separated. An hour later the crystals were removed and washed with water. After drying, *benzyl α-hydroxyimino-β-oxobutyrate* had m. p. 76—78°; the yield was theoretical. A small sample recrystallized from benzene–*cyclo*hexane gave needles, m. p. 79—79·5° (Found: C, 60·0; H, 4·9. $C_{11}H_{11}O_4N$ requires C, 59·7; H, 5·0%).

Benzyl α-Amino-β-oxobutyrate Hydrochloride.—Stannous chloride dihydrate (100 g.) was dissolved in a mixture of ethanol (30 ml.) and hydrochloric acid (100 ml.; *d* 1·18) contained in a 2 l. flask, placed in a water-bath (16°) and fitted with a mechanical stirrer. To the solution was added finely powdered benzyl α-hydroxyimino-β-oxobutyrate (44·24 g., 0·2 mole) at such a rate that the temperature was just below 30°. When all the oxime had been added and the temperature had started to fall, the bath was warmed to 30°, stirring being continued for 1 hr. after all the solid had dissolved (extraction with light petroleum at this stage showed that less than 1% of the oxime remained). The solution was then cooled to 10° and ether (1 l.) was added. After addition of most of the ether the phases separated and the ethereal layer, which contained most of the stannous and stannic chloride, was discarded. After addition of water (100 ml.) the aqueous layer was again extracted with ether (3 × 500 ml.); at this stage the aqueous solution contained no tin, as judged by treatment with hydrogen sulphide. The yield of crystalline material depended on the speed at which water and excess of acid could be removed; titration showed that initially the yield of material having pK' 5·4 (see below) was theoretical. After ether-extraction, therefore, the liquid was transferred to an evaporating basin and left overnight in a desiccator (1 mm. Hg) containing sodium hydroxide. The crystals which resulted were removed by flotation with dry ether, followed by filtration and washing with dry ether in a filter-funnel protected from atmospheric moisture. (Only by this method could the product be obtained free from excess of hydrogen chloride and solid impurities. The crystals pass

readily into the ethereal phase on swirling of the aqueous suspension with ether; the resulting ethereal suspension can then be decanted before the crystals settle at the interface.) The desiccation and flotation were then repeated with the aqueous mother-liquor. After five such fractionations the yield of *benzyl α-amino-β-oxobutyrate hydrochloride* was 39·4 g. (81%) of colourless needles, m. p. 135—137° (decomp.), p*K'* 5·4 (Found: C, 53·9; H, 5·8; N, 6·0; Cl, 14·5. $C_{11}H_{13}O_3N$,HCl requires C, 54·2; H, 5·8; N, 5·8; Cl, 14·6%).

Crystalline material obtained by rapid and complete removal of water, rather than by flotation into ether, was found by titration and titrimetric determination of chloride to contain 71% of the desired ester hydrochloride, exactly one equivalent of excess of hydrogen chloride, and 11·6% of an unidentified solid, insoluble above pH 2 and in organic solvents, which charred above 400° without melting. The pure dry hydrochloride is stable for several weeks, but the impure dry solid darkens in a few days. Solutions of the hydrochloride in hydrochloric acid slowly decompose, forming benzyl chloride (identified by its odour, and by extraction with ether to give an oil of b. p. 175—180°) and aminoacetone hydrochloride (identified chromatographically).

Benzyl 3 : 6-*Dimethylpyrazine*-2 : 5-*dicarboxylate*.—To a solution of the foregoing hydrochloride (2·44 g., 0·01 mole) in water (25 ml.) was added one equivalent of N-sodium hydroxide. The solution was left exposed to the air for a week, with occasional shaking. The white crystalline precipitate was removed, dried, and recrystallized from ether–light petroleum (b. p. 60—80°), yielding 1·66 g. (88%) of the desired *ester*, as colourless needles, m. p. 108°, $\lambda_{max.}$ 288 mμ (log ε 4·06) in methanol (Found: C, 69·9; H, 5·3; N, 7·5. $C_{22}H_{20}O_4N_2$ requires C, 70·2; H, 5·4; N, 7·4%).

Ethyl α-Chloroacetamido-β-oxobutyrate.—Crystalline ethyl α-hydroxyimino-β-oxobutyrate [9] (15·92 g., 0·10 mole) and chloroacetic anhydride (34·2 g., 0·20 mole) were dissolved in acetic acid (50 ml.). This mixture was shaken under hydrogen for 5 hr. with palladized charcoal [12] (4 g.), by which time the theoretical quantity of hydrogen had been absorbed. After removal of the catalyst the acetic and chloroacetic acid and excess of anhydride were removed at 5 mm. from an oil-bath whose final temperature (120°) was maintained for 1 hr. The dark brown residue was dissolved in a hot 10% (v/v) solution (100 ml.) of ethanol in water and treated with charcoal. After filtration and cooling the water was extracted with chloroform (6 × 25 ml.). The chloroform extracts were dried and concentrated, and boiling light petroleum (b. p. 80—100°) was added. The solution, on slow cooling, yielded yellow crystals, m. p. 76—78°. These were recrystallized twice from ether, giving colourless needles (14·6 g., 66%) of the *chloroacetamido-ester*, m. p. 80—81° (Found: C, 43·3; H, 5·5; N, 6·7; Cl, 16·2. $C_8H_{12}O_4NCl$ requires C, 43·4; H, 5·5; N, 6·3; Cl, 16·0%).

Benzyl α-Chloroacetamido-β-oxobutyrate.—Benzyl α-amino-β-oxobutyrate hydrochloride (21·95 g., 0·09 mole) was added to a solution of chloroacetic anhydride (17·1 g., 0·10 mole) in acetic acid (100 ml.). To the mixture was added anhydrous sodium acetate (24·6 g., 0·3 mole), whereupon the temperature rose to 60°. The next day ether (250 ml.) was added and the precipitated sodium salts were removed by filtration. The ethereal solution was transferred to a separatory funnel and shaken with dilute sodium hydroxide solution; sufficient of the latter was added to bring the pH of the aqueous phase to between 5 and 6 one hour after the last addition. The aqueous phase was then separated and extracted twice with ether; the combined ethereal phases were washed with sodium hydrogen carbonate solution and finally with water. The ether was removed and the residue crystallized from methanol–water as light yellow clusters of needles, m. p. 97—98° (15·3 g., 60%). Recrystallized after solution in hot (90°) water, *benzyl α-chloroacetamido-β-oxobutyrate* formed colourless needles, m. p. 98° (Found: C, 55·3; H, 5·1; N, 4·9; Cl, 12·0. $C_{13}H_{14}O_4NCl$ requires C, 55·0; H, 5·0; N, 4·9; Cl, 12·5%).

Ethyl α-Acetamido-β-oxobutyrate.—This compound was prepared by catalytic reductive acetylation of the crystalline oxime as described.[9,22] The product, purified by recrystallization from dry ether–light petroleum (b. p. 60—80°) at −10°, had m. p. 48° (Albertson *et al.*[9] give m. p. 46—49°; Wiley and Borum [22] give m. p. 46—47·5°). By titration in water with sodium hydroxide, the apparent p*K'* corresponding with the ionization of the enol was found to be 8·72 (Found: equiv., 187·2. Calc. for $C_8H_{13}O_4N$: equiv., 187·2); by spectrophotometric titration a value of 8·1 was found [11] for the corresponding chloroacetyl compound.

Benzyl α-Benzyloxycarbonylamino-β-oxobutyrate.—Benzyl α-hydroxyimino-β-oxobutyrate (0·10 mole) was reduced with stannous chloride, and the reaction mixture was extracted with

[22] Wiley and Borum, *J. Amer. Chem. Soc.*, 1948, **70**, 1666.

ether, as described above. The residual aqueous solution was kept at 0° while small quantities of benzyl chloroformate (total 15 ml.) and 10N-sodium hydroxide (total 10 ml.) were added alternately in 2 hr. with vigorous stirring. The mixture was left overnight at room temperature and during the next day further small quantities of sodium hydroxide solution were added until the pH remained above 6. A precipitate (of the unidentified solid already mentioned) was allowed to remain, and the mixture was set aside for two weeks, crystallization of the desired product then commencing. After removal of crystals for seeding, the mixture was extracted with ether (5 × 100 ml.); the combined ethereal extracts were washed with dilute sodium carbonate and water, dried, and concentrated to about 50 ml. On addition of light petroleum (b. p. 100—120°) and seeding, yellow needles (m. p. 75—76°) were formed very slowly. After recrystallization from the same solvents and then twice from ethanol–water, *benzyl α-benzyloxycarbonylamino-β-oxobutyrate* formed colourless needles (18·8 g., 55% from the oxime), m. p. 77—77·5° (Found: C, 66·9; H, 5·7. $C_{19}H_{19}O_5N$ requires C, 66·8; H, 5·6%).

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