The Synthesis of δ-Succinamidolævulic Acid and Related Compounds.

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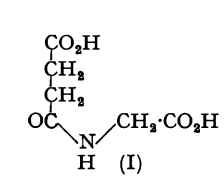
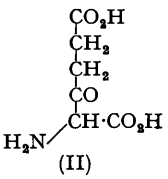
δ-Chloro- and δ-*N*-substituted derivatives of lævulic acid have been prepared. The conversion of succinimido-compounds into the corresponding *N*-substituted succinamic acids has been studied.

Succinamido[α-^{14}C]acetic acid, δ-[^{15}N]aminolævulic acid hydrochloride, and δ-succinamidolævulic acid have been synthesised in order to study their possible rôles in porphyrin biosynthesis.

PREVIOUS work on the biosynthesis of protoporphyrin has shown that, for each molecule formed, eight molecules of glycine and eight molecules of a compound derivable from α-oxoglutarate are required (Wittenberg and Shemin, *J. Biol. Chem.*, 1950, **185**, 103; Muir and Neuberger, *Biochem. J.*, 1950, **47**, 97). On the basis of work with isotopically labelled acetate and succinate, Shemin and Kumin (*J. Biol. Chem.*, 1952, **198**, 827) postulated an asymmetric derivative of succinic acid as the compound which condenses initially with glycine; they also suggested that succinyl-coenzyme A might be the required "active" succinate. For many years it has been considered likely that the first tetrapyrrole formed is uroporphyrin. Each pyrrole ring in this compound carries carboxyl-bearing side chains in the two β-positions, and Neuberger, Muir, and Gray, (*Nature*, 1950, **165**, 948) suggested a mechanism of porphyrin biosynthesis, according with the isotope data then available, by which the first pyrrolic compound formed was such a dicarboxylic pyrrole (V) with free α-positions. It was assumed in this theory that the methene-bridge carbon atoms (derivable from the α-carbon atom of glycine) were condensed with the free α-positions at a later stage in order to account for the occurrence only of series I and III porphyrins in Nature, with the latter predominant.

Accordingly, we set out in 1952 to synthesise a number of compounds formally derivable from glycine condensed with one or two molecules of succinic acid. By *N*-succinylation succinamidoacetic acid (I), and by α-*C*-succinylation α-amino-β-oxoadipic acid (II) would be formed. The latter compound would be expected to lose the glycine carboxyl group readily to give δ-aminolævulic acid (III). By addition of a second molecule of succinic acid, (I) or (III) could be converted into δ-succinamidolævulic acid (IV). This compound, by a reductive


(I)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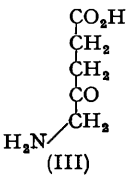
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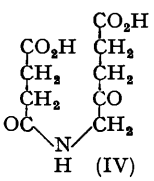
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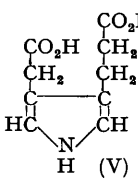
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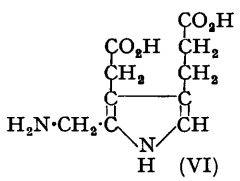
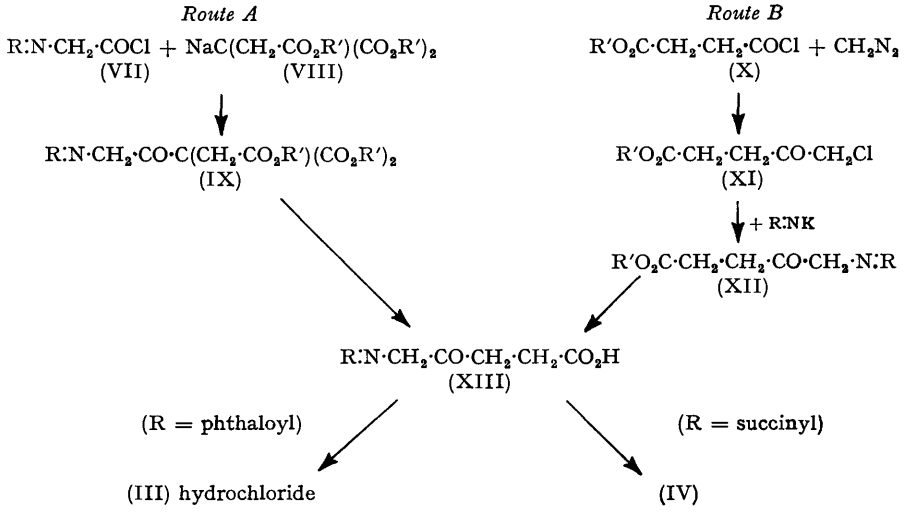

(VI)

self-condensation, might then form the pyrrole (V). Alternatively, a different pyrrole (VI) having the structure assigned to porphobilinogen by Cookson, Rimington, and Kennard (*Nature*, 1953, **171**, 875), might arise from a Knorr-type condensation of two molecules of (III). The aminomethyl α-substituent (which has arisen from glycine) would then be expected to give rise to the methene carbon atoms of the resulting uroporphyrin. It was thus desirable to be able to prepare (I), (III), and (IV) labelled either with ^{15}N or with ^{14}C at any position in the molecule, but especially in the position corresponding to the α-carbon atom of glycine, *viz.*, succinamido[α-^{14}C]acetic acid and [δ-^{14}C]lævulic acid derivatives.

A brief summary of the syntheses described below has been given in a preliminary communication (Neuberger and Scott, *Nature*, 1953, **172**, 1093). Only one of these compounds, (III), turned out to be a precursor (Shemin and Russell, *J. Amer. Chem. Soc.*, 1953, **75**, 4873; Neuberger and Scott, *loc. cit.*) and an outline of syntheses of labelled (III) was also given by Shemin and Russell. The synthesis of *labelled* (IV) was never eventually undertaken, since it was eliminated as a possible precursor by use of a test procedure not requiring isotopic labelling (Neuberger and Scott, *loc. cit.*), but succinamido[α-^{14}C]acetic acid and [^{15}N]aminolævulic acid hydrochloride were prepared.

Uniquely δ-substituted derivatives of lævulic acid are almost unknown. Windaus *et al.* (*Ber.*, 1921, **54**, 2745) prepared δ-benzamidolævulic acid by a Bamberger fission of ethyl β-4(5)-glyoxalylpropionate; Wynn and Corwin (*J. Org. Chem.*, 1950, **15**, 203) prepared the hydrochloride of (III) in low yield by reduction of methyl δ-hydroxyimino-acetylacrylate. As far as we are aware no other δ-substituted derivatives have been described; the βδ-dihalogeno-derivatives are of course well known. It was at once apparent that a Dakin and West reaction between glycine and succinic anhydride might lead directly to (IV). This was attempted under a variety of conditions, but no crystalline material could be isolated, nor was there any evidence that the reaction had proceeded in the desired direction. Considering first (III) and (IV), two main lines of approach were then explored; these are set out schematically below.



By route A, an imidoacetyl chloride (VII) was condensed with an appropriate malonic ester derivative (VIII), followed by removal of the ester groups and decarboxylation, to give the δ-imidolævulic acid (XIII); an imido[α-^{14}C]acetyl chloride would thereby yield a [δ-^{14}C]lævulate. The imide ring would then either be opened with alkali (XIII; R = succinyl) to give (IV), or else be completely hydrolysed to the hydrochloride of (III). It is known that the ester groups of acylalkylmalonates cannot in general be removed by hydrolysis without splitting the whole molecule in such a way as to reverse the condensation (Bowman, *J.*, 1950, 325; Fonken and Johnson, *J. Amer. Chem. Soc.*, 1952, **74**, 831). Following therefore Bowman's method (*loc. cit.*), benzyl sodioethane-1 : 1 : 2-tricarboxylate (VIII; R' = CH_2Ph) was condensed with phthaloyl- or succinyl-glycyl chloride. On catalytic reductive de-esterification and decarboxylation a low yield (34%) of the phthalimido-compound (XIII; R = phthaloyl) was obtained. With the succinimido-derivative (IX; R = succinyl) uptake of hydrogen was low, and a crystalline compound could not be isolated, nor was an alternative method (Bowman and Fordham, *J.*, 1952, 3945) employing 2 : 3-dihydropyran (VIII; R' = tetrahydropyran-2-yl) more fruitful.

Route B was preferred for preparation of both (III) and (IV). Methyl δ-chlorolævulate

(XI; R′ = Me) was characterised by hydrolysis to the free acid and by conversion into thiazole derivatives. The melting points of these compounds, particularly that of the free base, did not agree with those reported by Ziegler (*J. Amer. Chem. Soc.*, 1944, **66**, 744). His synthesis was therefore repeated, giving products whose properties, including the *X*-ray diffraction pattern, agreed with those of the thiazoles prepared from (XI). The α-chloro-ketone (XI) was condensed with potassium phthalimide or succinimide in anhydrous dimethylformamide (cf. Sheehan and Bolhofer, *ibid.*, 1950, **72**, 2786). We found (at least with α-halogeno-ketones) that anhydrous potassium carbonate and succinimide may be used in this condensation, in place of the less available anhydrous potassium succinimide, without loss in yield (cf. Ing and Manske, *J.*, 1926, 2348). The succinimide ring opens readily on addition of alkali, as was shown by plotting pH change against time after addition of sodium hydroxide to the ester (XII; R = succinyl, R′ = Me). The free acid (IV) was isolated crystalline after treatment of a solution of the disodium salt with an acidic ion-exchange resin. It is necessary to hydrolyse the ester first with cold hydrochloric acid, since the ketonic ester (XII) is unstable to excess of alkali. However, when this procedure was applied to methyl succinimidoacetate, acid hydrolysis of the ester was found to proceed much less readily, and attempts to isolate the free acid (I) after treatment with sodium hydroxide gave a clear " glass " which appeared to be a loosely bound polymer of glycine and succinic acid. By treating succinimidoacetic acid with baryta and removal of the latter as the sulphate, crystalline (I) was obtained.

Experimental

M. p.s were determined with a Kofler apparatus.

Anhydrous dimethylformamide was prepared in 80% yield by formylation of dimethylamine with chloral (cf. Blicke and Chi-Jung Lu, *J. Amer. Chem. Soc.*, 1952, **74**, 3933). It was fractionated in a vacuum-jacketed Widmer column, material distilling at 153·5—154° being collected. Traces of water in the dimethylformamide lower the yields from Gabriel condensations in this solvent (cf. Cox and Warne, *J.*, 1951, 1896).

Succinyl- and Phthaloyl-glycyl Chlorides (VII).—Glycine was fused with succinic (Scheiber and Reckleben, *Ber.*, 1913, **46**, 2412) or phthalic (Drechsel, *J. pr. Chem.*, 1883, **27**, 418) anhydride, and the cooled product was treated with excess of thionyl chloride. After distillation of the unchanged thionyl chloride with added dry toluene at reduced pressure, the imidoacetyl chloride was recrystallised from benzene–light petroleum (b. p. 60—80°); (VII; R = succinyl), yield 60%, m. p. 79—81° (Scheiber and Reckleben, *loc. cit.*, give 76°); (VII; R = phthaloyl), yield 90%.

δ-Phthalimidolævulic Acid (XIII; R = phthaloyl).—Phthaloylglycyl chloride (44·7 g., 0·2 mole) was brought into reaction with the sodio-derivative of benzyl ethane-1 : 1 : 2-tricarboxylate (VIII; R′ = benzyl) (Bowman, *J.*, 1950, 325) (0·22 mole), this being followed by debenzylation in dry ethyl acetate. After two changes of catalyst (palladised strontium carbonate) uptake of hydrogen was slightly in excess of theoretical. On decarboxylation in boiling toluene and removal of the solvent in an oil-bath, a pale yellow viscous material remained. After 3 weeks, crystallisation commenced and on working up the material by Bowman's procedure 18 g. (34%) of crude product were obtained. Recrystallised from ethyl acetate, twice from water, and twice from benzene, *δ-phthalimidolævulic acid* melted at 158·5° (Found: C, 60·0; H, 4·4; N, 5·1. $C_{13}H_{11}O_5N$ requires C, 59·77; H, 4·25; N, 5·4%). A small quantity of the compound was esterified with ethanol–hydrogen chloride, and the resulting *ethyl δ-phthalimidolævulate*, recrystallised from hot (80°) water, had m. p. 79° (Found: C, 62·2; H, 5·35; N, 4·8. $C_{15}H_{15}O_5N$ requires C, 62·3; H, 5·2; N, 4·85%).

β-Methoxycarbonylpropionyl Chloride (X; R′ = Me).—Succinic anhydride (100 g., 1 mole) was refluxed with excess (95 c.c.) of dry methanol, heating being continued for 3·5 hr. after a clear solution had been obtained. On removal of the excess of methanol *in vacuo*, crystalline methyl hydrogen succinate was obtained in theoretical yield (cf. Riegel and Lilienfeld, *J. Amer. Chem. Soc.*, 1945, **67**, 1273). After recrystallisation from benzene–*cyclo*hexane, the half-ester (132 g., 1 mole) was treated with excess of thionyl chloride (93 c.c.) and set aside for 18 hr. The mixture was then warmed to 45° for 2 hr., 100 c.c. of dry toluene were added, and the excess of thionyl chloride and toluene removed *in vacuo* at 50°. On fractional distillation (10 cm. Vigreux column) the ester-chloride distilled at 63—65°/3 mm. (131 g., 87%).

Methyl δ-Chlorolævulate (XI; R′ = Me).—To a solution of diazomethane (0·76 mole) in dry ether (1270 c.c.), cooled to below −5°, was added with mechanical stirring a solution of the fore-

going chloride (56 g., 0·37 mole) in ether (200 c.c.) during 2 hr. The mixture was allowed to warm to room temperature overnight. The diazo-ketone had then partly separated as an oil which disappeared on addition of chloroform (200 c.c.). A small amount of fluffy precipitate was removed by filtration into a 2-l. three-necked flask. This was fitted with a reflux condenser, mechanical stirrer, and gas bubbling tube. Dry hydrogen chloride was passed in, resulting in a brisk evolution of nitrogen, and refluxing of the ether; passage of gas was continued for 1·5 hr. after visible evolution of nitrogen had ceased. The solution was left for 48 hr. at 5°, then concentrated to about 400 c.c. *in vacuo* from a bath at room temperature. It was then washed with water (3 × 100 c.c.) to remove the hydrogen chloride, and the combined water washings were extracted with ether (6 × 100 c.c.), since the ketone ester is quite soluble in water. The combined ether extracts were dried ($MgSO_4$) and the solvent was removed. The residual material distilled at 92—94°/2—3 mm., 79—80°/1 mm. (12-cm. Vigreux column), yielding 34·7 g. (57% based on the acid chloride) of pure *methyl δ-chlorolævulate* (Found: C, 43·9; H, 5·5; Cl, 21·7. $C_6H_9O_3Cl$ requires C, 43·8; H, 5·5; Cl, 21·5%).

Ethyl δ-chlorolævulate (XI; R′ = Et), prepared in a similar way, had b. p. 106—112°/1—2 mm.

The methyl ester (2·2 g.) was added to 6 c.c. of 7N-hydrochloric acid; solution took place almost immediately. The mixture was left for 28 hr. at 18° and taken to dryness *in vacuo*. The residue was left overnight *in vacuo* over potassium hydroxide, and then crystallised twice from dry ether–light petroleum (b. p. 60—80°), giving plates of the free *acid*, m. p. 72—73° (Found: C, 40·2; H, 4·75; Cl, 24·1. $C_5H_7O_3Cl$ requires C, 39·9; H, 4·7; Cl, 23·5%).

3-*Aminothiazole Derivatives*.—Methyl δ-chlorolævulate (1·45 g.) was dissolved in 1 c.c. of ethanol; thiourea (0·8 g.) in water (1 c.c.) was added, and the mixture shaken for 1 hr. The solution became warm and deposited crystals of the thiazole ester hydrochloride. On addition of ammonia solution (*d* 0·88; 1·2 c.c.) the crystals dissolved, but those of the free base appeared on cooling. After two crystallisations from 50% methanol, *methyl* β-3-*amino*-4(5)-*thiazolylpropionate* melted at 94—96° (Found: C, 45·3; H, 5·2; N, 15·3; S, 17·1. $C_7H_{10}O_2N_2S$ requires C, 45·15; H, 5·4; N, 15·05; S, 17·2%). The amino-ester was treated with 20% hydrochloric acid on a boiling-water bath. The crystals of β-3-amino-4(5)-thiazolylpropionic acid hydrochloride which formed on cooling were filtered off and recrystallised from 2N-hydrochloric acid; they had m. p. 244—246° (decomp.), with some darkening at 241° (Ziegler, *loc. cit.*, gives m. p. 243—244°) (Found: C, 34·4; H, 4·1; N, 12·85. Calc. for $C_6H_8O_2N_2S,HCl$: C, 34·5; H, 4·35; N, 13·4%). The hydrochloride (0·63 g.) was suspended in water (5 c.c.), and dilute aqueous ammonia was added until the solution was just alkaline to litmus, then one drop of dilute hydrochloric acid was added (pH = 5). The solution was concentrated, and the free base filtered off. After recrystallisation from water to constant m. p., β-3-amino-4(5)-thiazolylpropionic acid had m. p. 195—196°. A sample prepared by Ziegler's method had m. p. 195—197°, mixed m. p. 195° (Ziegler, *loc. cit.*, gives m. p. 213—214°). Comparison of the *X*-ray diffraction pattern of crystals of the free base (m. p. 196°) prepared by these two routes showed the following interplanar spacings, in order of decreasing intensity: 3·38, 4·85, 6·37 (from the chloro-ketone); 3·38, 4·87, 6·24 (Ziegler's route). It was concluded that the two substances were identical.

Methyl δ-[^{15}N]*Phthalimidolævulate* (XII; R = phthaloyl, R′ = Me).—Potassium phthalimide (30·7 atoms % excess ^{15}N; 10·35 g., 0·0555 mole) was dissolved in 50 c.c. of anhydrous dimethylformamide; to this was added methyl δ-chlorolævulate (9·137 g., 0·0555 mole) which was washed in with 5 c.c. of dimethylformamide. The mixture was shaken for ½ hr., then warmed at 60° for an hour. After isolation (cf. Sheehan and Bolhofer, *loc. cit.*) and recrystallisation from boiling water (2 l.), needles of *methyl* δ-[^{15}N]*phthalimidolævulate* had m. p. 96—97° (9·6 g.); a further 1·2 g. (m. p. 96—97°) were obtained on evaporation of the mother-liquor to 250 c.c., and treatment with charcoal; the total yield was 70·5% (this rather low yield may have been due to traces of moisture in the potassium [^{15}N]phthalimide; with non-isotopic material yields were generally over 80%) (Found: C, 61·0; H, 4·9; N, 4·9. $C_{14}H_{13}O_5N$ requires C, 61·1; H, 4·8; N, 5·1%). Ethyl δ-phthalimidolævulate was prepared similarly (80% yield); after two crystallisations from hot (80°) water, it had m. p. 79°, undepressed on admixture with the same compound prepared by route A.

Methyl δ-Succinimidolævulate (XII; R = succinyl, R′ = Me).—Powdered succinimide (9·9 g., 0·10 mole) was suspended in dimethylformamide (25 c.c.); on gentle warming, most of the solid went into solution. To this was added methyl δ-chlorolævulate (16·456 g., 0·10 mole), washed in with solvent. Anhydrous potassium carbonate (13·82 g., 0·10 mole) was added (in small portions to minimise the possibility of ring-opening by excess of carbonate) from a

stoppered weighing bottle, the mixture being shaken after each addition. When most of the carbonate had been added, heat was evolved (a transitory orange colour may develop, depending mainly on the purity of the succinimide). The last of the carbonate was washed in, and the flask stoppered and left overnight, reaction being completed by warming to 60° for 1 hr. Sheehan and Bolhofer's procedure (*loc. cit.*), designed for the isolation of phthalimido-compounds, was followed except that in place of the alkali wash the organic layer was washed twice with 5% sodium hydrogen carbonate; these washings were then extracted twice with chloroform. On evaporation of the dried chloroform extracts at reduced pressure (14 mm.) on a water-bath at 90° (a temperature high enough to remove residual dimethylformamide, but not sufficient to cause loss of succinimido-ester), the contents of the flask solidified spontaneously on cooling; the yield of crude product was 88% (85—90% when anhydrous potassium succinimide was used); the pale brown crystals had m. p. 61—62°. The crude solid was recrystallised twice from ether–light petroleum (b. p. 40—60°) (the hot solution required to be well seeded by adding a suspension of crystals in light petroleum), giving needles of *methyl δ-succinimidolævulate*, m. p. 75·5° (Found: C, 53·1; H, 5·8; N, 6·1. $C_{10}H_{13}O_5N$ requires C, 52·85; H, 5·8; N, 6·2%). The compound is remarkably soluble in cold water. Light absorption ($\lambda_{max.}$ 2675—2685 Å; $\varepsilon_{max.}$ 35·6) in aqueous solution is of the order expected from the ketone carbonyl group (cf. Braude, *Ann. Reports*, 1945, **42**, 112). By following the change of pH potentiometrically, on adding one equiv. of sodium hydroxide to a 0·1M-solution, it was found that opening of the imide ring was virtually complete after 1 hr. at 20°. From this solution a crystalline compound, presumably the sodium salt of methyl δ-succinamidolævulate, was the sole product. If two equivalents of alkali were added, after a few hours a brown " oil " separated on evaporation of most of the water at room temperature, and the reducing properties of the ketone were lost.

δ-Succinimidolævulic Acid (XIII; R = succinyl).—Methyl δ-succinimidolævulate (11·358 g., 0·05 mole) was dissolved in 7N-hydrochloric acid (25 c.c.) and left at room temperature for 7 days, by which time a faint brown colour had appeared. The mixture was then warmed to 40° and treated with charcoal, filtered, and left at 0° for 2 days, yielding needles, m. p. 125—128°. Mineral acid was removed by distillation at reduced pressure (10 mm.) from a bath at 30°; the yield of crude product was theoretical. Recrystallisation twice from chloroform–*cyclo*hexane gave *δ-succinimidolævulic acid* as stellate clusters of needles (9·8 g., 92%), m. p. 129° (Found: C, 50·8; H, 5·4; N, 6·3. $C_9H_{11}O_5N$ requires C, 50·7; H, 5·2; N, 6·6%).

δ-Succinamidolævulic Acid (IV).—δ-Succinimidolævulic acid (8·527 g., 0·04001 mole) was weighed into a 50-c.c. Emich filter beaker fitted with a grade-3 sintered filter. Water (10 c.c.) was added, followed by 20 c.c. of 2N-sodium hydroxide from a burette; a pink colour developed until most of the acid was in solution, whereupon the colour faded. A further 20 c.c. of alkali were added slowly (total 0·0800 mole), whereupon a marked yellow colour developed. Two hours after addition of the alkali, the mixture was warmed for 5 min. on a bath at 40°, then Zeokarb 225 (14 g.) was added. The mixture was left at 20° for 4 hr. with occasional shaking and then filtered, treated with charcoal at 40° for 15 min., and refiltered, and the acid allowed to crystallise as water was slowly removed in a vacuum-desiccator, giving a quantitative yield of crude product, m. p. 180—190°. Fractional crystallisation from water gave rectangular white plates of *δ-succinamidolævulic acid*: 6·2 g., m. p. 197—199°; 2·4 g., m. p. 185—190°; total yield 92% (Found: C, 46·6; H, 5·8; N, 6·2.. $C_9H_{13}O_6N$ requires C, 46·8; H, 5·7; N, 6·1%). The disodium salt of the acid becomes yellow on melting and loses water; a solution of the melt in methanol gives an immediate intense violet colour in the cold with Ehrlich's reagent, with an absorption band at 553 mμ. When kept at the m. p. until effervescence has ceased, the material darkens and crystals appear which char above 260° without melting. This change may indicate formation of a pyrrole and is being investigated further.

δ-[^{15}N]*Aminolævulic Acid Hydrochloride* (III).—By refluxing (IV), (XII), or (XIII) for 8 hr. with 7N-hydrochloric acid (10 c.c./g. of imide), the amino-acid hydrochloride could be prepared in theoretical yield. The ^{15}N-labelled amino-acid was prepared by hydrolysis of methyl δ-[^{15}N]-phthalimidolævulate. On cooling, phthalic acid crystallised and was filtered off; after removal of the solvent by distillation *in vacuo*, the residue was recrystallised from dry methanol–ethyl acetate; it then had m. p. 145° (decomp.) (Wynn and Corwin, *loc. cit.*, give m. p. 144—147°). Light absorption: $\lambda_{max.}$ 2665 Å; $\varepsilon_{max.}$ 23·0, in water; after addition of 2 equiv. of sodium hydroxide and exposure to the air: $\lambda_{max.}$ 2760, $\varepsilon_{max.}$ 2000, in accordance with the expected formation at alkaline pH of a 2 : 5-disubstituted pyrazine (Hartley and Dobbie, *J.*, 1900, **77**, 846, give $\lambda_{max.}$ 2710 for 2 : 5-dimethylpyrazine); this solution was pale yellow and gave no colour with Ehrlich's reagent.

Methyl Succinimidoacetate.—Methyl bromoacetate (21·4 g., 0·14 mole) was treated with

succinimide (14·5 g., 0·144 mole) and potassium carbonate (20·0 g., 0·145 mole) in dimethylformamide (25 c.c.) by the procedure described. The resulting compound (19·2 g., 80%, m. p. 85—90°) was recrystallised from dry ether–light petroleum (b. p. 60—80°), giving needles of *methyl succinimidoacetate*, m. p. 96—97° (subl.) (Found: C, 49·0; H, 5·4; N, 8·1. $C_7H_9O_4N$ requires C, 49·1; H, 5·3; N, 8·2%). The ester is readily soluble in cold water.

Succinamidoacetic Acid (I).—Methyl succinimidoacetate (10·43 g., 0·061 mole) was dissolved in 7N-hydrochloric acid (100 c.c.) and left at 37° for 3 days (hydrolysis by hydrochloric acid was incomplete after a week at 20°, in contrast to the corresponding lævulate). The solution was taken to dryness *in vacuo* in a bath at 40°, and the flask left in a high vacuum over sodium hydroxide for 2 days. The resulting succinylglycine was dissolved in about 500 c.c. of boiling dry benzene; on cooling, transparent hexagonal plates separated, m. p. 115° (Scheiber and Reckleben, *loc. cit.*, give 113°). On working up the mother-liquors, the total yield was 8·61 g., 90%. This acid was dissolved in a little water and 1·95 equiv. of baryta were added during 2 hr.; after 1 hr. the mixture was heated to 70° on a water-bath. After cooling, the barium ions were precipitated with sulphuric acid, and most of the sulphate removed by centrifuging. The solution was now taken to dryness *in vacuo* on a bath at 50°. After desiccation, the white residue was extracted several times with boiling dioxan. The solution was filtered hot, and boiling *cyclo*hexane added, giving rectangular plates of *succinamidoacetic acid* (58%), m. p. 146° after a second recrystallisation (Found: C, 41·3; H, 4·9; N, 7·9. $C_6H_9O_5N$ requires C, 41·2; H, 5·2; N, 8·0%). Succinamido[α-^{14}C]acetic acid was prepared by this method, starting with bromo[α-^{14}C]acetic acid (0·10 mole), recrystallised from dry light petroleum (b. p. 60—80°); the bromoacetic acid was esterified with diazomethane; the overall yield was 40%.

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