Researches on Acetylenic Compounds. Part LI. The Syntheses of γ-Methyleneglutamic Acid and γ-Methyleneglutamine.*

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The structures proposed for γ-methyleneglutamic acid and for γ-methyleneglutamine are confirmed by rational syntheses of the racemic acids. The routes employed render these substances readily accessible.

Recently the isolation of an amino-dicarboxylic acid and its half-amide from ground-nut seedlings was described (Done and Fowden, *Biochem. J.*, 1952, **51**, 451). These compounds, the main ninhydrin-active substances present, were assigned structures (I; R = OH and NH_2, respectively), and their presence has since been reported in hops (Harris, *Chem. and Ind.*, 1954, 244) and in tulip bulbs (Zacharius, Pollard, and Steward, *J. Amer. Chem. Soc.*, 1954, **76**, 1961). The corresponding α-keto-acids occur in tulip leaves (Towers and Steward, *ibid.*, p. 1959), and also in ground-nut seedlings (Fowden and Webb, *Biochem. J.*, 1955, **59**, 228). In the latter plant, these acids play an important role in nitrogen transport (*idem*, *Ann. Bot.*, 1954, **18**, 72, 417). The decarboxylation product from the acid (I), *i.e.*, the monocarboxylic acid (II), occurs in ground-nut seedlings (Fowden and Done, *Biochem. J.*, 1953, **55**, 548), and the closely related lactone (III) was isolated by Cavallito and Haskell (*J. Amer. Chem. Soc.*, 1946, **68**, 2332) from *Erythronium americanum*. There is thus some evidence that α-methylene-acids of this type are of importance in plant

* Part L, *J.*, 1955, 1874.

metabolism; to facilitate biochemical work and to confirm the structures assigned, the synthesis of compounds (I; R = OH and NH_2) was attempted.

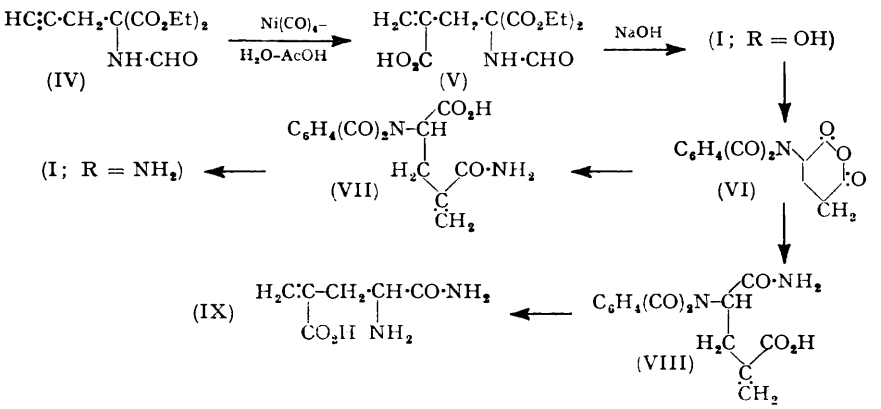
$H_2C{:}C(COR)\cdot CH_2\cdot CH(NH_2)\cdot CO_2H$ (I) $\quad H_2C{:}C(CO_2H)\cdot CH_2\cdot CH_2\cdot NH_2$ (II) $\quad H_2C{:}C\cdot CH_2\cdot CH_2$ (III) CO—O

Diethyl 1-formamidobut-3-yne-1 : 1-dicarboxylate (IV) (Gershon, Meek, and Dittmer, *ibid.*, 1949, **71**, 3573) was treated with nickel carbonyl in aqueous-ethanolic acetic acid (Jones, Shen, and Whiting, *J.*, 1950, 230); a typical exothermal reaction took place, and the Reppe carboxylation product (V) was formed in 45% yield. Vigorous alkaline hydrolysis, followed by the removal of sodium ions by passage through ZeoKarb-216 resin, gave an acidic solution, which was evaporated. Extraction of the residue with alcohol removed the *N*-formyl derivative of (I; R = OH), leaving the free amino-acid (I; R = OH) undissolved : the former probably arose by re-formylation during the isolation.

Natural γ-methyleneglutamic acid is probably the L-isomer, although its optical activity has not yet been reported. On treatment with mineral acid it gives the racemate, best distinguished by the lower yield of carbon dioxide given on enzymic decarboxylation (Fowden, personal communication). Both acids of natural origin soften at about 204°, as did the synthetic acid, and no depression was observed on admixture; the racemic acids of natural and synthetical origin gave identical *X*-ray powder diagrams and identical and detailed infrared spectra as Nujol suspensions. In addition, the three amino-dicarboxylic acid specimens were inseparable chromatographically.

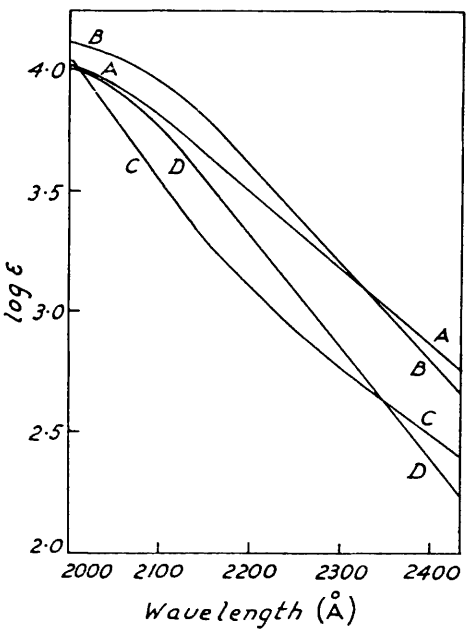
The synthetic method employed is rational, since the Reppe carboxylation is now well documented (Reppe, *Annalen*, 1953, **582**, 1) and the double bond in α-methylene-acids shows no tendency to rearrange into the main chain even under drastic conditions (Cason, Allinger, and Williams, *J. Org. Chem.*, 1953, **18**, 842).

For the preparation of the half-amide (I; R = NH_2) a method modelled on the conversion of glutamic acid into glutamine was sought, though hydrogenolysis of benzyl groups obviously could not be employed. Treatment of the acid (I; R = OH) with phthalic anhydride in pyridine, and subsequently with acetic anhydride (King and Kidd, *J.*, 1949, 3315), gave the corresponding phthalimido-anhydride (VI) in 70% yield. The analogous derivative of glutamic acid was shown by King and Kidd (*loc. cit.*) to give a



74% yield of the phthaloylglutamine on treatment with ammonia in dry ether–dioxan, although some of the isomeric half-amide may have been formed and eliminated by crystallisation. In the present case, the carbonyl group on which attack by ammonia was desired is conjugated with the methylene group, and lower reactivity, relative to the carbonyl group adjacent to the phthalimido-grouping, is therefore to be expected. The product, obtained in excellent yield and analytically satisfactory, melted at 189—196°, not sharpened by repeated crystallisation. Removal of the phthaloyl group (see below) gave a deliquescent amorphous solid from which no homogeneous product could be isolated. It was concluded that a mixture of amides (VII) and (VIII) has been obtained,

and, since crystallisation had failed to separate it, counter-current partition between butan-1-ol and a saturated phosphate buffer (pH 6·2) was investigated, fractions being analysed spectrophotometrically at 3000 Å. Although concentrated solutions, initially supersaturated, had to be employed, the available 24-tube apparatus readily separated the two components. The slower-moving component (47% from VI), m. p. 203—205°, had pK 5·18 in 60% ethanol (cf. 4·93 for phthalimidoacetic acid in the same solvent), and was clearly the desired half-amide (VII). The other isomer (20% from VI), m. p. 201—204°, had pK 5·99, agreeing well with pK 6·38 for methacrylic acid, under the same conditions, when allowance is made for the acid-strengthening effects of the phthalimido- and amino-carbonyl groups, and was therefore (VIII). The ultraviolet absorption spectra were almost identical; infrared spectra were different but not diagnostically useful, because of the multiplicity of bands in the 1600—1800-cm.$^{-1}$ region.



A, γ-Methyleneglutamine (I; R = NH_2).
B, Glycine plus methacrylamide.
*C, γ-Methylene*isoglutamine (IX).
D, Glycine amide plus methacrylic acid.
(*All in* H_2O.)

Treatment of the phthaloyl derivative (VII) with hydrazine in neutral aqueous solution at 20° (King and Kidd, *loc. cit.*) and isolation of the methanol-insoluble portion of the product gave a crystalline half-amide (A) in 24% yield. In boiling ethanol (Sheehan and Corey, *J. Amer. Chem. Soc.*, 1952, **74**, 4555) phthalhydrazide was formed in good yield, but no crystalline amino-acid could be isolated. It was now necessary to prove (*a*) that (A) was the racemic half-amide (I; R = NH_2), and (*b*) that, apart from optical isomerism, it was identical with the natural half-amide. From analytical data, and from its ultraviolet absorption spectrum, which at least proved that the $H_2C{=}\overset{|}{C}{\cdot}\overset{|}{C}{=}O$ chromophore was present, (A) must be either (I; R = NH_2) or the isomer (IX). Assuming, from the pK measurements that the two phthaloyl half-amides were correctly identified, the method of formation barely permits the structure (IX), though rearrangements through cyclic intermediates are common in derivatives of succinic and glutaric acids (cf., *inter al.*, Battersby and Robinson, *J.*, 1955, 259). The ultraviolet absorption spectrum (Figure), though indecisive, agrees better with the model mixture (glycine + methacrylamide) for (I; R = NH_2) than with that (glycine amide + methacrylic acid) for (IX). Nevertheless attempts were made to prepare the authentic isomer (IX) from the less acidic phthaloyl-half-amide. The reaction with hydrazine was much less satisfactory in this case, giving amorphous products which were shown by paper chromatography to be complex mixtures; however, after several weeks a small quantity of crystalline material was isolated. This substance was very soluble and could not be freed from inorganic contaminants, but paper chromatography indicated that only one substance was present capable of giving a colour with ninhydrin or with chlorine followed by potassium iodide and starch (Rydon and Smith, *Nature*, 1952, **169**, 922). Dr. L. Fowden (personal communication) confirmed that it was separable from (A) chromatographically, and also found that it gave a substance inseparable from γ-methyleneglutamic acid on acid hydrolysis. Its ultraviolet absorption spectrum agrees with that expected for the amide (IX), and we conclude that it was in fact a specimen of the latter, containing about 20% of inorganic salts. Since the two phthaloyl-amides thus give different products with hydrazine, it may be assumed that no rearrangement is involved, and that (A) is therefore the isomer (I; R = NH_2).

The proof that this was the racemate corresponding to the natural amide was obtained indirectly, since powder *X*-ray patterns were quite different, and the infrared spectra of the two specimens (perforce determined in Nujol suspensions) differed so profoundly that scepticism in other respects seemed essential. Paper chromatography and enzymic hydrolysis were employed, with results, detailed in a preliminary publication (Wailes, Whiting, and Fowden, *Nature*, 1954, **174**, 130), which leave no doubt as to the chemical identity of the two substances. As might be expected, the isomeric amide (IX) did not undergo enzymic hydrolysis.

In earlier attempts to synthesise these compounds, 2-aminopent-4-ynoic acid (Gershon, Meek, and Dittmer, *loc. cit.*) proved to be inert to nickel carbonyl (cf. the similar unreactivity of pent-4-ynoic acid; Jones, Whitham, and Whiting, *J.*, 1954, 1865). Its phthaloyl derivative was prepared and converted into a well-crystallised methyl ester which, despite satisfactory analytical data and a normal infrared spectrum, melted over a 10° range. This ester reacted exothermally with nickel carbonyl, but the acidic product did not crystallise.

Experimental

M. p.s were determined on the Kofler block. Ultraviolet absorption data were determined with a Unicam SP 500 spectrophotometer, and infrared spectra with a Perkin–Elmer double-beam Model 21 spectrophotometer. Dissociation constants (quoted as pK_a) were determined with a pH meter (Cambridge Instrument Co. Ltd.) and are probably accurate to ± 0.05.

Diethyl 3-Carboxy-1-formamidobut-3-ene-1 : 1-dicarboxylate (V).—Diethyl 1-formamidobut-3-yne-1 : 1-dicarboxylate (Gershon, Meek, and Dittmer, *loc. cit.*) (115 g.) was heated, with stirring, in ethanol (500 c.c.), water (30 c.c.), and acetic acid (75 c.c.) at 65°. Nickel carbonyl (40 g., 30 c.c.) under ethanol (10 c.c.) was added dropwise and after an initial induction period a typical exothermal ("Type A") reaction began (Jones, Shen, and Whiting, *J.*, 1951, 48). The temperature rose to 75° and the colour changed to bright green. The temperature was maintained at 70—75° by addition of nickel carbonyl and when reaction had ceased ether (*ca.* 100 c.c.) was added and the excess of carbonyl was removed by distillation of all material boiling below 50°. The residue was poured into dilute sulphuric acid and extracted with ether, the acid fraction being isolated through potassium hydrogen carbonate. Crystallisation from benzene–light petroleum (b. p. 60—80°) afforded the *α-methylene-acid* (61·5 g., 45%) as leaflets, m. p. 140—144° (Found: C, 50·2; H, 5·8; N, 5·15. $C_{12}H_{17}O_7N$ requires C, 50·15; H, 5·95; N, 4·9%).

γ-Methyleneglutamic Acid (I; R = OH).—The above acid (53 g.) was heated in a solution of sodium hydroxide (40 g.) in water (800 ml.) under reflux for 4·5 hr. After cooling, an equal volume of water was added and the sodium was removed by passage through ZeoKarb-216 (activated with 2N-hydrochloric acid; 6 × 52 cm.). Elution with water was continued until the pH of the eluate had risen to 5. The whole of the eluate (*ca.* 6 l.) was evaporated under reduced pressure to 600 c.c., then treated with charcoal and evaporated further. Crystallisation then soon commenced and the product was collected by alternate filtration and evaporation. When the volume had been reduced to 100 c.c., 20 g. of a white solid had been isolated (material collected subsequently was highly coloured and impure, and was discarded). The equivalent weight of the solid was considerably lower than that of the desired acid, so it was extracted with boiling ethanol (100 c.c.). A white crystalline residue (13·0 g., 45%) of DL-γ-methyleneglutamic acid (I; R = OH) remained. This was sufficiently pure to use in the next stage (equiv., 155. Calc. for $C_6H_9O_4N$: equiv., 159), and had m. p. 204° (softens) (Done and Fowden, *loc. cit.*, give softening point 204° with the same rate of heating). For analysis it was recrystallised from water as small prisms softening at 204° (Found: C, 44·95; H, 5·35; N, 8·6%; equiv., 158. Calc. for $C_6H_9O_4N$: C, 45·3; H, 5·7; N, 8·8%). Evaporation of the ethanol extract gave a crystalline residue (5·5 g.) which appeared to be the *formyl derivative* [Found: equiv., 93·3. $C_5H_7ON(CO_2H)_2$ requires equiv., 93·5], and was not investigated further.

Phthaloyl-γ-methyleneglutamic Anhydride (VI).—γ-Methyleneglutamic acid (13·0 g.) and phthalic anhydride (12·5 g.; recrystallised from acetic anhydride) were boiled together in dry pyridine (200 c.c.) for 7·5 hr. The solvent was evaporated under reduced pressure, leaving a viscous oil which dissolved in warm acetic anhydride (100 c.c.); the solution was heated on the steam-bath for a further 10 min. On cooling, colourless leaflets of the *anhydride* (VI) [15·5 g., 70% from (I; R = OH)] separated, having m. p. 222—224° (Found: C, 62·2; H, 3·55; N, 5·2.

$C_{14}H_9O_5N$ requires C, 62·0; H, 3·35; N, 5·15%). The infrared spectrum showed bands at 970 and 1630 cm.$^{-1}$ (characteristic of $CH_2{:}CR{\cdot}CO$) and carbonyl bands at 1706, 1755, 1774, and 1804 cm.$^{-1}$.

Phthaloyl-γ-methylene-glutamine (VII) *and* -iso*glutamine* (VIII).—The anhydride (VI) (15·5 g.) was dissolved in warm dry dioxan (200 c.c.), and an excess of anhydrous ammoniacal ether was added slowly with shaking and occasional cooling, the ammonium salts of the half-amides separating. After 15 min. the solid was filtered off, dissolved in the minimum of water (*ca.* 40 c.c.), and acidified to Congo-red with 5N-hydrochloric acid. After several hours at 0° the separated solid (14·7 g., 89%) was collected; it had m. p. 189—196°, not improved by repeated crystallisation from water or aqueous acetone.

Countercurrent partition separation. The mixed phthalimido-amides (4·0 g.) were dissolved in butanol (80 c.c.) which had been equilibrated with M-phosphate buffer solution (pH 6·2) and introduced in 40-c.c. portions into the first two tubes of a 24-tube countercurrent partition apparatus (see Craig and Craig in Weissburger, "Technique of Organic Chemistry," Vol. III, New York, 1950). The lower layer in each tube consisted of 80 c.c. of the above buffer solution, and two fractions of butanol were transferred through the apparatus just ahead of the amide solution to ensure complete saturation of the aqueous layer. Fractions of the upper phase were collected by the single-withdrawal technique until spectroscopic analysis at 3000 Å showed that the faster-moving amide was being eluted from the apparatus. The upper phases of all of the tubes were then analysed spectroscopically at 3000 Å.

Phthaloyl-γ-methyleneglutamine (VII). The contents of tubes 1—7 were combined and the lower layer was acidified with phosphoric acid and extracted with the upper. After being washed with a little water, the butanol layer was evaporated under reduced pressure and the residue was crystallised twice from aqueous methanol, giving prisms (2·1 g., 47% from the anhydride) of the *half-amide*, m. p. 203—205° (Found: C, 58·05; H, 4·0; N, 10·1. $C_{14}H_{12}O_5N_2$ requires C, 58·35; H, 4·2; N, 9·7%). The infrared spectrum (Nujol suspension) included bands at 1638, 948, and 968 cm.$^{-1}$ and carbonyl bands at 1664, 1714, and 1780 cm.$^{-1}$. In 60% ethanol at 15° the pK was 5·18.

*Phthaloyl-γ-methylene*iso*glutamine* (VIII). Tubes 13—24 were worked up similarly. Two crystallisations from aqueous methanol afforded needles (0·9 g., 20% from the anhydride) of the *amide*, m. p. 201—204° (Found: C, 58·05; H, 4·2; N, 9·5%). The infrared spectrum (Nujol suspension) showed bands at 932, 966, and 1630 cm.$^{-1}$, and carbonyl peaks at 1706 and 1775 cm.$^{-1}$. The pK in 60% ethanol at 15° was 5·99.

γ-Methyleneglutamine (I; R = NH_2).—Phthaloyl-γ-methyleneglutamine (VII) (1·6 g.) was dissolved in aqueous sodium carbonate (0·30 g. in 10 c.c. water), and hydrazine hydrate (1 mol., 0·56 c.c. of a solution containing 0·495 g. per c.c.) was added. The mixture was kept at room temperature for 3 days, then acidified with 5N-hydriodic acid and cooled to 0° for several hours. Phthalhydrazide was removed and the filtrate was neutralised (pH meter) with 5% sodium carbonate solution and evaporated almost to dryness at 40° (bath-temp.). Dry methanol (*ca.* 50 c.c.) was then added and the solution was cooled to −10° for 24 hr. On filtration *γ-methyleneglutamine* (0·21 g., 24%) was obtained as colourless plates, m. p. 171—173° (decomp.), unchanged on recrystallisation (Done and Fowden, *Biochem. J.*, 1952, **51**, 451, give m. p. 173° for the naturally occurring amide) (Found: C, 45·55; H, 6·15; N, 17·65. $C_6H_{10}O_3N_2$ requires C, 45·55; H, 6·35; N, 17·7%).

*γ-Methylene*iso*glutamine* (IX).—Phthalolyl-γ-methylene*iso*glutamine (VIII) (0·80 g.) was treated as above, and the crude amide was precipitated from the mixture with sodium iodide by trituration with absolute ethanol. Chromatography of the residue in butanol–acetic acid–water (Partridge, *Biochem. J.*, 1948, **42**, 238) and development with chlorine and starch–iodide (Rydon and Smith, *loc. cit.*) showed the presence of four compounds of different R_F values. Attempted crystallisation from aqueous methanol, propan-2-ol, or acetone did not effect purification, but when an aqueous-ethanol solution was kept for several weeks a small quantity (35 mg.) of crystalline material separated (m. p. 174°) (Found: N, 13·8%). Appreciable amounts of inorganic contaminants were still present, but paper chromatography gave only one spot after development with ninhydrin (reddish-brown) or with chlorine–potassium iodide.

2-*Phthalimidopent*-4-*ynoic Acid*.—2-Aminopent-4-ynoic acid (0·72 g.) (Gershon, Meek, and Dittmer, *loc. cit.*) and phthalic anhydride (0·92 g., 1 mol.) were heated under reflux in pyridine (10 ml.) for 2 hr. The solution was evaporated under reduced pressure, the residue was taken up in benzene, and the acid fraction was isolated. Crystallisation from benzene–light petroleum (b. p. 60—80°) gave the *acid* (0·96 g., 62%) as needles, m. p. 158—161° (Found: C, 63·95; H, 4·15; N, 5·9. $C_{13}H_9O_4N$ requires C, 64·2; H, 3·75; N, 5·75%). The *methyl ester*, prepared in

methanol (13 ml.) containing concentrated sulphuric acid (0·15 ml.) at 20° for 48 hr., crystallised from aqueous methanol as needles, m. p. 97—107°, not sharpened by recrystallisation (Found : C, 65·2; H, 4·4; N, 5·4. $C_{14}H_{11}O_4N$ requires C, 65·35; H, 4·3; N, 5·45%).

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