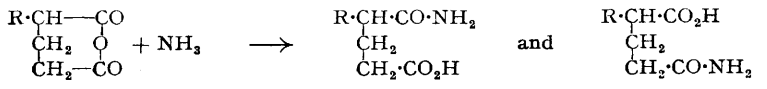
518. *The Influence of Substituent Groups on Ring Scission. Part I. The Ring Scission of Some Unsymmetrically Substituted Glutaric Anhydrides.*

By John C. Roberts and Keith Selby.

A method, employing partition chromatography, has been developed for the quantitative separation of the mixture of isomeric amic acids obtained when an unsymmetrically substituted glutaric anhydride reacts with ammonia. α-Phenylglutaric anhydride, dissolved in dioxan, yields at 20° with ammonia 63 ± 2% of the α-amic acid. Under the same conditions α-*p*-nitrophenylglutaric anhydride gives 46 ± 2% of the α-isomer. Five new arylglutaramic acids have been synthesised by unambiguous methods. The K_a' values for four arylglutaramic acids have been determined.

When an unsymmetrically substituted cyclic acid anhydride reacts with an alcohol, ammonia, or a substituted ammonia, two isomeric products are possible. For example, an α-substituted glutaric anhydride and ammonia may yield both the α- and the γ-amic acid :



Many examples of this type of reaction have been investigated, but in no case have both of the possible isomers been isolated quantitatively, and it should be noted that, at least in some cases, differences in the ease of isolation of the two products may lead to a false concept of the proportion of the isomers originally present. Anschütz *et al.* (*Annalen*, 1907, **354**, 117) have stated that, in the case of phenylsuccinic anhydride, the amino- or substituted amino-residue attaches itself to the carbonyl group corresponding to the " weaker " carboxyl group in the parent dicarboxylic acid, *i.e.*, to the carboxyl group further removed from the phenyl substituent. Unfortunately, these authors have not recorded the yields of the amic or substituted amic acids obtained, but have merely stated that the reaction products were homogeneous. King *et al.* (*J.*, 1949, 3315; 1951, 243) have shown that phthaloylglutamic anhydride possesses the remarkable property of giving very high yields (74—91%, crude products) of γ-derivatives when the ring is opened by ammonia, amino-compounds, or alcohols. However, the evidence available indicates that in most cases a mixture of isomers is obtained. For example, Le Quesne and Young (*J.*, 1950, 1954) have shown that *N*-carbobenzyloxyglutamic anhydride, with a variety of reagents, furnishes mixtures of α- and γ-derivatives (for further examples see Melville, *Biochem. J.*, 1935, **29**, 179; Bergmann, Zervas, and Fruton, *J. Biol. Chem.*, 1936, **115**, 606; Barry and Twomey, *Proc. Roy. Irish Acad.*, 1947, **51**, *B*, 152; Boothe *et al.*, *J. Amer. Chem. Soc.*, 1949, **71**, 2310; Roberts and Shaw, *J.*, 1950, 2842.)

There appears to be little doubt that the nature of the group which is unsymmetrically substituted in the anhydride ring markedly influences the composition of the mixture of isomers produced on ring scission. In order to obtain more precise information on this point we investigated quantitatively the reactions between ammonia and some glutaric anhydrides containing simple substituents in the α-position. This paper records our results with α-phenyl-, α-*p*-nitrophenyl-, and α-*p*-benzamidophenyl-glutaric anhydrides.

It became necessary to develop a method for the quantitative separation of the isomeric amic acids produced. Since they contained the same functional groups, it was considered that the ordinary process of partition chromatography (using water and an organic solvent) would not be successful, as such compounds would have almost identical distribution coefficients (Martin, *Ann. Reports*, 1948, **45**, 269). However, it appeared probable that the two isomers would differ in acidic strength, owing to the different relative positions of the substituent group and the carboxyl group, and this led us to expect that a special type of chromatographic analysis might be successful. The use of an immobile aqueous phase of constant pH has been shown to be advantageous in the separation of mixtures of bases or of acids (Craig, Golumbic, Mighton, and Titus, *J. Biol. Chem.*, 1945, **161**, 321; Moyle, Baldwin, and Scarisbrick, *Biochem. J.*, 1948, **43**, 308; Roberts and Selby, *J.*, 1949, 2785). Eventually we found that an efficient separation of the two amic acids, produced either from α-phenyl- or from α-*p*-nitrophenyl-

glutaric anhydride, could be obtained by using an immobile aqueous phase of pH 7·76 and a mobile phase of butanol–chloroform. It was not possible to obtain a satisfactory chromatogram with the *p*-benzamidophenylglutaramic acids because of their adverse solubility properties.

Further, it became necessary to devise an unequivocal synthesis for each amic acid and to make a prior determination of their chromatographic behaviour. γ-Carbamyl-α-phenylbutyric acid was efficiently synthesised by the following route:

$$Ph{\cdot}CH(CO_2Et)_2 \xrightarrow{Cl{\cdot}[CH_2]_2{\cdot}CN} Ph{\cdot}C(CO_2Et)_2{\cdot}[CH_2]_2{\cdot}CN \xrightarrow[-CO_2]{\text{hydrolysis,}} Ph{\cdot}CH(CO_2H){\cdot}[CH_2]_2{\cdot}CN \xrightarrow[(H_2SO_4)]{H_2O} Ph{\cdot}CH(CO_2H){\cdot}[CH_2]_2{\cdot}CO{\cdot}NH_2$$

The isomeric γ-carbamyl-γ-phenylbutyric acid was obtained by the method of Wideqvist (*Svensk Kem. Tids.*, 1942, **54**, 34), who records m. p. 168°. However, the acid we obtained

FIG. 1.

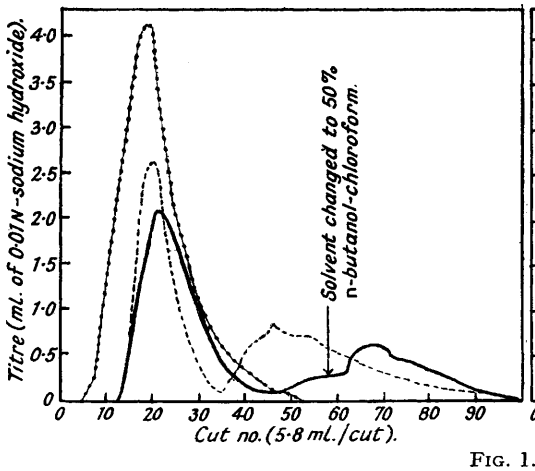


FIG. 2.

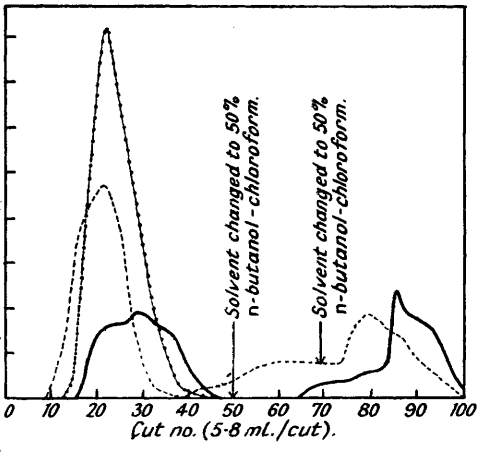


FIG. 1.

Immobile phase: 2M-*phosphate buffer solution*, pH 7·76 (*glass electrode*). *Mobile phase:* 30% (v/v) n-*butanol in chloroform at start of each run.* · — · — · — · — · γ-*Carbamyl-γ-phenylbutyric acid* (147 *mg.*). — — — — *A mixture of γ-carbamyl-γ-phenylbutyric acid* (51 *mg.*; $K_a' = 3{\cdot}63 \times 10^{-5}$ *at* 25°) *and γ-carbamyl-α-phenylbutyric acid* (51 *mg.*; $K_a' = 8{\cdot}32 \times 10^{-5}$ *at* 25°). ———— *Unknown mixture of phenylglutaramic acids* (101·5 *mg.*) *obtained by reaction of ammonia with α-phenylglutaric anhydride in dry dioxan at* 20°. *Proportion of γ-carbamyl-γ-phenylbutyric acid found* = 63 ± 2%.

FIG. 2.

Immobile phase: 2M-*phosphate buffer solution*, pH 7·76 (*glass electrode*). *Mobile phase:* 30% (v/v) n-*butanol in chloroform at start of each run.* · — · — · — · γ-*Carbamyl-γ-p-nitrophenylbutyric acid* (127 *mg.*). — — — — *A mixture of γ-carbamyl-γ-p-nitrophenylbutyric acid* (86 *mg.*; $K_a' = 3{\cdot}47 \times 10^{-5}$ *at* 25°) *and γ-carbamyl-α-p-nitrophenylbutyric acid* (82 *mg.*; $K_a' = 2{\cdot}34 \times 10^{-4}$ *at* 25°). ———— *Unknown mixture of p-nitrophenylglutaramic acids* (101 *mg.*) *obtained by reaction of ammonia with α-p-nitrophenylglutaric anhydride in dry dioxan at* 20°. *Proportion of γ-carbamyl-γ-p-nitrophenylbutyric acid found* = 46 ± 2%.

had m. p. 180° when prepared either by this method or by the alternative method, involving a chromatographic separation, from α-phenylglutaric anhydride and ammonia. The α-*p*-nitrophenyl- and α-*p*-benzamidophenyl-glutaric anhydrides and the corresponding α- and γ-amic acids were also synthesised (see below).

The appropriate anhydride, dissolved in dry dioxan, was treated with ammonia at 20°. The mixture of the free amic acids was isolated quantitatively from the ammonium salts produced and was analysed chromatographically. Each acid was isolated and was identified by comparison with its synthetic analogue.

α-Phenylglutaric anhydride yielded a mixture containing 63 ± 2% of γ-carbamyl-γ-phenylbutyric acid and 37 ± 2% of γ-carbamyl-α-phenylbutyric acid (Fig. 1); α-*p*-nitrophenylglutaric anhydride gave 46 ± 2% of γ-carbamyl-γ-*p*-nitrophenylbutyric acid and 54 ± 2% of the isomer (Fig. 2).

The figure of ±2 denoting the maximum possible error in the analyses is an estimate based

on two considerations. First, the amic acids were isolated (as a mixture) in >99% yield from the original reaction mixture. Secondly, using known mixtures for chromatography, we found that the titre for the acid in the first band was >98% in each case. (Total recovery of material was >96%.) The composition of the mixture was calculated from the amount of material in the first band. Although the titre did not fall absolutely to zero during the separation of the phenylglutaramic acids (Fig. 1), only 1% of the total acids was eluted between cuts 43 and 47, and cut 45 was chosen as the dividing point. The reproducibility of the analytical figures is shown by the following typical series of results for the percentage of γ-carbamyl-γ-phenylbutyric acid when in admixture with its isomer: (i) 63·3, (ii) 63·7, (ii*a*) 63·3, where (i) and (ii) were obtained in experiments starting from different batches of anhydride and (ii*a*) was the result of a second chromatographic analysis on the same mixture of amic acids as was used in (ii).

The K_a' values, for the isomeric amic acids concerned, corresponded with their relative positions on the chromatograms (Figs. 1 and 2), and it is noteworthy that the greater difference in the K_a' values of the isomers in the case of the *p*-nitrophenylglutaramic acids led to a better separation (Fig. 2).

Anschütz's generalisation, for phenylsuccinic anhydride, does not therefore apply to the α-substituted glutaric anhydrides which we have investigated. Owing to the limited number of reliable results available we consider it inadvisable, at this stage, to advance any generalisations or theoretical considerations concerning the influence of substituent groups on ring scission. However, it is of interest that, in the case of the two α-arylglutaric anhydrides which we have investigated and in which any steric interference may be considered constant, a smaller proportion of the α-amic acid is formed when the α-substituent possesses a greater electron-attracting capacity.

Experimental.

Ethyl (2-Cyanoethyl)phenylmalonate.—Ethyl phenylmalonate (*Org. Synth.*, Coll. Vol. II, p. 288) (1 mol.), in an ethanolic solution containing sodium ethoxide (1 mol.), was condensed with β-chloropropionitrile (prepared in 82% yield from acrylonitrile and dry hydrogen chloride) (1·1 mols.). The product, isolated in the usual way, distilled at 186°/2·5 mm. The oily distillate subsequently crystallised. Recrystallisation from light petroleum (b. p. 40—60°) gave *ethyl (2-cyanoethyl)phenylmalonate* as colourless, irregular prisms, m. p. 41° (32%) (Found: C, 66·9; H, 6·7; N, 4·7. $C_{16}H_{19}O_4N$ requires C, 66·4; H, 6·6; N, 4·8%).

γ-Cyano-α-phenylbutyric Acid.—The foregoing ester (1 mol.) was shaken overnight with a 2·5% solution of potassium hydroxide (3·2 mols.) in dry ethanol. Potassium carbonate was slowly deposited. The mixture was heated under reflux for 2 hours on the steam-bath (in order to complete the hydrolysis) and then cooled and filtered. The filtrate, after neutralisation, concentration (by evaporation of the alcohol), acidification, and extraction with ether, yielded a yellow oil which rapidly solidified. Two crystallisations from ether–light petroleum (b. p. 40—60°) followed by recrystallisation from water gave *γ-cyano-α-phenylbutyric acid* as colourless prisms, m. p. 87° (67%) (Found: C, 70·1; H, 6·0; N, 7·1. $C_{11}H_{11}O_2N$ requires C, 69·8; H, 5·9; N, 7·4%). Decarboxylation had therefore occurred spontaneously after hydrolysis.

γ-Carbamyl-α-phenylbutyric Acid.—A solution of γ-cyano-α-phenylbutyric acid (1 mol.) in concentrated sulphuric acid (6 mols.) was kept for 8 hours at room temperature and was then poured on ice. The semi-solid product was filtered off, washed, and dried (m. p. 58°). Recrystallisation proved difficult. However, preliminary purification (by trituration with ether) followed by slow evaporation of an ethanolic solution of the residue yielded the desired *acid* as square plates, m. p. 60—61° (40%) (Found: C, 63·5; H, 6·5; N, 6·6. $C_{11}H_{13}O_3N$ requires C, 63·8; H, 6·3; N, 6·8%).

Ethyl α-Cyano-α-phenylglutarate.—Prepared by Wideqvist's method (*loc. cit.*), this was a colourless oil, b. p. 187—188°/3 mm. Wideqvist records b. p. 197—198°/8 mm.

γ-Cyano-γ-phenylbutyric Acid.—This acid was obtained by hydrolysis (and simultaneous decarboxylation) of the foregoing ester (1 mol.) with a 10% solution of potassium hydroxide (3·2 mols.) in absolute ethanol and crystallised from carbon tetrachloride as regular octahedra, m. p. 58° (yield, 62%). Wideqvist records m. p. 61°. It had been hoped to devise a more convenient synthesis of this acid by way of ethyl γ-cyano-γ-phenylbutyrate, but two attempts to prepare this ester from benzyl cyanide (1 mol.) and ethyl β-iodopropionate (1·1 mols.), sodamide (1 mol.) being used as condensing agent (cf. Bodroux, *Compt. rend.*, 1910, **151**, 234), were unsuccessful. Dry ether and dry benzene were used as solvents but only a very little product of the correct b. p. was obtained, together with much tarry matter which may have originated from ethyl acrylate produced by the removal of hydrogen iodide from ethyl β-iodopropionate.

γ-Carbamyl-γ-phenylbutyric Acid.—This acid was prepared from the nitrile (as described above for the α-phenyl isomer) and recrystallised from hot water (charcoal) as fine, colourless needles, m. p. 180° (yield, 90%). Wideqvist records m. p. 168° (Found: C, 63·8; H, 6·3; N, 6·5. Calc. for $C_{11}H_{13}O_3N$: C, 63·8; H, 6·3; N, 6·8%).

α-Phenylglutaric Anhydride.—Ethyl (2-carbethoxyethyl)phenylmalonate was obtained (yield, 42·5%) by the usual procedure from ethyl phenylmalonate and ethyl β-iodopropionate and had b. p.

198°/2 mm. (Fichter and Merckens, *Ber.*, 1901, **34**, 4175, give b. p. 219—221°/13 mm.). A trial hydrolysis with 10N-hydrochloric acid, as suggested by these authors, was unsuccessful. Hydrolysis of the ester (1 mol.) was achieved by heating it under reflux with a 10% solution of potassium hydroxide (9·5 mols.) in absolute ethanol. The product was isolated in the usual manner and decarboxylation to α-phenylglutaric acid was completed at 180°. The acid was then heated under reflux with an excess of acetic anhydride. Removal of acetic acid and the excess of acetic anhydride by evaporation *in vacuo* followed by crystallisation of the residue from dry carbon tetrachloride yielded the desired anhydride as colourless rectangular prisms, m. p. 95° (60% calc. on the ester). Fichter and Merckens (*loc. cit.*) record m. p. 95°.

γ-Cyano-γ-p-nitrophenylbutyric Acid.—(Preliminary experiments established that benzyl cyanide could be converted into *p*-nitrophenylacetamide, m. p. 190°, by nitration of the phenyl nucleus followed by partial hydrolysis of the cyano-group.) γ-Cyano-γ-phenylbutyric acid (17 g.) was dissolved in fuming nitric acid (*d* 1·50; 120 g.) at 10°. The solution was kept at this temperature for 10 minutes and then poured into water, whereupon a small amount of solid separated. The mixture was neutralised with 40% sodium hydroxide solution. Addition of a slight excess over the theoretically required quantity of dilute sulphuric acid liberated the product which was obtained, by extraction with ether and evaporation of the solvent, as a yellow oil. This oil, when scratched under light petroleum (b. p. 100—120°), solidified. Two crystallisations from water and successive recrystallisation from (i) dibutyl ether and (ii) chloroform–carbon tetrachloride yielded *γ-cyano-γ-p-nitrophenylbutyric acid* as colourless cubes, m. p. 112° (78%) (Found: C, 56·7; H, 4·4; N, 12·1. $C_{11}H_{10}O_4N_2$ requires C, 56·4; H, 4·3; N, 12·0%). The acid dissolves in 2N-sodium hydroxide solution with production of a blood-red colour. Oxidation of this acid, by boiling it under reflux for ½ hour with an excess of alkaline (sodium carbonate) potassium permanganate solution, yielded *p*-nitrobenzoic acid (m. p. and mixed m. p. with an authentic specimen 238°).

γ-Carbamyl-γ-p-nitrophenylbutyric Acid.—This acid was prepared from the foregoing γ-cyano-acid in the usual way, and the glutinous product was rendered completely solid by shaking it with ether to remove the soluble impurities. Recrystallisation from water gave an almost theoretical yield of *γ-carbamyl-γ-p-nitrophenylbutyric acid* as plates, m. p. 180° (Found: C, 52·9; H, 5·0; N, 11·1%; equiv., by titration, 251. $C_{11}H_{12}O_5N_2$ requires C, 52·4; H, 4·8; N, 11·1%; equiv., 252).

γ-Cyano-α-p-nitrophenylbutyric Acid.—This *acid* was prepared by nitration of γ-cyano-α-phenylbutyric acid, the same method being used as that described above for γ-cyano-γ-*p*-nitrophenylbutyric acid. The product was obtained by pouring the reaction mixture into water. Recrystallisation from aqueous ethanol gave the acid as colourless, hexagonal plates, m. p. 126° (38%) (Found: C, 56·2; H, 4·0; N, 12·0%). (Extraction with ether of the filtrate from the diluted reaction mixture yielded a further 33% of crude material which could be used directly in the next stage.) The acid of m. p. 126° yielded *p*-nitrobenzoic acid when oxidised with potassium permanganate in alkaline solution.

γ-Carbamyl-α-p-nitrophenylbutyric Acid.—This *acid* was obtained from the foregoing cyano-acid in the manner already described; it crystallised from water in colourless cubes, m. p. 170° (yield, almost theoretical) (Found: C, 52·7; H, 5·0; N, 11·0%; equiv., 252).

α-(p-*Nitrophenyl*)*glutaric Anhydride.*—Several methods for preparing α-*p*-nitrophenylglutaric acid were investigated, *viz.*, (*a*) from α-phenylglutaric acid by nitration; (*b*) from γ-carbamyl-γ-*p*-nitrophenylbutyric acid by three routes: (i) by alkaline hydrolysis; (ii) by treatment with nitrous acid (cf. Bouveault, *Bull. Soc. chim.*, 1892, **9**, 368; Sudborough, *J.*, 1895, **67**, 602); (iii) by acid hydrolysis. Of these methods only (*b*) (iii) gave a reasonable yield of the desired product. The amic acid (3·75 g.) was heated under reflux for 15 minutes with 70% (w/w) sulphuric acid (10 ml.). When the hydrolysate was poured into water an almost black, amorphous solid was obtained. A solution of this material in aqueous sodium carbonate was boiled with decolorising charcoal and filtered. The filtrate, on acidification and cooling, gave the desired acid (2·2 g., 59%) as a light yellow, ether-soluble solid, m. p. 138°. α-p-*Nitrophenylglutaric anhydride*, prepared from the acid by treatment with acetic anhydride in the usual way, was insoluble in cold chloroform, carbon tetrachloride, and light petroleum, sparingly soluble in ether, and moderately soluble in benzene and dioxan. Crystallisation from dry light petroleum (b. p. 60—80°) (charcoal) or from dry benzene yielded the anhydride as pale yellow needles, m. p. 136° (42%) (Found: C, 56·2; H, 3·9; N, 5·8. $C_{11}H_9O_5N$ requires C, 56·2; H, 3·9; N, 6·0%). The anhydride was insoluble in cold 10% sodium hydrogen carbonate solution and was only very slowly soluble in cold 2N-sodium hydroxide solution, with which it gave a yellow colour.

γ-p-Aminophenyl-γ-carbamylbutyric Acid.—After a trial hydrogenation of *p*-nitrophenylacetic acid with subsequent benzoylation to *p*-benzamidophenylacetic acid (m. p. 205°), γ-carbamyl-γ-*p*-nitrophenylbutyric acid (2·5 g.) was hydrogenated in ethanolic solution at room temperature and slightly more than atmospheric pressure, Raney nickel being used as catalyst. The uptake of hydrogen was almost theoretical for one nitro-group. A little water was added and the mixture was boiled and filtered. Evaporation *in vacuo* of the filtrate to dryness yielded the amino-acid (2 g., 91%) as a yellow amorphous solid. (The presence of the amino-group was confirmed by the usual test.)

γ-p-Benzamidophenyl-γ-carbamylbutyric Acid.—The amino-acid (1·5 g.) was benzoylated by shaking it for 15 minutes with benzoyl chloride (1·5 ml.) in an excess of 10% sodium hydrogen carbonate solution. The reaction mixture was acidified and the product was freed from benzoic acid by extraction of the latter with ether. Repeated recrystallisation from aqueous ethanol yielded *γ-p-benzamidophenyl-γ-carbamylbutyric acid* as colourless needles, m. p. 234° (41%) (Found: C, 66·0; H, 5·4; N, 8·7%; equiv., 328. $C_{18}H_{18}O_4N_2$ requires C, 66·3; H, 5·6; N, 8·6%; equiv., 326). This amic acid was insoluble in chloroform and butanol; it was sparingly soluble in dioxan and in β-ethoxyethanol.

α-p-*Benzamidophenyl-γ-carbamylbutyric Acid.*—This *acid* was prepared in a similar manner from γ-carbamyl-α-*p*-nitrophenylbutyric acid and recrystallised from very dilute alcohol as rosettes of needles, m. p. 207° (yield, 35%) (Found: C, 66·1; H, 6·0; N, 8·6%). The solubility properties of this amic acid were similar to those of its isomer.

α-p-*Benzamidophenylglutaric Acid and Anhydride.*—(i) *From* γ-p-*benzamidophenyl-γ-carbamylbutyric acid.* The acid was boiled under reflux with 70% sulphuric acid for 30 minutes and the benzoic acid produced was removed by steam-distillation. The residual solution was neutralised with sodium hydroxide solution, and the α-*p*-aminophenylglutaric acid (in solution as its disodium salt) was then benzoylated and isolated in the usual way. The benzoylated amino-acid, after crystallisation from aqueous ethanol, was obtained (yield, 56%) as rosettes of needles, m. p. 194°. Treatment of this acid with acetic anhydride and crystallisation of the product from acetic anhydride–benzene gave α-p-*benzamidophenylglutaric anhydride* as minute, colourless cubes, m. p. 208° (yield, 85%) (Found: C, 69·4; H, 4·6; N, 4·9. $C_{18}H_{15}O_4N$ requires C, 69·9; H, 4·9; N, 4·5%). It was insoluble in 10% sodium hydrogen carbonate solution.

(ii) *From* α-p-*nitrophenylglutaric acid.* The acid (10 g.), dissolved in ethanol (150 ml.), was hydrogenated at 100 atm. pressure by aid of Raney nickel as catalyst. The amino-acid was isolated, benzoylated, and converted into the anhydride in the usual ways. The product (yield, 41·5%) was identical with that obtained by method (i).

Determination of K_a' *Values of the Substituted Glutaramic Acids.*—Values of K_a' for four of the acids (see Figs. 1 and 2) were determined by electrometric titration, a glass electrode and a Cambridge pH Meter being used.

Preparation of the Solution for Chromatographic Analysis.—A stream of dry ammonia was passed for 30 minutes into a solution of the anhydride (500 mg.) in dry dioxan (50 ml.). The reaction vessel was contained in a thermostat (20°) and was shaken intermittently. A little water was used to remove some of the product which adhered to the thermometer and inlet tube, and the aqueous dioxan was distilled *in vacuo*. To the dried ammonium salts was added an exact equivalent of dilute sulphuric acid solution required to liberate the free amic acids which were obtained in the dry state, together with ammonium sulphate, by evaporation *in vacuo*. The residue was warmed with dry acetone, and the remaining ammonium sulphate filtered off and washed with more acetone. The combined filtrate and washings were made up to 100 ml. with the same solvent. Titration of a portion of this solution with 0·01N-alkali showed, in each case, that not more than 1% of material had been lost by this stage. Moreover, the acetone solution was shown to be free from sulphate ion. A measured volume of the acetone solution (containing *ca.* 100 mg. of material) was evaporated to dryness *in vacuo* and the residue was analysed chromatographically.

Chromatographic Procedure.—Separations of the isomeric amic acids were achieved, in each case, by employing a column of kieselguhr with an immobile phase of 2M-phosphate buffer solution and a mobile phase of *n*-butanol–chloroform. The buffer solution, the optimum pH for which had been determined by previous experiments as 7·76, within narrow limits, was mixed with purified "Supercel" kieselguhr to form a gel containing 50% (w/w) of the aqueous solution. Approx. 30 g. of this material were made into a slurry in the appropriate mobile phase and introduced into a Pyrex tube, 30 cm. long and 1·5 cm. in diameter. Packing was facilitated by means of a perforated brass disc, fitted with a long handle, which was used first to agitate the gel to remove entrapped air and then to pack the material by slowly tamping down successive layers to give a column of length 20 cm. The upper surface of the gel was protected with a thin layer of glass wool and the column was then washed with 200 ml. of the mobile phase, previously equilibrated with the appropriate buffer solution. The sample for analysis (*ca.* 100 mg.) was taken up in the minimum amount of the mobile phase and the solution was loaded on to the top of the column in the normal manner. The chromatogram was developed with *n*-butanol–chloroform mixtures under positive pressure; the apparatus was designed to enable the solvent reservoir to be refilled without releasing the pressure on the top of the column. An automatic fraction-cutting machine of the gravity type (Randall and Martin, *Biochem. J.*, 1949, **44**, Proc., ii) collected cuts of 5·8 ml. each, which were titrated with 0·01N-sodium hydroxide to cresol-red (Moyle, Baldwin, and Scarisbrick, *loc. cit.*). A graph was constructed of titre, after correction for a blank value, against cut number. This enabled the amount of material in each peak to be determined and hence the proportion of the two amic acids in the original solution to be calculated (Figs. 1 and 2).

Isolation of the Amic Acids after Chromatography.—The titrated cuts corresponding to one peak were combined, and dilute sulphuric acid, exactly equivalent to the total titre of sodium hydroxide, was added. The solution was evaporated to dryness *in vacuo* and the product dried *in vacuo* over phosphoric oxide. The amic acid was then obtained by extraction with hot dry acetone, and filtration and evaporation of the acetone solution, followed by recrystallisation of the residue from a suitable solvent.

Identification of the Products after Chromatography.—The structures of the two phenylglutaramic acids and of the two *p*-nitrophenylglutaramic acids were established by comparison (m. p. and mixed m. p.) with their respective samples which had been synthesised by unequivocal routes. Identity was proved in all four cases. It is noteworthy that the γ-carbamyl-γ-phenylbutyric acid, isolated by chromatographic analysis, had m. p. 180° alone or after admixture with the material synthesised by Wideqvist's method. The authenticity of our compound and the correctness of its m. p. were thus established.

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