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N,N-Phthaloyl-L-glutamic Acid and Some Derivatives

R. STUART TIPSON

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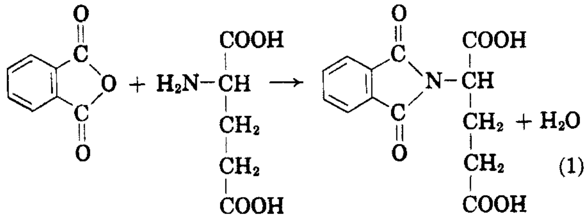
The reaction of L-glutamic acid with phthalic anhydride at 145–150°, followed by treatment with boiling water, has been shown to give rise to a separable mixture of N,N-phthaloyl-L-glutamic acid, N,N-phthaloyl-DL-glutamic acid, phthalic acid, and L-pyroglutamic acid. The reaction of N,N-phthaloyl-L-glutamic 1,5-anhydride with sodium methoxide in methanol has been studied; conditions for avoiding racemization are given. The 1- and 5-methyl esters of N,N-phthaloyl-L-glutamic acid have been obtained crystalline, and some physical properties, not previously recorded, are given for these and related compounds.

By the interaction of equimolar proportions of phthalic anhydride (I) and L-glutamic acid (II) during 15 minutes in a bath at 180–185°, followed by recrystallization from water, Billman and Harting[1] obtained a 45% yield of an "optically active" N,N-phthaloylglutamic acid (2-phthalimidoglutaric acid). On the other hand, King and Kidd[2] repeated the preparation and isolated "a product with feeble optical activity" which, *on recrystallization*, afforded N,N-phthaloyl-DL-glutamic acid (IIIa; yield, not given). They therefore decided[2] "that their synthesis had led to racemisation," but apparently overlooked the possibility that they had discarded (in the mother liquors) any N,N-phthaloyl-L-glutamic acid (IIIb) simultaneously formed. They also stated[2] that "at 130–140° the reaction appeared to be incomplete, the product not being purifiable by recrystallisation from water." A permissible inference is that the reaction was thought to proceed solely according to equation (1), to yield IIIa, despite the fact that many other possibilities can be envisaged.



I II (L-form) IIIa (DL-form) IIIb (L-form)

Shortly thereafter, however, Sheehan and Bolhofer[3] described the successful preparation, in 54% yield, of optically active N,N-phthaloyl-L-glutamic 1,5-*anhydride* by conducting the condensation at 140–150° for 20 minutes and then treating with acetic anhydride at 100°, but the optically active product was of unknown optical purity. (DeWald and Moore[4] have used similar 1,5-anhydride for preparing pure 1-methyl ester of IIIb.) Now, King and Kidd[2] had prepared the 1,5-anhydride of IIIb by a different method, but had failed to record its specific rotation. We therefore repeated their preparation[2] of the 1,5-anhydride of IIIb, measured its specific rotation, and, from the value observed, calculated that Sheehan and Bolhofer's anhydride[3] had contained 5.9% of the D-form, *i.e.*, 11.8% of DL and 88.2% of L.

This result cast doubt on King and Kidd's conclusions,[2] and consequently, reinvestigation of the supposed reaction mentioned (equation 1) was deemed desirable. Under our conditions, the following products were isolated: IIIb (47.4%); IIIa (9.1%); L-pyroglutamic acid (5-oxo-L-proline; 16.0%); II (15.5%); unidentified (12.0%, by weight). The total yield of IIIa plus IIIb was 56.5% (by weight) and this contained 16.1% of the DL-material; these results agree quite closely with those for Sheehan and Bolhofer's anhydride.[3] A method for separating much of the DL from the L form of the acid (by fractional recrystallization) was devised, and a product containing 97% of IIIb (and 3% of IIIa) was readily obtained. Recording of infrared absorption spectra (see Fig. 1) proved particularly useful in this and the subsequent work, permitting speedy decisions (as to identity of products) which could scarcely have been made by other methods at present available.

In dilute aqueous alkali, the N,N-phthaloyl group is readily split to the N-(*o*-carboxybenzoyl) group. This observation may prove useful; however, it renders King and Kidd's measurements[2] of optical rotations in such a solvent of no significance, since the concentration and the elapsed time after dissolution were not recorded.

The 1("α")-methyl ester of IIIb is an intermediate in one successful route[4] for the chemical synthesis of 6-diazo-5-oxo-L-norleucine (an antibiotic and tumor-inhibitory substance). Treatment of 97% L-anhydride (from 97% IIIb) with slightly less than one equivalent of sodium methoxide in methanol, at −40°, caused no racemization; and

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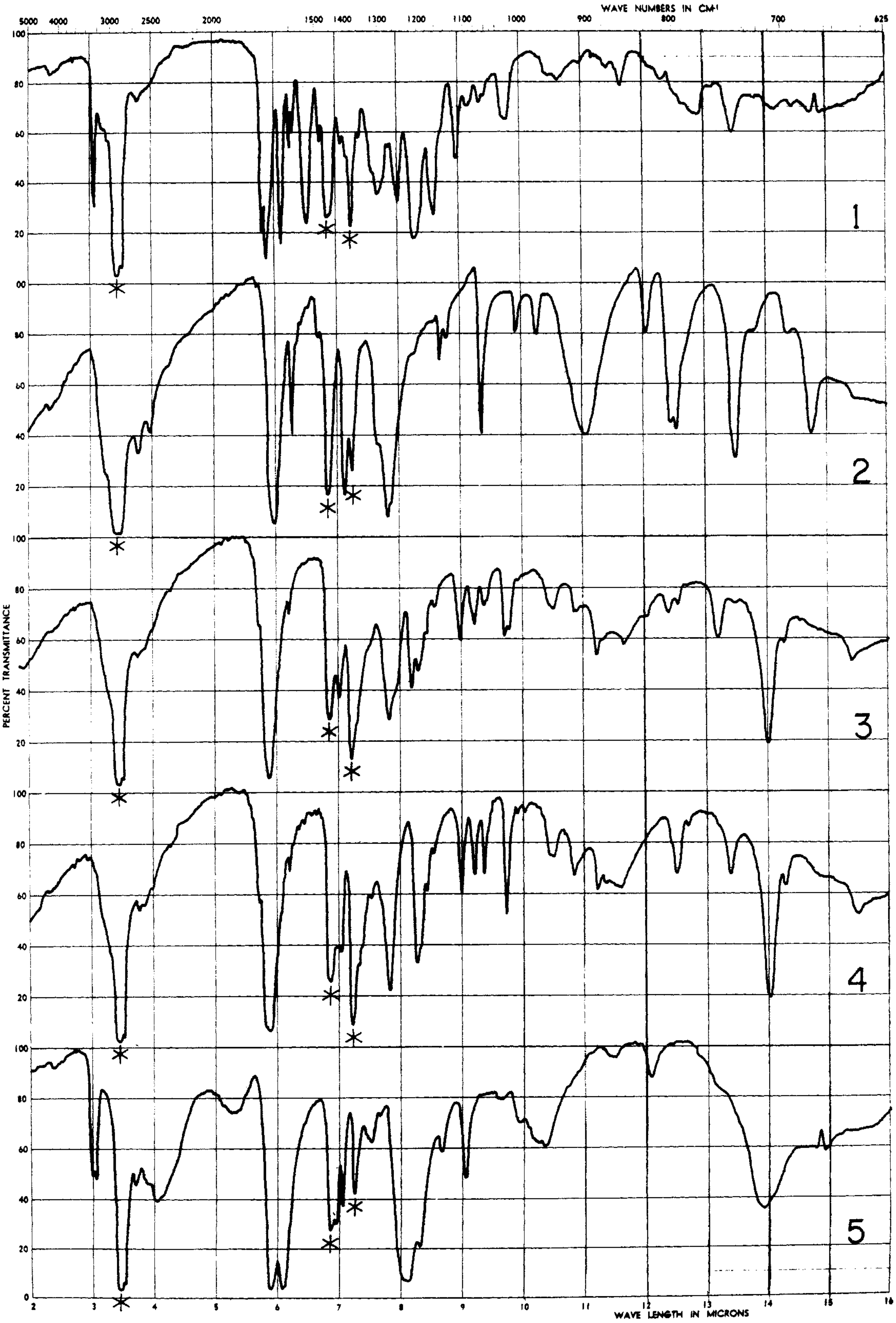
[1] Billman and Harting, *J. Am. Chem. Soc.*, **70**, 1473 (1948).

[2] King and Kidd, *J. Chem. Soc.*, 3315 (1949).

[3] Sheehan and Bolhofer, *J. Am. Chem. Soc.*, **72**, 2469 (1950).

[4] DeWald and Moore, *Abstracts Papers Am. Chem. Soc.*, 129th meeting, 13M (1956).



the 3% of crystalline 1-methyl ester of IIIa was readily separated from the crystalline 1-methyl ester of IIIb. [In contrast, treatment of authentic 1,5-anhydride of IIIb with one equivalent of sodium methoxide in methanol at 5° caused extensive racemization (*ca.* 46%), although some 1-methyl (and 5-

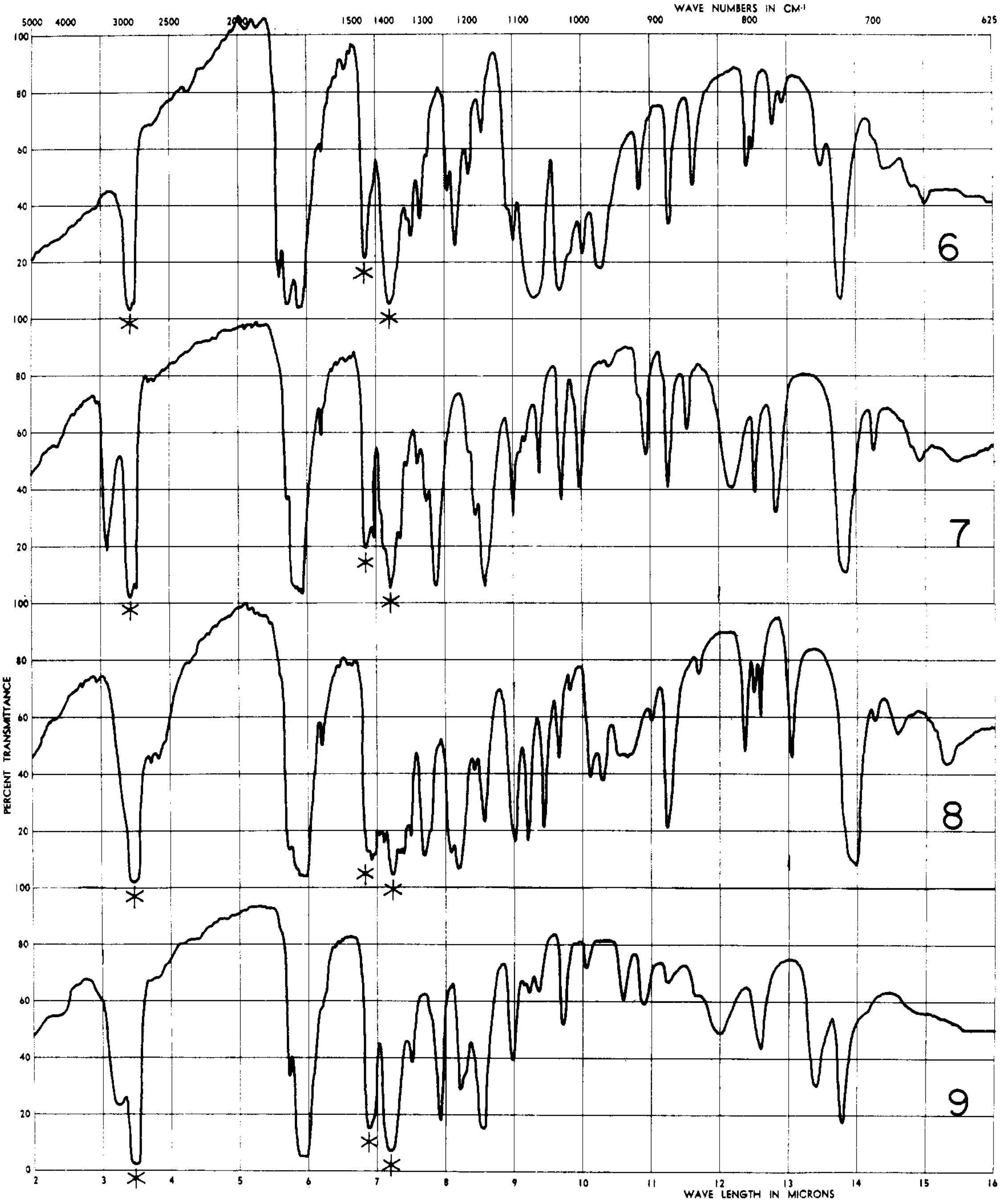


FIG. 1. INFRARED ABSORPTION SPECTRA OF (1) DIETHYL N-(*o*-CARBOXYBENZOYL)-L-GLUTAMATE, (2) PHTHALIC ACID, (3) N,N-PHTHALOYL-L-GLUTAMIC ACID, (4) N,N-PHTHALOYL-DL-GLUTAMIC ACID, (5) L-PYROGLUTAMIC ACID, (6) N,N-PHTHALOYL-L-GLUTAMIC 1,5-ANHYDRIDE, (7) 1-METHYL N,N-PHTHALOYL-L-GLUTAMATE, (8) 1-METHYL N,N-PHTHALOYL-DL-GLUTAMATE, AND (9) 5-METHYL N,N-PHTHALOYL-L-GLUTAMATE. (Asterisks indicate bands of Nujol.)

methyl) ester of IIIb was isolated.] Because of the ready separability of the 1-methyl esters of IIIa and IIIb by fractional recrystallization, prepurification of Sheehan and Bolhofer's anhydride[3] (88% L) is therefore unnecessary in preparing samples of pure 1-methyl ester of IIIb.

For comparison with the 1-methyl esters of IIIa[3] and IIIb, and with the 5("γ")-methyl ester[3] of IIIa, a sample of crystalline 5-methyl ester of IIIb was needed. Although Sheehan and Bolhofer[3] had prepared this ester, they had failed to obtain it crystalline; the preparation[3] was therefore repeated and the 5-methyl L-ester was crystallized. The melting points of these four esters are of interest: *1-ester*, of IIIa,[3] 154–155°; of IIIb, 137–138°; *5-ester*, of IIIa,[3] 119.5–120.5°; of IIIb, 118–120°. Slight contamination can markedly affect the melting points, and the 1- and 5-methyl esters of IIIb have quite similar optical activities; however, these two esters may be readily distinguished by their infrared absorption spectra and by their apparent dissociation constants. The effect of the N,N-phthaloyl group on the apparent dissociation constant of L-glutamic acid[5] and esters is given[6] in Table I.

TABLE I

EFFECT OF N,N-PHTHALOYL GROUP ON APPARENT DISSOCIATION CONSTANT OF L-GLUTAMIC ACID AND SOME ESTERS

		pK_a'	
Compound	Solvent[a]	First —COOH	Second —COOH
L-Glutamic acid[5]	Water	2.2	4.3
	A[6]	2.85	4.85
	Water[6]	2.1	4.3
1-Ethyl ester[5]	Water		3.9
5-Ethyl ester[5]	Water	2.2	
N,N-Phthaloyl-L-glutamic acid[6]	A	4.1	5.9
	Water	3.1	5.1
1-Methyl ester[6]	A		5.5
	Water		4.7[b]
5-Methyl ester[6]	A	4.0	
	Water	3.2[b]	

[a] Solvent A is 50% (by volume) aqueous methanol. [b] Estimated.[7]

EXPERIMENTAL

Diethyl N-(o-carboxybenzoyl)-L-glutamate. This ester was prepared as described by King and Kidd[2]; yield, almost quantitative (lit.,[2] 84%). For recrystallization, 44 g. was dissolved in 200 ml. of benzene at 65°, the hot solution was filtered, and 70 ml. of hexane was added to the cooled filtrate, giving a mass of minute crystals. Benzene (150 ml.) was added and the suspension was filtered; the colorless crystals were washed with 10 ml. of benzene and dried; wt., 35.0 g.; m.p. 95–97° (lit.[2] m.p., 94°, optical rotation not recorded); $[\alpha]_D^{22}$ +8.0° (*c*, 1.067, in chloroform). The infrared absorption spectrum of the product (see Fig. 1, 1) agreed with the proposed structure.

Diethyl N,N-phthaloyl-L-glutamate. This ester was prepared from the preceding compound by King and Kidd's method[2] *b*; yield, almost quantitative (King and Kidd[2] did not record their yield).

N,N-Phthaloyl-L-glutamic acid (IIIb). The preceding ester (22 g.) was hydrolyzed by dissolving it in 225 ml. of glacial acetic acid, adding 55 ml. of concentrated hydrochloric acid, and boiling under reflux during 2 hours. The solution was evaporated to *ca.* 50 ml., affording 2.0 g. of colorless crystals which, from the melting point and infrared absorption spectrum (Fig. 1, 2), proved to be phthalic acid. The filtrate was evaporated under diminished pressure, with occasional addition of water, until such addition gave a faint opalescence. Colorless crystals of N,N-phthaloyl-L-glutamic acid then separated; wt., 8.5 g.; m.p., 158–159° (cloudy; clear at 160.5°); pK_a 4.1 and 5.9 (in 50%, by vol., aqueous methanol),[6,7] 3.1 and 5.1 (in water).[6] Evaporation of the mother liquor to dryness, followed by treatment with 6 volumes of cold water afforded a further crop; wt., 2.1 g. Its infrared absorption spectrum (Fig. 1, 3) agreed with the proposed structure and, moreover, indicated absence of phthalic acid. The compound had $[\alpha]_D^{21}$ −23.8° (*c*, 1.007, in 0.33 *N* sodium carbonate solution) soon after dissolution, but this solvent (recommended by King and Kidd)[2] is unsuitable for the determination since it causes fission of the N,N-phthaloyl group; on keeping the solution, its optical rotation gradually becomes less negative, and peaks in the ultraviolet absorption spectrum [$E_{1\,cm}^{1\%}$ 993.0 at 222 mμ, and 51.2 at 299 mμ] disappear after 24 hours at room temperature. This decomposition does not occur if 95% ethyl alcohol is used as the solvent; $[\alpha]_D^{21}$ −42.6° (*c*, 1.033, in 95% ethyl alcohol); $E_{1\,cm}^{1\%}$ 1,370 (218 mμ), 331.6 (shoulder at 240 mμ), 62.92 (292.5 mμ); all unchanged on keeping.

N,N-Phthaloyl-L-glutamic 1,5-anhydride. This compound was prepared as described by King and Kidd;[2] yield, 88.4% (lit. yield,[2] 67%). It had m.p. 196–198°; $[\alpha]_D^{21}$ −43.1° (*c*, 1.75, in dioxane); King and Kidd[2] found m.p. 195–196°, and did not record an optical rotation. Its infrared absorption spectrum (Fig. 1, 6) agreed with the proposed structure.

Action of sodium methoxide (in methanol) on N,N-phthaloyl-L-glutamic anhydride. The 1-methyl L-ester was prepared using the method described by Sheehan and Bolhofer[3] for the DL compound, but some racemization occurred and 5-methyl L-ester was simultaneously formed. [It was later found (see below) that racemization can be avoided by performing the esterification at −40°.] Treatment of 5.1 g. of N,N-phthaloyl-L-glutamic 1,5-anhydride gave a quantitative yield of mixed esters, affording a first crop consisting mainly of 1-methyl N,N-phthaloyl-DL-glutamate; colorless crystals; wt., 2.6 g.; m.p. 149–151°; pK_a' 5.5 (in 50%, by vol., aqueous methanol),[6,7] 4.7 (Est'd, in water) $[\alpha]_D^{23}$ −3.3° (*c*, 1.210, in absolute methanol). A second crop, displaying a different infrared absorption spectrum, was 1-methyl L-ester contaminated with some 5-methyl L-ester; it weighed 1.4 g.; m.p. 115–118°; pK_a' 4.8 (in 50% methanol),[6] 4.0 (Est'd, in water); $[\alpha]_D^{24}$ −45.3° (*c*, 1.149, in absolute methanol). It was recrystallized from ethyl acetate (8 vols.) by portionwise addition of hexane (14 vols.); m.p. and pK_a' value, practically unchanged; ϵ = 40,000 (at 219 mμ), 9,500 (at 240 mμ), and 1,800 (at 293 mμ), in absolute ethanol.[6] The ultraviolet absorption spectrum in 0.1 *N* hydrochloric acid was similar,[6] but in 0.1 *N* sodium hydroxide revealed rapid rupture of the N,N-phthaloyl group within a few minutes.

Anal. Calc'd for $C_{14}H_{13}NO_6$: C, 57.73; H, 4.50; N, 4.81; OCH_3, 10.66. Found: C, 57.59; H, 4.62; N, 4.87; OCH_3, 10.08.

The mother liquor afforded a third crop (wt., 0.6 g.) of m.p. 110–113°; $[\alpha]_D^{22}$ −46.1° (*c*, 1.129, in absolute methanol); its infrared absorption spectrum was practically identical with that of the 5-methyl L-ester described later. Evaporation of the final mother liquor gave a sirup which crystallized completely but was not further examined.

Direct preparation of N,N-phthaloyl-L-glutamic acid. A mixture of 14.8 g. of phthalic anhydride, 14.7 g. of L-glutamic acid, and 25 ml. of dry xylene was heated under reflux (Stark and Dean trap) in a glycerol bath at 180° (inner temp., 145–150°) until 1.4 ml. of water had been collected (1 hour) and a clear melt resulted. This was cooled, dissolved in 100 ml. of acetone, filtered from a trace of undissolved material, and the clear filtrate was evaporated to dryness under diminished pressure. Water (443 ml.) was added, giving a suspension of colorless crystals which was boiled under reflux until dissolved and then cooled, affording 6.6 g. of crystals. The mother liquor was evaporated to dryness, and the crystalline mass was treated with 146 ml. of water, giving a second crop of crystals, wt., 11.2 g., m.p. 154–158°. The mother liquor was evaporated to dryness and the material was treated with 71 ml. of water, giving a third crop of crystals, wt., 0.8 g.

By fractional recrystallization from water, a series of crops of crystals was obtained; these were identified from their melting points, optical rotation, and infrared absorption spectra: phthalic acid, 3.7 g.; *N,N*-phthaloyl-DL-glutamic acid, 0.9 g. (see Fig. 1, 4); N,N-phthaloyl-L-glutamic acid, 1.3 g.; mixture of IIIa and IIIb, 8.8 g. (and

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(5) Neuberger, *Biochem. J.* (*London*), **30**, 2085 (1936).

(6) Determination kindly performed by Dr. J. M. Vandenbelt of Parke, Davis and Company, Detroit, Michigan.

(7) Vandenbelt, Spurlock, Giffels, and Eash, *Science*, **121**, 646 (1955).

very water-soluble material, 9.6 g.). [Alternatively, the material was first extracted with water (5 vols.) at room temperature; the insoluble crystals were dried and then were extracted with 4 volumes of acetone at room temperature; the acetone solution then contained almost pure title compound. The acetone treatment removes phthalic acid, DL acid, and both.]

For purification, the last two crops of crystals (of IIIb, and IIIa + IIIb) were combined and recrystallized from 16 volumes of boiling water, giving 7.5 g. of crystals; m.p. 165–166°; $[\alpha]_D^{22}$ −34.4° (*c*, 1.179, in 95% ethanol). This material was extracted with 4 volumes of acetone at room temperature; the extract was evaporated to dryness, treated with 5 volumes of cold water, and filtered, giving 4.3 g. of colorless crystals, m.p. 161–163°; $[\alpha]_D^{23}$ −41.2° (*c*, 1.553, in 95% ethanol), indicating 3.3% of DL and 96.7% of L form of the title compound. (Further purification was not carried out, since, as shown later, even this degree of purity is not necessary if the acid is to be used for preparation of its 1-methyl ester.)

The *very water-soluble* material was treated with 5 volumes of acetone, affording 2.9 g. of colorless crystals, m.p. 140–145°; this proved to be slightly impure L-pyroglutamic acid[8] (5-oxo-L-proline). It was recrystallized from boiling 2-butanone, and then had the correct elementary analysis; m.p. 155–160°; $[\alpha]_D^{22}$ −13.1° (*c*, 1.447, in water). Its infrared absorption spectrum is given in Fig. 1, 5.

In a repetition of the experiment (scale, 0.2 mole), the product (54.9 g.) was separated into the following fractions: N,N-phthaloyl-L-glutamic acid (26.0 g.; 47.4%); N,N-phthaloyl-DL-glutamic acid (5.0 g.; 9.1%); phthalic acid (8.5 g.; 15.5%); L-pyroglutamic acid (8.8 g.; 16.0%); unidentified, very water-soluble (6.6 g.; 12.0% by weight).

N,N-Phthaloyl-L-glutamic 1,5-anhydride (96.3% L). This was prepared from the acid (96.7% of L form), giving an 88.5% yield; m.p. 185–188°; $[\alpha]_D^{22}$ −41.6° (*c*, 1.155, in dioxane) indicating 96.3% of L form.

1-Methyl N,N-phthaloyl-L-glutamate. This ester was prepared, as before, from this anhydride (96.3% L, 5.1 g.), except that esterification was conducted at −40°, and only 90% of the theoretical amount of sodium methoxide was employed. The first crop was 1-methyl DL-ester (see Fig. 1, 8); it weighed 0.2 g., indicating absence of racemization The second crop (1-methyl L-ester) weighed 1.5 g.; m.p. 137–138°; pK_a' 5.5 (in 50% methanol),[6] 4.7 (Est'd, in water); ϵ = 40,000 (at 219 mμ), 9,500 (at 240 mμ), and 1,800 (at 293 mμ) in ethanol;[6] $[\alpha]_D^{23}$ −47.4° (*c*, 1.033, in absolute methanol); its infrared absorption spectrum (Fig. 1, 7) was identical with that of the previous sample of 1-methyl L-ester, except that it contained no bands indicative of slight contamination with 5-methyl L-ester.

Anal. Calc'd for $C_{14}H_{13}NO_6$: C, 57.73; H, 4.50; N, 4.81; OCH_3, 10.66. Found: C, 57.86; H, 4.60; N, 4.66; OCH_3, 10.36.

A third crop (1.7 g.) had m.p. 98–102°; $[\alpha]_D^{23}$ −43.4° (*c*, 1.243, in absolute methanol); and a fourth crop (1.0 g.) had m.p. 83–88°; $[\alpha]_D^{23}$ −41.4° (*c*, 1.185, in absolute methanol). Their infrared absorption spectra indicated that these last two crops consisted of 1-methyl L-ester and 5-methyl L-ester, respectively, but each was contaminated with some methyl ester of N-(*o*-carboxybenzoyl)-L-glutamic acid. The final mother liquor was evaporated to dryness, giving colorless crystals (1.2 g.) which were not further examined.

5-Methyl N,N-phthaloyl-L-glutamate. This ester was prepared from N,N-phthaloyl-L-glutamic 1,5-anhydride (96.3% L; 3 g.) as described by Sheehan and Bolhofer[3] for preparation of the oily 5-methyl L-ester, except that toluene was not used; yield of crude product, 3.0 g. (89%). It crystallized on evaporation of an ethereal solution, and was recrystallized from ethyl acetate (8 vols.) plus hexane (14 vols.), giving a first crop of colorless crystals (1.2 g.) having m.p. 122–138°; pK_a' 4.1 (in 50% methanol),[6] 3.3 (Est'd, in water); $[\alpha]_D^{21}$ −44.1° (*c*, 1.020, in absolute methanol). From its infrared absorption spectrum, it was 5-methyl L-ester, contaminated with a little racemate originating from the initial anhydride used. Addition of a further 10 ml. of hexane gave a second crop (0.6 g.); colorless crystals; m.p. 118–120°; pK_a' 4.0 (in 50% methanol),[6] 3.2 (Est'd, in water); $[\alpha]_D^{21}$ −45.1° (*c*, 1.008, in absolute methanol). Its infrared absorption spectrum (Fig. 1, 9), which was quite different from those of the 1-methyl L- and 1-methyl DL-esters, showed it to be purer than the first crop.

Anal. Calc'd for $C_{14}H_{13}NO_6$: C, 57.73; H, 4.50; N, 4.81. Found: C, 57.77; H, 4.66; N, 4.60.

On evaporation of the mother liquor, the residue solidified, but this material was not examined further.

PITTSBURGH 13, PENNSYLVANIA

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(8) Beilstein, *Handbuch der organischen Chemie*, **22**, 284; **EI**, 570; **EII**, 212.