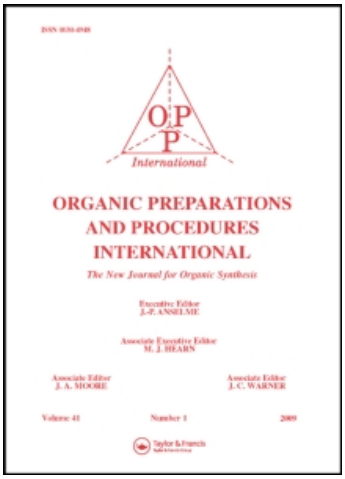

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AN IMPROVED SYNTHESIS OF L-γ-GLUTAMYL-4-NITROANILIDE

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OPPI BRIEFS

(By James A. Moore, Associate Editor)

AN IMPROVED SYNTHESIS OF L-γ-GLUTAMYL-4-NITROANILIDE

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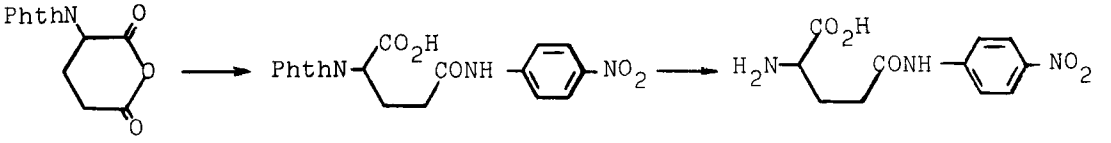
The preparation of L-γ-glutamyl-4-nitroanilide, a substrate for the estimation of serum γ-glutamyl transpeptidase activity, has been improved by modification of the published procedure.[1] Although it is commercially available, the cost of L-γ-glutamyl-4-nitroanilide restricts its routine clinical hepatic screening.[1,2]



I II III

Phth = Phthaloyl

EXPERIMENTAL

Mps. are uncorrected. Drying was carried out in a "Speedivac" freeze dryer Model 5PS, Edward's High Vacuum, Crawley, England.

Phthalyl-L-glutamic anhydride (I) was prepared by fusing finely ground L-glutamic acid (58.8 g) with phthalic anhydride (59.2 g). Initial heating was at 180° until effervescence began; then the temperature was lowered to 145° for a further 30 min., to give a pale yellow melt. Acetic anhydride (70 ml) was added to the cooled melt and the mixture was heated at 100-105° for 3 min. Xylene (210 ml) was added and on cooling to 4° for 12 hr., I was obtained as white crystals which, after drying in vacuo, melted at 199-201°, lit.[4] 199-201°.

Phthalyl-L-γ-glutamyl-4-nitroanilide (II). - The nitroanilide was prepared by dissolving I (53 g) and 4-nitroaniline (27.6 g) with stirring, in acetic acid (140 ml) at 60-65° in a water bath. The product began to crystallize after 5 min. and was completely precipitated after 35 min. at this temperature. The crystals were filtered from the hot solution, washed with n-propanol (50 ml) to remove residual acetic acid, and dried in vacuo to give II as yellow crystals, mp. 224-226°.

L-γ-glutamyl-4-nitroanilide (III). - This substrate was prepared by agitation of II (50 g) in methanol (1 l.) for 30 min. followed by filtration of the small amount of residual solids. The pH of the filtrate was adjusted to 8.2 by the addition of hydrazine hydrate (99-100%; 20 ml), to give a final hydrazine concentration of 0.4 M, and the mixture was left at 4° for 12 hr. The white crystalline product was filtered. A second

crop was obtained by seeding the mother liquor and leaving it for an additional 6 hr. at 4°. The combined solids were shaken vigorously with hydrochloric acid (0.5 M; 500 ml) and filtered. The filtrate was extracted with ethyl acetate (200 ml) to remove traces of 4-nitroaniline and the aqueous layer was neutralized by the gradual addition of solid sodium hydrogen carbonate. On approaching neutrality a dense, white, crystalline precipitate was formed and the mixture was left for 2 hr. at 4° to complete the precipitation. The solid was filtered, washed with ethanol (50 ml) and dried *in vacuo* to give 33 g (98%) of L-γ-glutamyl-4-nitroanilide as a pale yellow powder, mp. 184-186°, lit.[5] 186-188°. The product was stored at -20° in an anhydrous state to prevent hydrolysis. Batches of substrate prepared by this method have been in routine clinical use for several months in the estimation of γ-glutamyl transpeptidase activity and have been found most satisfactory.

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