Synthesis of a Vinylog of *p*-Aminobenzoylglutamic Acid

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In an attempt to prepare a folic acid vinylog, a vinylog of *p*-nitrobenzoylglutamic acid has been prepared using an alkyl carbonic-carboxylic acid-mixed anhydride. This intermediate nitro vinylog was reduced in the presence of Raney nickel to give a vinylog of *p*-aminobenzoylglutamic acid.

IN PURSUING a program dealing with the preparation of a vinylog of folic acid (pteroylglutamic acid), a relatively large quantity of *p*-nitrocinnamoylglutamic acid and *p*-aminocinnamoylglutamic acid was desired. Since the method (1) reported in the literature for preparing *p*-nitrobenzoylglutamic acid involved the condensation of *p*-nitrobenzoyl chloride and glutamic acid, it was believed that the condensation of *p*-nitrocinnamoyl chloride and glutamic acid would give the vinylog corresponding to *p*-nitrobenzoylglutamic acid. The preparation of *p*-nitrocinnamoylglutamic acid by this technique has been reported (2) to give a 37% yield of a pale yellow material melting at 206–208°. The modified Schotten-Baumann described by Siegart and Day (3) for the preparation of *p*-nitrobenzoylglutamic acid when applied to this study failed to improve the yields. In both procedures, *p*-nitrocinnamic acid and glutamic acid can be recovered in considerable quantity. This might have been anticipated due to the much higher degree of reactivity of *p*-nitrocinnamoyl chloride than that of *p*-nitrobenzoylchloride.

At this point, our attention was turned to the use of alkyl carbonic-carboxylic acid-mixed anhydrides for the preparation of amides (4). The use of this method of synthesis has become of increasing importance, especially in the synthesis of peptides (5). This reaction was readily adapted to the synthesis of *p*-nitrocinnamoylglutamic acid.

The reaction of the anhydride of *p*-nitrocinnamic acid and ethyl chloroformate with the diethyl ester of glutamic acid proceeded smoothly in diethylene glycol dimethyl ether (diglyme) or tetrahydrofuran (THF) at 0–5°. The diethyl ester of glutamic acid was employed to avoid possible interaction with the carboxylic groups. Hydrolysis of the diethyl *p*-nitrocinnamoylglutamate obtained in 50 to 73% yield gave a pale yellow product identical to that reported by Carrol (2).

The use of a solution of ammonium sulfide (6) for the selective reduction of the nitro group of cinnamic acids appears to be inadequate here since its basic character and the high reaction temperature (85–90°) is sufficient to split the amide linkage. For the conversion of the diethyl *p*-nitrocinnamoylglutamate to diethyl *p*-aminocinnamoylglutamate, the procedure of Blout (7) was found to be effective. In Blout's work, it was demonstrated that Raney nickel does effectively catalyze the hydrogenation of aromatic nitro groups in preference to aliphatic double bonds conjugated with a benzoid ring, and that it was possible to prepare in good yields amino cinnamic acids and esters from the corresponding nitro compound. However, if 2 to 3 Gm. (wet wt.) of Raney nickel were used, as in Blout's original work, an oil was the only product isolated. If, on the other hand, 1 to $1^1/_2$ Gm. (wet wt.) of Raney nickel were employed, a yellow solid substance was obtained; this proved to be diethyl *p*-aminocinnamoylglutamate. In the latter case, the rate of hydrogen absorption was very slow. Adkins (8) has reported that the use of sufficient quantities of Raney nickel can be employed to reduce esters of certain amino acids to amino alcohols. This may, in part, account for the undesirable oil which was isolated when 2 or 3 Gm. (wet wt.) of Raney nickel was employed.

As the similarity in physical and chemical properties of a compound and its vinylog is well known (9), it has become of interest to learn whether or not vinylogs of folic acid will demonstrate similar biological activity. At this time, the only significant observation occurring among these vinylogous substances has to do with one of their physical properties, *viz.*, melting points (Table I).

EXPERIMENTAL

Diethyl *p*-Nitrocinnamoyl-DL-glutamate.—The general method of Sam (10) was utilized with some modification. The procedure of Pandya (11, 12) was employed for preparing *p*-nitrocinnamic acid. Diethyl glutamate was prepared by the method of Chiles and Noyes (13). To a stirred and cooled

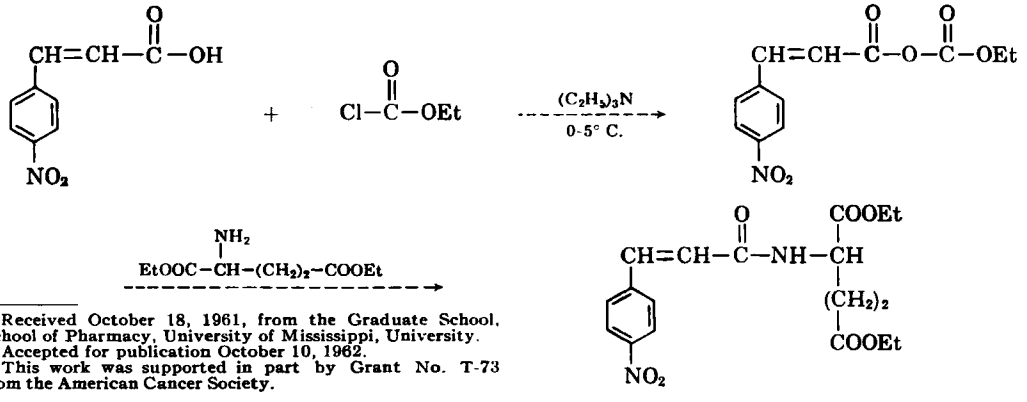

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TABLE I.—COMPARISON OF MELTING POINTS

Parent Compound	M.p., ° C.	Vinyl Compound	M.p., ° C.[a]
p-Nitrobenzoylglutamic acid	112.5 to 113.5	*p*-Nitrocinnamoylglutamic acid	205–207
Diethyl *p*-nitrobenzoylglutamate	96	Diethyl *p*-nitrocinnamoylglutamate	97–99
p-Aminobenzoylglutamic acid	172–173	*p*-Aminocinnamoylglutamic acid	193.8 to 196.6
Diethyl *p*-aminobenzoylglutamate	143–144	Diethyl *p*-aminocinnamoylglutamate	115.5 to 117

[a] Melting points are corrected.

mixture of 20 Gm. (0.103 mole) of *p*-nitrocinnamic acid in 1 L. of diglyme there was gradually added 10.4 Gm. (0.103 mole) of triethylamine. The resulting solution was maintained at 0–5° during the addition of 13 Gm. (0.128 mole) of ethyl chloroformate. After the mixture had been maintained at 0° for 30 minutes there was added 20.0 Gm. (0.146 mole) of diethyl DL-glutamate dissolved in 100 ml. of diglyme; the temperature was maintained at 0 to 5°. The contents of the reaction vessel were allowed to warm to room temperature (32°) and stirred at that temperature for 6 hours. A white crystalline material (triethylamine hydrochloride) was collected by filtration. The yellow solution was allowed to stand overnight at room temperature. Five-hundred milliliters of distilled water was then added to this yellow solution, followed by the addition of chipped ice until precipitation was complete. There was obtained 25.7 Gm. (66.0%) of diethyl *p*-nitrocinnamoyl-DL-glutamate. After recrystallization to analytical purity from an ethanol-water solution, a m.p. of 97–99° was observed.

Anal.—Calcd. for $C_{18}H_{22}N_2O_7$: C, 57.14; H, 5.82; N, 7.14. Found: C, 57.32; H, 5.60; N, 7.46.

Diethyl *p*-Aminocinnamoyl-DL-glutamate.—Ten grams (0.026 mole) of diethyl *p*-nitrocinnamoyl-DL-glutamate was suspended in 50 ml. of absolute ethanol in a pressure bottle and approximately 1 to 1.5 Gm. (wet wt.) of Sponge Nickel Catalyst[1] was added. The bottle was flushed with hydrogen and then shaken mechanically until the rate of hydrogen uptake had decreased tenfold (approx. 24 hours). The catalyst was filtered off and ice-water added to the filtrate. Upon chilling, a pale yellow solid formed. The product obtained in yields of 64–77% melted in the range of 115.5 to 117°.

Anal.—Calcd. for $C_{18}H_{24}N_2O_5$: C, 62.06; H, 6.90; N, 8.05. Found: 62.16; H, 7.12; N, 8.05.

p-Aminocinnamoyl-DL-glutamic Acid.—One gram (0.003 mole) of diethyl *p*-aminocinnamoyl-DL-glutamate was suspended in 25 ml. of 1 *N* NaOH and stirred overnight at room temperature. The resulting solution was chilled and acidified to pH 3–4 with concentrated hydrochloric acid. A cloudy solution resulted but no precipitation occurred. To this solution was added about 1 Gm. of sodium chloride. Upon stirring, a yellow-brown solid appeared. This material was collected on a sintered-glass filter-funnel. Recrystallization from hot water to which a few milliliters of ethanol were added gave a yellow product. This material was observed to melt in the range of 193.8 to 196.6°.

Anal.—Calcd. for $C_{14}H_{16}N_2O_5$: C, 57.53; H, 5.53; N, 9.58. Found: C, 57.54; H, 5.57; N, 9.38.

The presence of a dicarboxylic acid was also confirmed by the determination of its neutralization equivalent.

[1] Division Chemical Co., Cincinnati, Ohio.

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ERRATUM

In the paper titled "Chromatography and Electrophoresis of Phenothiazine Drugs" (1), the last sentence of paragraph two under *Methods* on page 1169 should be changed to read: "The dissolved substances were applied in amounts of 5 to 10 mcg. . . ." Additionally, the last clause of the last sentence of the first full paragraph on page 1170 should be changed to read: ". . . , when concentrations over 10 mcg. per spot were applied."

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