DINITROPHENYL PEPTIDES

I. PREPARATION AND PROPERTIES OF SOME DINITROPHENYL GLYCYL DIPEPTIDES

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ABSTRACT

Some dinitrophenyl glycyl peptides have been prepared from dipeptides and from dinitrophenyl glycyl chloride, and the products suitably characterized. Electrophoresis gave good separation of those differing by more than two carbon atoms.

Dinitrophenyl peptides prepared from protein or peptide hydrolyzates by Sanger's method (1) are usually characterized by chromatographic identification of their hydrolysis products. Where direct comparison with authentic dinitrophenyl peptides has been made, this was based only on chromatographic behavior (2, 3, 4). Comparison on the basis of other physical properties, such as melting point, specific rotation, etc., would require the synthesis of authentic dinitrophenyl peptides, preferably by several independent methods, and determination of their physical properties. Rhinesmith (5) has recently reported the synthesis of dinitrophenyl-DL-valyl peptides from dinitrophenyl-DL-valyl chloride, but did not report their physical properties or compare them with products from any other preparative method. Also, yields were calculated from spectral data, and analysis was based on chromatographic and spectral estimation of the hydrolysis products.

We have therefore undertaken a systematic study of the preparation and properties of dinitrophenyl peptides, in particular the dinitrophenyl derivatives of glycylglycine, glycyl-L-alanine, glycycl-L-valine, and glycyl-L-leucine by two procedures found to be the most suitable. Separation of these peptide derivatives from one another and from dinitrophenyl glycine by electrophoresis is also reported.

EXPERIMENTAL

Dinitrophenyl Glycine

This compound was prepared by the method of Levy and Chung (6) using sodium bicarbonate instead of sodium carbonate as condensing agent.

Dinitrophenyl Glycyl Chloride

Dinitrophenyl glycine (1 g) and thionyl chloride (10 ml) were heated 1 hour under reflux, and the excess thionyl chloride removed by distillation under vacuum. The yellow crystalline residue was heated under reflux with carefully dried benzene to complete solution; on cooling, the product crystallized as large yellow needles. Yield 1 g (92.5% theory), m.p. 129–130°. Anal. Calc. for $C_8H_6N_3O_5Cl$: C, 36.96, H 2.33, N 16.17, Cl 13.66. Found: C 37.42, H 2.62, N 15.29, Cl 12.57. The preparation of this compound was studied previously (5) but lower yields were obtained and its melting point was not reported.

Dinitrophenyl Peptides

The dinitrophenyl peptides made directly from the dipeptides and from the acid chloride were identical, and distinct from dinitrophenyl glycine, as established by mixed melting point determination. When they were made from dinitrophenyl glycyl chloride, little if any racemization occurred, and sodium hydroxide was unsuitable as acid binder.

A. From the Dipeptides

The dipeptide and 2,4-dinitrofluorobenzene (equimolar quantities) were added to 15% (w/v) sodium bicarbonate solution (8.7 ml/mmole of peptide), and the mixture stirred 2 hours at room temperature. Immediate acidification with hydrochloric acid precipitated the product as a yellow solid, which was filtered and washed with water.

B. From Dinitrophenyl Glycyl Chloride

A solution of the acid chloride (1 mmole) in dry benzene (6–8 ml) was added slowly with stirring to an ice-cold solution of the amino acid (1 mmole) in 3.5% (w/v) aqueous sodium carbonate (18 ml) during 2 hours. The stirring was continued for a further 2 hours at ice-bath temperature, whereupon the benzene layer was removed and the aqueous layer acidified with hydrochloric acid to precipitate the dinitrophenyl peptide. With all the peptides except glycyl glycine, a darker-colored impurity appeared during the initial stages of acidification, before precipitation of the product. This impurity was easily removed by careful addition of acid until no further impurity separated, followed by decantation; the remainder of the acid was then added immediately.

Dinitrophenyl Glycylglycine

The solid was recrystallized by dissolving in ethanol and adding just enough water for crystals to form, which were filtered and dried 4 hours at 104°. *Method A*—91–92% yield, m.p. 194–195°, optically inactive in 95% ethanol. *Method B*—54–55% yield, m.p. 194–195°, optically inactive in 95% ethanol. Anal. Calc. for $C_{10}H_{10}N_4O_7$: C 40.03, H 3.38, N 18.8, equiv. 298.14. Found: C 39.44, H 3.64, N 16.72, equiv. 299.4.

Dinitrophenyl Glycyl-L-alanine

Method A—Recrystallization and drying were as described for dinitrophenyl glycylglycine; 75–76% yield, m.p. 175–176.5°, $[\alpha]_D^{21}$ −23.4±2° (2.77% in 95% ethanol). *Method B*—Recrystallization had to be as described for dinitrophenyl glycyl-L-valine; drying was as for method A; yield 44–45%, m.p. 175–176.5°, $[\alpha]_D^{21}$ −23.4±2° (2.75% in 95% ethanol). Anal. Calc. for $C_{11}H_{12}N_4O_7$: C 42.31, H 3.87, N 17.98, equiv. 312.27. Found: C 41.73, H 3.77, N 18.01, equiv. 317.1.

Dinitrophenyl Glycyl-L-valine

This compound was recrystallized by dissolving in ethanol,* diluting with a large volume of water, evaporating to small bulk at room temperature,† and repeating the entire process as often as necessary to reach a constant melting point; it was dried 4 hours at 90°.

Method A—44–45% yield, m.p. 132–134°, $[\alpha]_D^{22}$ −21.2±2° (1.18% in 95% ethanol). *Method B*—44–45% yield, m.p. 131–132°, $[\alpha]_D^{22}$ −20.4±2° (1.22% in 95% ethanol). Anal. Calc. for $C_{13}H_{16}N_4O_7$: C 45.59, H 4.74, N 16.38, equiv. 340.3. Found: C 45.29, H 4.96, N 14.9, equiv. 337.0.

Dinitrophenyl Glycyl-L-leucine (Monohydrate)

Recrystallization was as for the glycyl-L-valine derivative, and it was dried overnight at room temperature over phosphorus pentoxide. *Method A*—46–47% yield, m.p. 98–100°, $[\alpha]_D^{22}$ −29.8±2° (1.01% in 95% ethanol). *Method B*—57–58% yield, m.p. 96–98°, $[\alpha]_D^{22}$ −29.1±2° (1.03° in 95% ethanol). Anal. Calc. for $C_{14}H_{20}N_4O_8$: C 45.16, H 5.41, N 15.05, equiv. 373.32. Found: C 45.82, H 5.24, N 15.11, equiv. 364.3–369.1.

Carbon, hydrogen, and nitrogen analyses were by Geller Laboratories, Bardonia, N.Y.

Electrophoresis

A Reco model E 800-2 open strip apparatus was used, at 300 v and 5 ma for 20 hours at 21°, with 0.02 *M* borate buffer (pH 9.11) and Whatman No. 1 paper, 50×20 cm. A bridge support was placed under the paper near the positive plate, to ensure that the compounds (in anionic form) would be migrating on a rising incline. The initial spots were placed near the opposite end, avoiding the part of the paper held to the cooling surface by unavoidable capillary action.

The movements of dinitrophenyl glycine and of the products, separately and in various admixtures with one another, were studied. The glycine derivative moved much further than the dipeptide derivatives, and the latter showed good separation of those differing by more than two carbon atoms. Distances moved by the dinitrophenyl derivatives of glycine, glycylglycine, glycyl-L-alanine, glycyl-L-valine, and glycyl-L-leucine were 17.5, 14, 13.2, 10.7, and 10.5 cm respectively.

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**The ethanol must not be warmed.*
†Air must not be bubbled through the liquid.