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A MILD CONVERSION OF THE 2,4-DINITROPHENYL-GLYCYL-MOIETY TO A DERIVATIVE OF 6-NITROBENZIMIDAZOL-1-OXIDE

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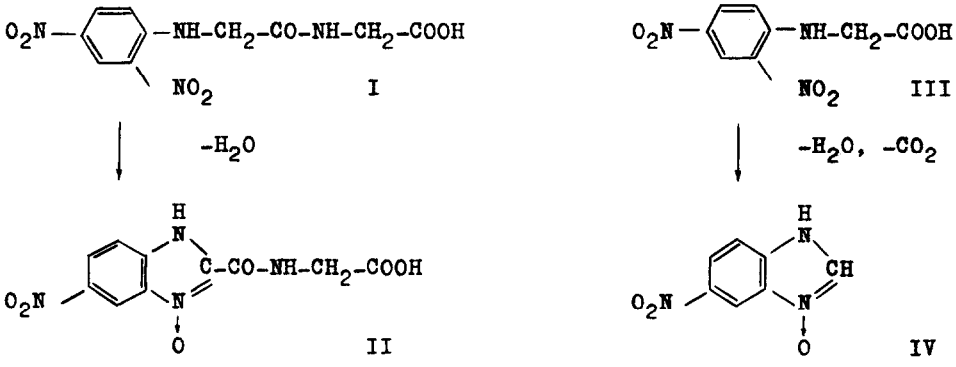
Peptides, containing 2,4-dinitrophenylglycine as N-terminal moiety at pH 8.3-9.6 and 37° undergo a slow conversion into derivatives of 6-Nitrobenzimidazole, as shown on the scheme below:



A solution of 500 mg of DNP-Gly-Gly(I) in 100 ml of 1 M triethylammonium-carbonate buffer (pH 8.3) was kept in the dark at 37°. A change in UV absorption appeared after 24 hr and after 7 days a quite different spectrum was recorded (Fig.1). The maximum at 350 nm characteristic of the 2.4-dinitroanilino group had disappeared and a new one at 295 nm with a shoulder at 330 nm emerged. The pH of the mixture was now 9.1. Paper elec-

trophoresis (pH 5.6, 1 hr, 27 v/cm) revealed a new pale yellow spot which migrated 10.4 cm to the anode whereas DNP-Gly-Gly moved 8.2 cm to the anode. The mixture was acidified to pH 2.2 with HCl and kept for 12 hr at 4°. The precipitate was collected, washed with 10 ml of ice water, and recrystallized from 50 ml of 95 per cent ethanol. Yield - 275 mg (58.6%). Repeated crystallization gave 208 mg of II as pale yellow crystals, m.p.250-251°. Found: C 42.97; H 3.14, N 19.97%. Calcd. for $C_{10}H_8N_4O_6$:C 42.86, H 2.87, N 20.00%. λ_{max} in water,pH 3.5 - 281 nm, pH 6.5 - 297 nm, in 95% ethanol - 300 nm. Spectrophotometric titration revealed a group with pK_a 5 in addition to carboxyl C-terminal glycine which explained the increased electrophoretic mobility. The IR spectrum of II contained the following characteristic bands: 1665 cm^{-1} (amide I), 1570 cm^{-1} (amide II), 1720 cm^{-1} (CO of carboxyl), 3400-2700 cm^{-1} (OH of carboxyl), 1520 and 1350 cm^{-1} (NO_2 group). Among hydrolysis products (5.7 n HCl, 22 hr, 105°) glycine and 6-nitrobenzimidazol-1-oxide (IV) were identified.

Essentially the same changes were observed in UV spectra of DNP-Gly-Gly-X, (X = Arg, Lys, Val-Arg, Phe-Arg, Ile-Arg), DNP-Gly-Arg and DNP-Gly-Phe-Phe-Arg. No effect was observed with DNP-peptides containing N-terminal amino acids other than glycine, e.g. with DNP-Pro-Phe-Arg and

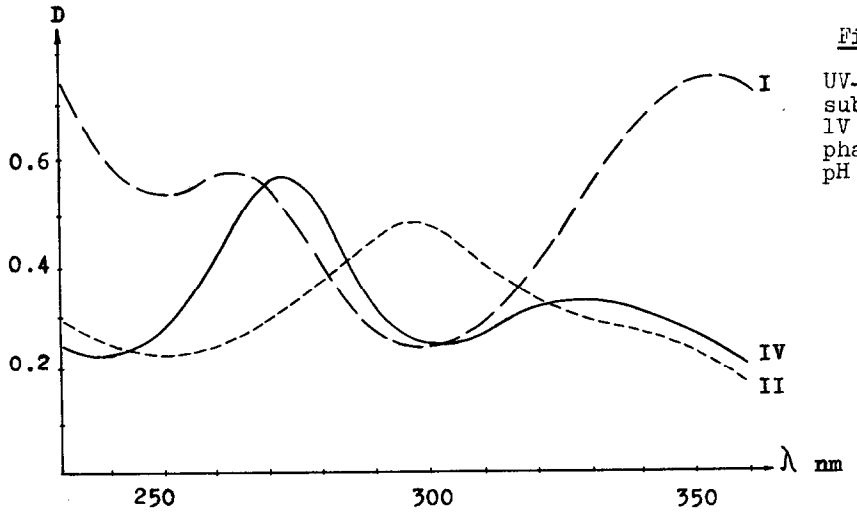


Fig.1

UV-spectra of substances I,II, IV (0.2 M phosphate buffer, pH 8).

DNP-Ala-Asn. UV spectrum of the latter remained unchanged after 45 days at 37°. These data emphasize the importance of methylene group of N-terminal glycine for described reaction.

Analogous conversion of DNP-glycine (III) proceeds rather slowly which can be ascribed to the influence of the free carboxylate group. A solution of 500 mg of DNP-glycine in 50 ml of 0.2 M phosphate buffer, pH 8.5, was heated for 72 hr at 90° and acidified to pH 2 with HCl. The precipitate was collected, washed on the filter with 6x2 ml of cold water and crystallized from 50 ml of 95% ethanol. Yield - 270 mg (72.5%). Recrystallization from ethanol gave 200 mg of the substance with m.p. 268.5-269°. Found: C 46.90, H 2.62, N 23.74%. Calcd. for $C_7H_5N_3O_3$: C 46.93, H 2.81, N 23.45. The IR spectrum of the substance coincides with that of 6-nitrobenzimidazol-1-oxide, obtained as described (1).

The properties of IV obtained as above and of 6-nitrobenzimidazole-1-oxide compared below ascertain their identity.

	Substance from DNP-Gly	6-Nitrobenzimidazol-1-oxide	
		our measurements	:Lit. data (1)
UV maxima at pH 2	234, 282	234, 283	230, 278
pH 4-6	257, 300 (sh)	256, 300 (sh)	258, 300 (sh)
pH 8	273, 330	274, 330	271, 325
in ethanol	242, 282		240, 296
pK_a	2.2; 5.9		2.2, 6.1
Electrophoretic mobility at pH 4.5	0	0	0
pH 2.3	4.1 cm	4.2 cm (to cathode)	-
pH 10.5	4.8 cm	5.0 cm (to anode)	-
Melting point	268.5-269°	269 - 270°	274°

Hence, under conditions specified above, cyclization of DNP-glycine with concomitant decarboxylation leads to 6-nitrobenzimidazol-1-oxide.

Formation of derivatives of 6-nitrobenzimidazole-1-oxide as a product of photodecomposition of DNP-amino acids has been described (2,3,4). It is tempting to suppose that the cyclization of DNP-glycyl moiety might explain the intensive decomposition of DNP-glycine which is known to limit the DNP-method of identification of N-terminal amino acids in peptides and proteins.

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