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SENSITIZED PHOTOOXIDATION OF N-BENZOYL HISTIDINE.

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The photooxidation of histidine residues has occasionally been applied to the investigation of the reaction mechanism of enzymes, in which the histidine residues were assumed to be involved in the active site[1, 2]. The mechanism of the photooxidation of the histidine residue in a protein and even of the monomeric histidine[3, 4] has not precisely been elucidated yet. The present authors attempted a precise investigation on the reaction mechanism of the photooxidation of N-benzoyl histidine which was found to be the most suitable derivative of histidine as the substrate of the reaction, because the products of the reaction could easily be detected by UV absorptions and isolated by extraction with organic solvents.

The solution of 36 mmoles of N-benzoyl histidine in 300 ml of water was oxidized in the presence of methylene blue at pH 11.0. Aeration was effected by rapid stirring of the solution and the illumination was provided by a reflector lamp of 300 W at a distance of 13 cm above the surface of the solution. The reaction mixture was worked up after 6 hr. of irradiation. The products were separated by ion exchange column chromatography using Dowex 50W-X4 (H^+) into group A which contained the products having no basic residues and group B which contained those having basic residues. On further separations of these groups by column chromatography through Dowex 1-X4 (formate) with elution by gradient of increasing formic acid concentration, group A gave nine products, Ia(15%), IIa(4%), IIIa(3%), IVa(8%), Va(3%), VIa(<1%), VIIa(<1%), VIIIa(1%) and IXa(<1%), whereas group B gave seven products, Xa(<1%), XIa(3%), XIIa(1%), XIIIa(6%), XIVa(5%), XVa(14%) and XVIa(9%). The group A, on application to a silica gel column chromatography, gave an additional product (XVIIa) in a yield of 2%, which had not been obtained by Dowex 1 column chromatography. Among these products isolated, Ia, IIa, IVa, Va, VIa, VIIa, VIIIa, XIa, XIIIa, XIVa, XVa and XVIIa were obtained as crystals appeared from methanol or aqueous methanol. Methylations with diazomethane of Ia, IIa, IVa, Va, XIIIa and XIVa yielded the corresponding methyl esters, Ib, IIb, IVa, Vb, XIIIb and XIVb, respectively.

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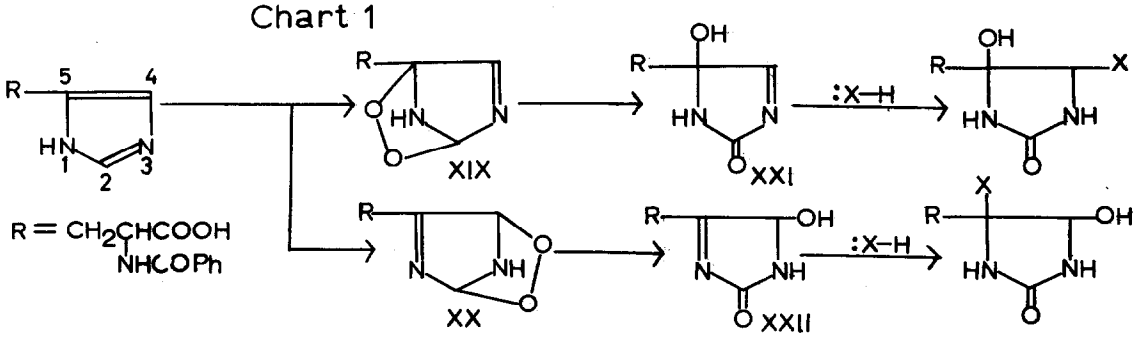
In TABLE, physical properties of the products, IIa, IIb, Va,Vb and XVIIa were summarized. Ia and IVa were identified with authentic samples of N-benzoyl aspartic acid and N-benzoyl asparagine[5] by mixed fusion, respectively.

Analogously to the related photooxidation in the furan[6], imidazole[4] and pyrrole[7], the photooxidation of N-benzoyl histidine should theoretically involved two types of hydrated imidazolone, XXI and XXII, which could be produced by cleavage of O-O bond and simultaneous prototropy of two types of endoperoxides, XIX and XX, respectively. Each of these hydrated imidazolones involved an electrophilic locus: the position 4 in XXI and the position 5 in XXII, which should receive the nucleophilic attack by several nucleophiles to give the following products.

Chart 1



The analysis of the product Va (see TABLE I) indicated the presence of a tertiary benzamido group[8] in UV spectrum, and a carboxyl group. The mass spectrum of the methyl ester of Va (Vb) showed that it could be easily dehydrated to give a double bond. From these characteristics and other analytic data, the structure of Va in Chart 2 was postulated to this compound. This compound (Va) should be produced by intramolecular attack of nonbonding electron pair of amido nitrogen at the 4 position of XXI. The compound XVIIa was found

Chart 2

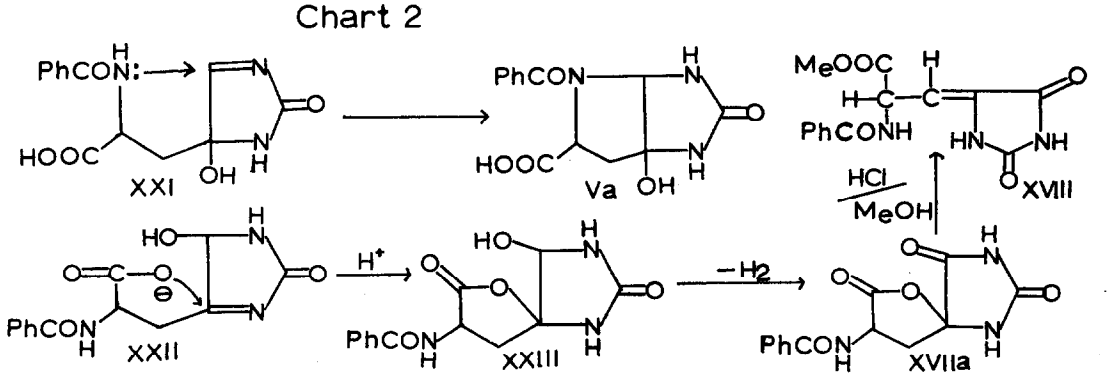
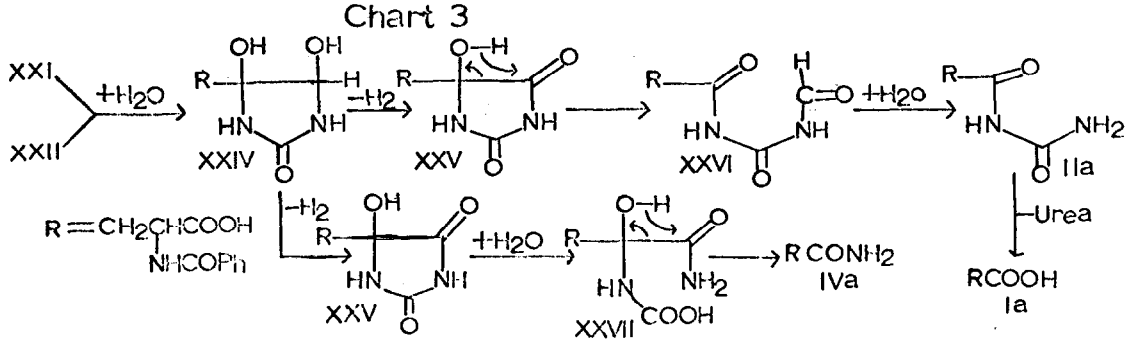


TABLE 1. Physical properties for the identifications of compounds, IIa, IIb, Va, Vb, XVIIa and XVIII.

compound	IIa	IIb	Va	Vb	XVIIa	XVIII
m.p. °C	217	218	170(dec.)	234	202(dec.)	163
molecular formula	$C_{12}H_{13}N_3O_5$	$C_{13}H_{15}N_3O_5$	$C_{13}H_{13}N_3O_5$	$C_{14}H_{15}N_3O_5$	$C_{13}H_{11}N_3O_5$	$C_{14}H_{13}N_3O_5$
UV (solvent) λmax: mμ (ε)	(H_2O) 228 (10500)	(MeOH) 228 (9700)	(H_2O) 228 (5000)	(MeOH) 228 (6400)	(MeOH) 230 (10900)	(MeOH) 230 (15500) 275 (7000)
IR (KBr) ν_{max}:cm^{-1}	3350, 1655 1525 (-CONH-) 1700 (-COOH) ($NHCONH_2$)	3360, 1655 1530 (-CONH-) 1750 (-COOMe) 1695 (-$NHCONH_2$)	3380, 1080 (-OH) 1710 (-COOH) 1690 (-NHCONH-) 1620 (-CON<)	3440, 1070 (-OH) 1725 (-COOMe) (-NHCONH-) 1650 (-CON<)	3340, 1650 1530 (-CONH-) 1780 (γ-lactone) 1805, 1760 1730 (hydantoin)	3370, 1640 1535 (-CONH-) 1730 (-COOMe) 1780, 1730 1695 (hydantoin)
mass spectrum m/e (% of base peak)	43 (29) 51 (23) 77 (74) 105 (100) 147 (17) 218 (15) 77=$C_6H_5^+$ 105=$C_6H_5CO^+$	50 (60) 51 (57) 77 (75) 105 (100) 174 (56) 218 (36) 234 (50) 293 (9)=M^+		51 (90) 77 (95) 105 (100) 219 (40) 228 (36) 246 (85) 287 (22) 305 (7)=M^+	44 (88) 51 (17) 77 (68) 105 (100) 140 (90) 245 (6)	51 (38) 77 (43) 105 (100) 198 (73) 244 (30) 302 (18) 303 (5)=M^+
nmr spectrum (d_6-DMSO) τ-value	7.10 (2H, d) (-CH_2-) 5.20 (1H, m) (-CH<) 2.90 - 2.05 (8H, m) (PhCO- and -$NHCONH_2$) 1.25 (1H, d) (-CONH-) -0.25 (1H, s) (-COOH)	7.10 (2H, d) (-CH_2-) 6.35 (3H, s) (-COOMe) 5.10 (1H, m) (-CH<) 2.90 - 2.05 (8H, m) (PhCO- and -$NHCONH_2$) 1.25 (1H, d) (-CONH-)	7.70 (2H, m) (-CH_2-) 5.60 (1H, m) (-CH<) 4.80 (1H, s) (-CH<) 4.60 (8H, broad) (HDO) 2.60 (7H, s) (PhCO- and -NHCONH-)	7.65 (2H, m) (-CH_2-) 6.35 (3H, s) (-COOMe) 5.50 (1H, m) (-CH<) 4.75 (1H, s) (-CH<) 2.55 (7H, s) (PhCO- and -NHCONH-)	7.15 (2H, d) (-CH_2-) 5.00 (1H, m) (-CH<) 2.65 - 2.00 (5H, m) (PhCO-) 0.90 (1H, s) (-NHCO-) 0.65 (1H, d) (-NHCO-) -1.40 (1H, s) (-CONHCO-)	6.30 (3H, s) (-COOMe) 4.60 (1H. m) 4.40 (1H, d) (>CH-CH=) 2.75 - 2.00 (5H, m) (PhCO-) 0.90 (1H, d) (-CONH-) -0.65 (1H, s) (-CONH-) -1.20 (1H, s) (-CONHCO-)

Chart 3



neutral, indicated a chracteristic IR spectrum to hydantoin derivative and gave the product (XVIII) by treatment with HCl - MeOH, which gave λ_{max}^{MeOH} 275 mμ. Together with analytic data given in the TABLE, the structures XVIIa and XVIII given in Chart 2 were proposed to these compounds. The compound XVIIa should be produced by an initial intramolecular attack by carboxylate at the 5 position of XXII to form an intermediate compound XXIII and followed dehydrogenation of the secondary hydroxyl group of XXIII. The compound (XVIII) must be produced from XVIIa by methanolysis and dehydration with methanolic HCl. The attack of H_2O at the 4-position of XXI or 5-position of XXII should give the same product having structure of XXIV. The subsequent dehydrogenation at the 4-position of XXIV should give a hydroxy imidazolidine-dione (XXV). This intermediate must be degradated in two directions, the one via XXVII gave N-benzoyl asparagine (IVa) and the other via XXVI N-benzoyl aspartyl urea (IIa). The benzoyl aspartic acid (Ia), isolated by this photooxidation should be produced from IIa by liberation of urea.

The products XIIIa, XIVa and XVa, which were also isolated as crystalline compounds, revealed each one basic dissosiation having pKa 5.6, 5.6, and 5.1, respectively. The titrimetrical estimation indicated that these three compounds have M.W. ca. 550 and must be dimeric products of the photooxidation. On alkali hydrolysis all of these three compounds liberated each one mole equivalent of N-benzoyl histidine to leave unstable compounds. The structures of these dimeric products are now under investigation.

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