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Triazines and Related Products. Part 23.[1] New Photo-products from 5-Diazoimidazole-4-carboxamide (Diazo-IC)

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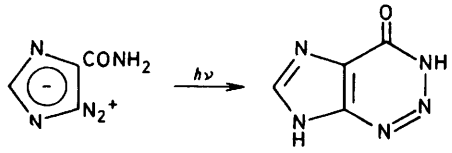
Photolysis of Diazo-IC in dilute aqueous solution affords products which differ according to the pH of the medium. At pH 1, or pH 7.4—12 the product is 2-azahypoxanthine (2): in the intervening pH range the product is 4-carbamoylimidazolium-5-olate (3). In the dark only 2-azahypoxanthine is formed. Photolysis of Diazo-IC in concentrated solution containing citric acid (1 mol equiv.) at pH 2.5 affords a maroon imidazolylazoimidazolium olate (8) and 2-azahypoxanthine.

5-DIAZOIMIDAZOLE-4-CARBOXAMIDE (Diazo-IC) (1) is an exceedingly reactive and toxic compound which covalently labels reactive moieties at the active sites of a range of enzymes.[2] The compound is photosensitive and previous investigators[3,4] have shown that it readily cyclises to imidazo[4,5-*d*][1,2,3]triazin-7(6*H*)-one hydrate, usually known as 2-azahypoxanthine (2). However, contrary to former interpretations we have found that photo-transformations of Diazo-IC are markedly influenced by pH. When photolyses are conducted under weakly acidic conditions a hitherto unreported photo-product is formed.

Decomposition of Diazo-IC in diffuse laboratory light was examined in a series of buffers (conc: 1 mg/100 ml) giving a pH range of 1—12. Changes were monitored by u.v.–visible spectrophotometry. At pH 1 and at pH



7.4 and above the spectral changes were indicative of the formation of 2-azahypoxanthine (2) as originally deduced.[3,4] [Note: 2-azahypoxanthine and other photo-products and reference compounds are amphoteric and the positions of their absorption bands varies according to pH (Table 1). Photolysates were compared with reference spectra recorded at the appropriate pH.] In the intervening pH range in diffuse light (see Figure 1 for the decomposition at pH 2.5) the spectral changes were inconsistent with the formation of 2-azahypoxanthine and a new stable photo-product was formed with $\lambda_{max.}$ 236 and 276 nm quite different from the spectrum of 2-azahypoxanthine at the same pH. Exposure of solutions to direct sunlight did not qualitatively effect the photolysis, but merely accelerated the rate of degradation.

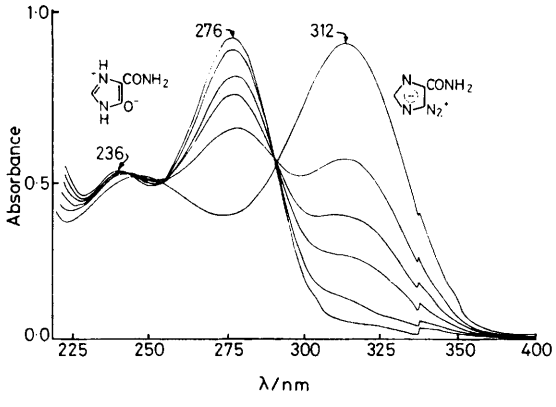


FIGURE 1 Decomposition of 5-diazoimidazole-4-carboxamide at pH 2.5 in diffuse light recorded at 15 min intervals.

Diazo-IC also cyclised in the dark (see Figure 2 for the decomposition at pH 2.5) and the product was exclusively 2-azahypoxanthine in the entire pH range 1—12. The rate of cyclisation accelerated as the pH increased; at pH 12 cyclisation was nearly instantaneous.

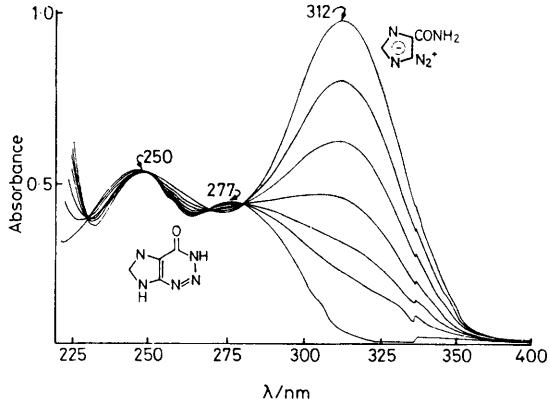


FIGURE 2 Decomposition of 5-diazoimidazole-4-carboxamide at pH 2.5 in the dark recorded at 30 min intervals

The new photo-product ($\lambda_{max.}$ 236 and 276 nm) formed from Diazo-IC at weakly acid pH was spectroscopically identical to an authentic sample of 4-carbamoylimidazolium-5-olate (3).[5-7] This imidazole is the aglycone of bredinin (4) an antibiotic isolated from *Eupenicillium brefeldianum M*-2166.[8-10]

Our proposed mechanistic interpretation of these observations which accounts for the pH-dependence of the products is presented in the Scheme. The u.v. spectrum of Diazo-IC exhibits a substantial hypsochromic shift at pH 1 compared to pH 2.5 (Table 1). Evidently

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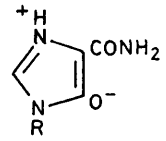
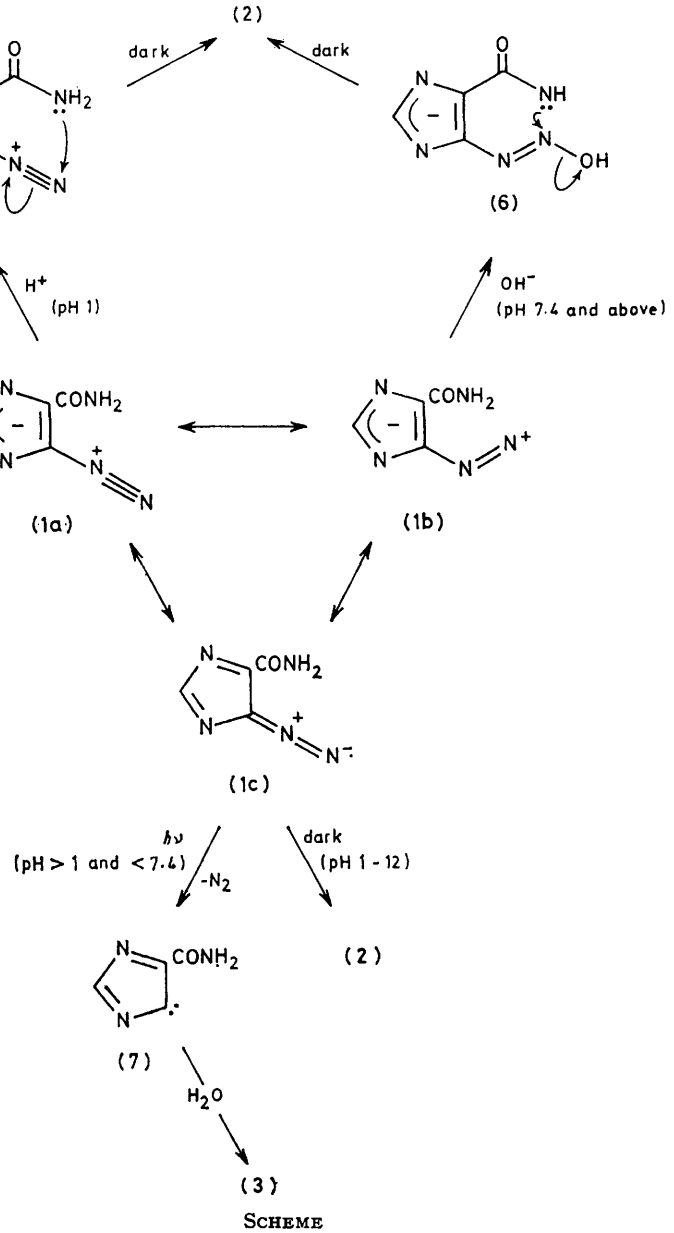
at pH 1 Diazo-IC exists as the protonated imidazole diazonium ion (5) which is trapped in an intramolecular cyclisation by the carboxamide group with the formation



(3) R = H
(4) R = β-D-ribofuranosyl

of 2-azahypoxanthine (2). At pH values 7.4 and above where 2-azahypoxanthine is also formed, the reactive species is probably the imidazole diazohydroxide (6). Analogous cyclisations of diazotised anthranilamides to 1,2,3-benzotriazin-4(3*H*)-ones are known to be promoted by alkali.[11] Both sequences leading to 2-azahypoxanthine are ' dark ' reactions, albeit accelerated by light.

However, in the pH range between 1 and 7.4 Diazo-IC exists as an internally-compensated zwitterion for which diazonium [*e.g.* (1a) and (1b)] and diazo [*e.g.* (1c)] contributions have been invoked.[12] In the dark this neutral species cyclises to 2-azahypoxanthine in agreement with published reports.[3,12] Because the formation of 4-carbamoylimidazolium-5-olate (3) in the same pH range is an *exclusively photochemical process* and cannot be mimicked by treating Diazo-IC in acid media with copper catalysts known to promote conversion of arenediazonium salts to phenols we propose that the photoreaction at



SCHEME

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TABLE 1

Effect of pH on the electronic absorption spectra ($\lambda_{max.}$ in nm) of imidazole derivatives

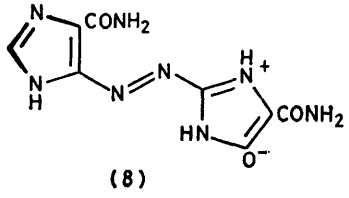
Compound	pH 1 [a]	pH 2.5	pH 7.4	pH 10.15
5-Diazoimidazole-4-carboxamide (1) [b]	237, 293	246, 312	245,* 310	*c*
2-Azahypoxanthine (2) [d,e]	250, 277	250, 277	250, 288	254, 294
4-Carbamoylimidazolium-5-olate (3) [b,f]	236, 275	236, 276	236, 276	236, 276
5-Aminoimidazole-4-carboxamide (9) [b,g]	239, 264	241, 263	230,* 265	230,* 265

[a] For details of buffers see Table 2. [b] Sample dissolved directly in buffer. [c] Compound immediately cyclises to 2-azahypoxanthine (see Table 2). [d] Sample dissolved in a minimum of DMSO and diluted with buffer. [e] Hydrate (ref. 12). [f] Ref. 7. [g] Hydrochloride salt obtained from Aldrich Chemical Company, Inc.

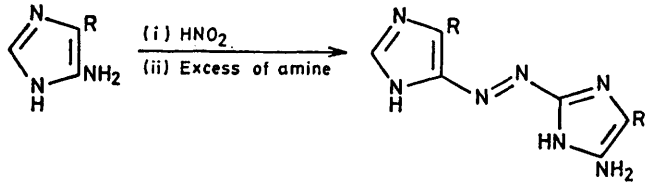
* Inflexion.

pH 1—7.4 involves a reactive carbene intermediate (7) generated from the diazo-mesomer (1c). This carbene is quenched by water to give the observed product (3). Heterolytic displacement of the diazonium group by water in an S_N1 or S_N2 reaction, or a homolytic process, are plausible but less likely alternatives.

A concentrated aqueous solution of Diazo-IC (0.1 g in 100 ml) containing citric acid (1 mol equiv.) with a pH of *ca.* 2.5, rapidly developed a red colour when exposed to sunlight. The colour development was accompanied by effervescence. After 2 h, a deep maroon product started to deposit from the solution reaching a maximum yield of 25% after 10 h. The maroon photoproduct was identified as the imidazolylazoimidazolium olate (8) since it was identical with the compound independently prepared by coupling Diazo-IC with authentic 4-carbamoylimidazolium-5-olate in neutral aqueous medium.



(8)



(9) R = $CONH_2$
(10) R = CN

(11) R = $CONH_2$
(12) R = CN

Other activated imidazoles readily undergo coupling at C-2 with diazonium compounds. When 5-aminoimidazole-4-carboxamide (9) or 5-aminoimidazole-4-cyanoimidazole (10) are diazotised in the presence of an excess of the amines autocoupling takes place with the formation of quantitative yields of the imidazolylazoimidazoles (11) and (12) respectively. These azo-dyes all showed an intense absorption for the azo-chromophore in their visible spectra (490—550 nm). However, those substrates where the imidazole ring is deactivated by a diazo-group as in Diazo-IC (1), or the masked diazo-group in 2-azahypoxanthine (2), do not couple at a range of pH values with either Diazo-IC or arenediazonium salts.

One further facet of the preparative-scale photolysis of Diazo-IC remained to be clarified. Following removal of the coloured photoproduct (8) the straw-coloured filtrate proved to contain only 2-azahypoxanthine (2) and *not* the imidazolium-olate (3) which was anticipated bearing in mind the pH (2.5) at the start of the photolysis. Initially it was considered possible that during the photolysis the pH had increased into the range (>7) known to favour 2-azahypoxanthine formation but this was not the case: the pH of the solution remained approximately constant. An explanation for this anomaly probably lies in the light-filtering effect of the intensely coloured solution. As the Diazo-IC is photohydrolysed the imidazolium-olate (3) thus formed immediately couples with unphotolysed Diazo-IC affording the azo-dye (8) which slowly precipitates from the mixture. However, traces of dissolved dye impart a deep colour to the supernatant solution and subsequent changes take place in a 'dark' environment. As had been previously noted in spectroscopic-scale photolyses Diazo-IC cyclises exclusively to 2-azahypoxanthine in the dark.

EXPERIMENTAL

Spectroscopic-scale Photolyses.—These photolyses were conducted in 1 cm quartz cuvettes. Concentrations of substrates were approximately 1 mg/100 ml in the appropriate buffer and spectral changes were monitored on a Unicam SP 8000 spectrometer with a Unicam SP 8005 programme controller operating in the repeat scan mode. Results are presented in Table 2.

Preparative-scale Photolysis of Diazo-IC.—5-Diazoimidazole-4-carboxamide (0.1 g) [12] was dissolved in dimethyl sulphoxide (2 ml), and the mixture was diluted with water (98 ml) containing citric acid (1 mol equiv.). The solution was illuminated by direct sunlight and developed a pink colouration after 5 min and a dark maroon solid started to precipitate after 2 h. After 10 h, the solid (0.025 g) was collected and shown to be identical (i.r.) to a specimen of 4-carbamoyl-2-(4-carbamoylimidazol-5-ylazo)imidazolium-5-olate (8) prepared below. The amber filtrate had $\lambda_{max.}$ 250 and 277 nm identical to that of a sample of 2-azahypoxanthine at pH 2.5.

The same two photo-products were produced when 5-diazoimidazole-4-carboxamide (0.1 g) was exposed to sunlight in buffer at pH 3.

4-Carbamoyl-2-(4-carbamoylimidazol-5-ylazo)imidazolium-5-olate (8).—To a solution of 4-carbamoylimidazolium-5-

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TABLE 2

Products [a] formed from the photodecomposition of 5-diazoimidazole-4-carboxamide (conc: 1 mg/100 ml) [b] at different pH values

Light conditions	pH 1	pH 2	pH 3	pH 5.2	pH 7.4	pH 8.5	pH 10.15	pH 12
A	(2)	(3)	(3)	(3)	(2)	(2)	(2)	(2)
B	(2)	*c*	(3)	(3)	*c*	(2)	(2)	*c*
C	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)

Light conditions: A, natural light with no direct sunlight; B, direct sunlight; C, in the dark.
Buffers: pH 1 and 2, Clark and Lub's potassium chloride–hydrochloric acid; pH 3, Sorensen's glycine I; pH 5.2 and 7.4, Sorensen's phosphate; pH 8.5, 10.15, and 12, Sorensen's glycine II.

[a] Products were identified by comparison of the final spectrum of the photolysate with those of reference samples at the same pH (Table 1). [b] Sample dissolved directly in buffer prior to photolysis. [c] Photolysis not conducted at this pH.

olate (0.127 g) [7] in water (15 ml) at 0—5 °C was added 5-diazoimidazole-4-carboxamide (0.137 g) in portions during 5 min. The red solution rapidly deposited a maroon precipitate of the *imidazolylazoimidazolium-olate* which was collected (0.18 g) after 2 h. The crude product crystallised from aqueous dimethylformamide as black microprisms, m.p. >350 °C (decomp.) (Found: C, 36.8; H, 2.7; N, 42.1. $C_8H_8N_8O_3$ requires C, 36.4; H, 3.0; N, 42.4%); $\lambda_{max.}$ (dimethylformamide) 550 nm; $\nu_{max.}$ (KBr) 3 375br (bonded NH, OH) and 1 640 cm^{-1} (C=O).

5-Amino-2-(4-carbamoylimidazol-5-ylazo)imidazole-4-carboxamide (11).—5-Aminoimidazole-4-carboxamide hydrochloride (0.02 mol) was dissolved in 1N-hydrochloric acid (60 ml) cooled to 0—5 °C and treated dropwise (during 15 min) with a solution of sodium nitrite (0.69 g, 0.01 mol) in water (5 ml). A deep red-violet colour rapidly formed followed by the deposition of a violet precipitate. The mixture was kept at 4 °C overnight and the precipitated *imidazolylazoimidazole* (1.9 g) collected. The product formed maroon crystals, m.p. >350 °C (decomp.) from dimethylformamide (Found: C, 36.1; H, 3.2; N, 37.9. $C_8H_9N_9O_2$ requires C, 36.5; H, 3.4; N, 38.1%) with $\lambda_{max.}$ (dimethylformamide) 490 nm; $\nu_{max.}$ (KBr) 3 400br (bonded NH) and 1 645 cm^{-1} (C=O).

The same imidazolylazoimidazole (85%) can be prepared by coupling 5-diazoimidazole-4-carboxamide with 5-aminoimidazole-4-carboxamide in sodium acetate buffered solution at 0—5 °C.

5-Amino-4-cyano-2-(4-cyanoimidazol-5-ylazo)imidazole (12) (*with C. P. Turnbull*).—A solution of 5-amino-4-cyanoimidazole (0.4 g) [13] in 2N-hydrochloric acid was added dropwise to a solution of sodium nitrite (0.26 g) in water (4 ml) at 0 °C during 30 min. Excess of nitrous acid was decomposed with urea and the resulting suspension of 4-cyano-5-diazoimidazole was added in portions to a solution of 5-amino-4-cyanoimidazole (0.4 g) in water (5 ml) containing sodium acetate trihydrate (3.0 g). The *azoimidazole* (70%) crystallized from aqueous dimethylformamide with m.p. >300 °C (decomp.) (Found: C, 40.3; H, 2.3; N, 57.0. $C_8H_5N_9$ requires C, 40.7; H, 2.1; N, 57.2%); $\lambda_{max.}$ (EtOH) 512 nm; $\nu_{max.}$ (KBr) 3 320 and 3 180 (NH) and 2 210 cm^{-1} (C≡N).

Attempted Preparation of 4-Carbamoylimidazolium-5-olate (3).—(i) 5-Diazoimidazole-4-carboxamide (0.7 g) was boiled in 1N-sulphuric acid (20 ml) for 10 min. The solution changed colour from red to yellow and a moderate effervescence was observed. After several weeks 2-azahypoxanthine hydrate (0.1 g) crystallised from the mixture. The mother liquor had $\lambda_{max.}$ 250 and 277 nm identical with the spectrum of an authentic sample of 2-azahypoxanthine hydrate in 1N-sulphuric acid.

(ii) A vigorous effervescence was observed when 5-diazoimidazole-4-carboxamide (0.5 g) in 30% sulphuric acid (10 ml) at 95 °C was treated with copper bronze (0.2 g). The pH of the solution was adjusted to 3.5 but spectroscopic examination of the filtrate did not reveal the presence of the imidazolium olate.

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