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A new light on the photo-decomposition of the antitumour drug DTIC

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The antitumour drug 5-(3,3-dimethyltriazen-1-yl)-imidazole-4-carboxamide, DTIC: NSC-45388 (I)† has found a limited rôle in the treatment of malignant melanoma (Carter & Friedman 1972; Comis 1976) and is formulated as a freeze-dried citrate salt containing mannitol. DTIC may be administered by i.v. injection or infusion, and it has been claimed (Baird & Willoughby 1978) that pain at the injection site is abolished if the drug is scrupulously shielded from light at all times. Aqueous solutions of the drug turn pink when exposed to light and in an effort to identify the products responsible for this discolouration the photo-reactions of DTIC have been re-examined.

Previous studies by Shealy et al (1962, 1968) showed that the drug rapidly decomposed in sunlight to produce dimethylamine and 5-diazoimidazole-4-carboxamide (Diazo-IC) (II) which subsequently cyclized to 2-azahypoxanthine (III). We now expand on our preliminary report of a reinvestigation of this degradation (Stevens & Peatey 1978) and show that the outcome is crucially influenced by pH.

Materials and methods

Compounds. DTIC-Dome was obtained from Dr J. G. Goodall (Miles Laboratories Ltd); 5-(3,3-dimethyltriazen-1-yl)imidazole-4-carboxamide was a gift from Dr Harry B. Wood, National Cancer Institute, Bethesda, Maryland, U.S.A.; and a sample of 4-carbamoylimidazolium-5-olate was provided by Dr Kimio Mizuno of Toyo Jozo Co. Ltd, Tokyo. 5-Aminoimidazole-4-carboxamide hydrochloride was purchased from Aldrich Chemical Company, Inc. Diazo-IC and 2-azahypoxanthine hydrate were prepared by the method of Shealy et al (1961); 4-carbamoyl-2-(4-carbamoylimidazol-5-ylazo) imidazolium-5-olate and 5-amino-2-(4-carbamoylimidazol-5-ylazo) imidazole-4-carboxamide were prepared by the method of Horton & Stevens (1981).

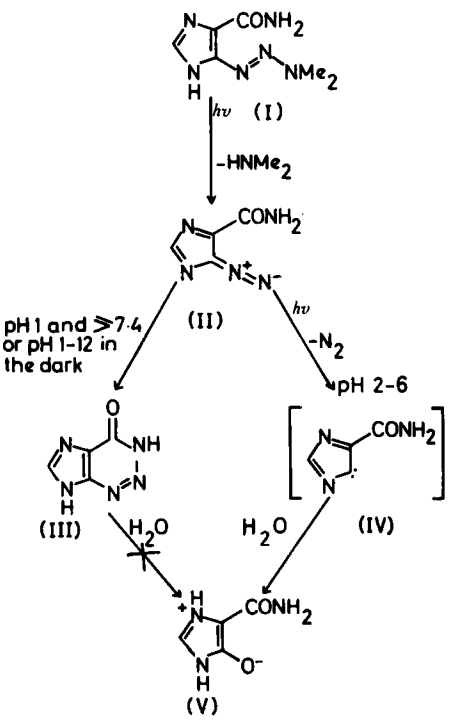
Buffers. pH 1 and 2: Clark and Lub's potassium chloride-hydrochloric acid. pH 3: Sørensen's glycine I. pH 5·2 and 7·4: Sørensen's phosphate. pH 8·5, 10·15 and 12: Sørensen's glycine II.

Photolysis experiments. For spectroscopic-scale photolyses, samples (concn: ca 0·01 mg ml^{-1}) were dissolved in buffers, transferred to 1 cm quartz cuvettes, sealed, and exposed to natural light with no direct sunlight (diffuse light) or to direct sunlight as appropriate. U.v.-visible spectra were recorded. When stability studies were conducted in the dark, the cuvette contents were protected by aluminium foil wrappings: alternatively, cuvettes remained within the sample compartment of the spectrometer and spectra were recorded at timed intervals using a program controller operating in the repeat scan mode.

Preparative-scale photolyses were conducted with samples (concn: 1 or 10 mg ml^{-1}) dissolved in water in 25 ml or 100 ml Pyrex graduated flasks exposed to sunlight passing through a south-facing window.

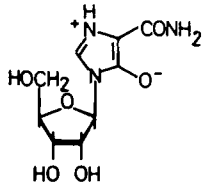
Results and discussion

Decompositions of DTIC-Dome (concn: ca 0·01 mg ml^{-1}) were examined in a series of buffers in the pH range 1–12; changes were monitored by u.v. spectroscopy. In confirmation of earlier reports the drug proved to be stable when stored in the dark, but in diffuse light at pH 1 and pH 7·4 and above the spectral changes indicated that 2-azahypoxanthine (III) was the final photo-product. However, when DTIC-Dome was dissolved in water at a similar spectroscopic concentration and exposed to diffuse light (Fig. 1) the spectral changes were *not* consistent with the formation of 2-azahypoxanthine since a new stable



* Correspondence.

† DTIC is also known as DTIC-Dome or Dacarbazine.



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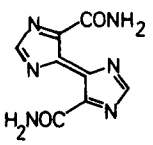

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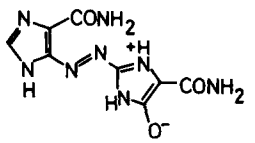

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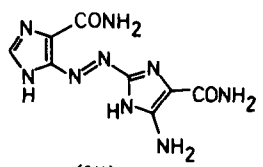

(IX)

photo-product was formed with λ_{max} 236 and 275 nm, quite distinct from the spectrum of 2-azahypoxanthine in water (Shealy et al 1961). The same product was formed from DTIC-Dome upon exposure to diffuse light in the pH range 2–6 and control experiments established that 2-azahypoxanthine was not the precursor of the new species. When a sample of pure 5-(3,3-dimethyltriazen-1-yl)-imidazole-4-carboxamide was subjected to a parallel series of degradations, similar results were obtained—that is, in diffuse light only 2-azahypoxanthine was formed at pH 1 and pH > 7·4, whereas in the intervening range (pH 2–6) the new photoproduct (λ_{max} 236 and 275 nm) emerged. Evidently the citric acid and mannitol in the formulated drug do not influence this decomposition (Table 1). Exposure of the aforementioned solutions to direct sunlight did not qualitatively affect the outcome, but merely accelerated the rate of degradation.

The new photo-product was spectroscopically identical (u.v. and i.r.) to an authentic sample of 4-carbamoylimidazolium-5-olate (V) (Schipper & Day 1952; Hayashi et al 1975). This betaine is the aglycone of the antibiotic bredinin (VI) which has been isolated from *Eupenicillium brefeldianum* M 2166 (Mizuno et al 1974). Bredinin inhibits L 5178Y mouse leukaemia cells in culture (Sakaguchi et al 1975a, 1976), other mammalian cell lines (Sakaguchi et al 1975b) and has immunosuppressant activity (Uchida et al 1979). Recent work on the chemistry of Diazo-IC (II) (Horton & Stevens 1981) implicates that compound as being the crucial intermediate on the reaction pathway from DTIC to the imidazolium olate (V). Since the conversion (II) → (V) is a purely photochemical transformation it has been tentatively suggested that the reactive carbene species (IV) may be generated by photodegradation of (II); this carbene is then quenched by water to afford the imidazolium olate (V).

The imidazolium olate (V) (λ_{max} 236 and 275 nm) is a colourless compound and clearly not responsible for the pink colouration of photo-decomposed solutions of DTIC in concentrated solutions. Discolouration was only observed when clinically realistic concentrations of DTIC-Dome (1 mg ml^{-1}) were exposed to light. Thus, in diffuse light, or more dramatically in direct sunlight, aqueous solutions of the drug were observed to effervesce and eventually deposit a maroon precipitate. Comparable concentrations of Diazo-IC containing citric acid (1 mol. equiv.) also developed an intense colour in sunlight whereas similar concentrations of Diazo-IC in distilled water, or water containing mannitol (1 mol equiv.) did not discolour. In both the former cases the yield of maroon photo-product reached a maximum (20–25%) after 10 h exposure to light.

The maroon product was not the anticipated bis-imidazole (VII), the dimer of carbene (IV), but was characterized as 4-carbamoyl-2-(4-carbamoylimidazol-5-ylazo)imidazolium-5-olate (VIII), since it was identical to a specimen of (VIII) prepared unequivocally by coupling Diazo-IC (II) with an authentic sample of the imidazolium-olate (V) (Horton & Stevens 1981). Therefore, this highly coloured dye (λ_{max} 550 nm in dimethylformamide), is the cause of the colour developed in light-exposed solutions of DTIC-Dome, but not apparently that producing discolouration in solutions prepared from drug which has been stored at elevated temperatures in the dark. This latter coloured species (λ_{max} 490 nm) is probably the related aminoimidazoazoimidazole (IX) which could be formed from Diazo-IC thermally-generated during storage by its coupling with traces of 5-aminoimidazole-4-carboxamide contaminating the sample.

Solutions of DTIC-Dome made up for injection (10 mg ml^{-1}) have pH 3–4 (information from DTIC-Dome Data sheet) in the range likely, on the basis of the foregoing work, to lead to the generation of 4-carbamoylimidazolium-5-olate (V) if they are carelessly exposed to light. Since there is a salvage pathway in mammalian cells whereby bredinin (VI) can be enzymically synthesized from the aglycone precursor (Mizuno et al 1975) it is an intriguing possibility that cytotoxic bredinin might be formed in vivo and add yet a further dimension to the complications bedevilling attempts to interpret the molecular basis of the mode of action of DTIC (Vaughan & Stevens 1978). That this possibility is, fortunately, remote can be inferred from a close examination of concentrated solutions of DTIC-Dome (10 mg ml^{-1}) which have been photo-decomposed in strong sunlight. Following removal of the maroon photo-product (VIII) spectral examination of the straw-coloured filtrate confirmed that it contained 2-azahypoxanthine (III) and *not* the imidazolium olate (V) which might have been anticipated considering the pH at the start of the photolysis. [The dimethylamine liberated during the transformation (I) → (II) does not increase the pH into a range >7·4 known to favour 2-azahypoxanthine

Table 1. Stable products* formed from the photo-decomposition of DTIC-Dome and pure 5-(3,3-dimethyltriazen-1-yl)-imidazole-4-carboxamide (pure DTIC) at different pH values.

Substrate	Light conditions	pH 1	pH 2	pH 3	pH 5·2	pH 7·4	pH 8·5	pH 10·15	pH 12
DTIC-Dome†	A	III	V	V	V	III	III	III	III
DTIC-Dome	B	III	V	V	V	III	III	III	III
DTIC-Dome	C	I	I	I	I	I	I	I	I
Pure DTIC‡	A	III	V	V	V	III	III	III	III
Pure DTIC	B	III	V	V	V	III	III	III	III
Pure DTIC	C	I	I	I	I	I	I	I	I

Light conditions. A: Natural light with no direct sunlight; B: Direct sunlight; C: In the dark.
* Products identified by comparison of the final u.v. spectrum of the photolysate with those of reference samples at the same pH (Horton & Stevens 1981).
† Sample dissolved directly in buffer before photolysis.
‡ Sample dissolved in DMSO and diluted with buffer prior to photolysis.

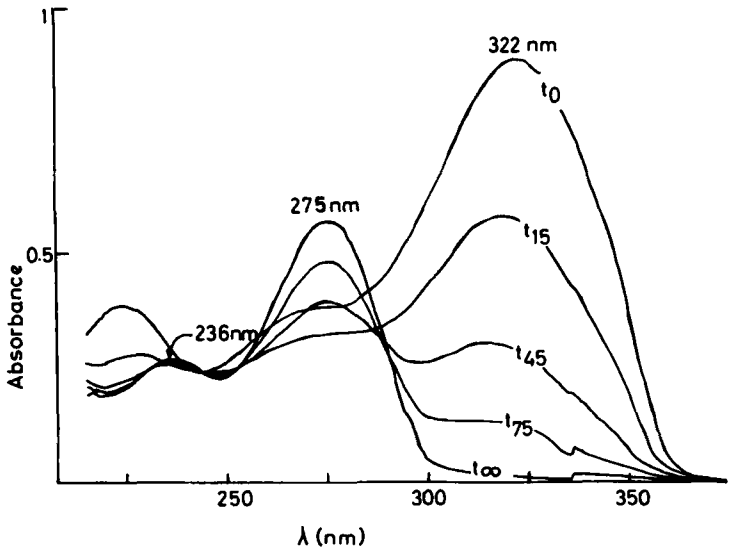

FIG. 1. Decomposition of DTIC-Dome in water (concn: 0·01 mg ml^{-1}) in diffuse light at 20 °C. Spectra were recorded at the start of the experiment (t_0) and after 15 min (t_{15}), 45 min (t_{45}), 75 min (t_{75}) and after prolonged exposure (t_∞).

formation. The pH of the photolysing mixture remains within the range 3–4 throughout.]

A simple explanation for these (apparently) contradictory results is as follows: as the DTIC photolyses and Diazo-IC concentration rises, some of the latter is photohydrolysed to the imidazolium-olate (V). The activated π-excessive imidazole ring of (V) is susceptible to electrophilic substitution and couples with unphotolysed Diazo-IC to afford the azo dye (VIII) which then slowly precipitates from solution. However, traces of dissolved dye impart a deep red colouration to the solution which acts as an effective filter inhibiting further photo-decomposition of residual Diazo-IC. Thus subsequent transformations of Diazo-IC take place essentially in a 'dark' environment. As has been observed previously in spectroscopic-scale studies Diazo-IC cyclises exclusively to 2-azahypoxanthine at pH 3–4 in the dark (Horton & Stevens 1981).

The toxicological implications for patients, who might receive photo-degraded DTIC containing the decomposition products reported in this paper, are unknown. Provided the drug is suitably protected from light during preparation *and* administration, and that these activities are supervised by someone cognisant with the extreme photosensitivity of the drug, no problems should arise. A new device has been described by Shükla (1980) which can be employed for the safe adminsitration of DTIC and other light-sensitive drugs.

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An examination of the 'wet dog' shake behaviour in rats produced by acute administration of sodium n-dipropylacetate

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Investigations into the mode of action of the anticonvulsant drug sodium n-dipropylacetate (DPA) have concentrated on its interactions with brain γ-aminobutyric acid (GABA) metabolism (for review see Kupferberg 1980). However, acute and chronic administration of DPA to rodents has been shown to elevate brain concentrations of 5-hydroxyindoleacetic acid (5-HIAA), possibly by stimulating 5-hydroxytryptamine (5-HT) turnover (Hwang & Van Woert 1979). Also, acute administration of DPA in rats causes 'wet dog' shakes (WDS) behaviour (de Boer et al 1977), a behavioural syndrome thought to be an expression of central 5-HT receptor activation (Bedard & Pycock 1977). These observations prompted us to examine the WDS behaviour produced by DPA in more detail, with particular regard to the possible involvement of GABAergic and 5-hydroxytryptaminergic systems.

Male Wistar rats, 150–250 g, were used. For behavioural observations animals were placed individually in an open-topped cage (50 cm × 30 cm × 25 cm) and left undisturbed for 5 min. DPA was then administered and the animal's behaviour observed for the following 30 min. WDS was scored as the number of whole-body shakes occurring during this period. The effects of various drug pretreatments on this behaviour were examined in separate groups of rats. A group of vehicle-pretreated controls was scored with each drug-pretreated group such that, during each experiment, drug-pretreated and control rats were observed alternately. One worker administered drugs and behaviour was scored by a second observer who was unaware of the pre-treatment status of individual rats. All drugs were administered by the intraperitoneal route, dissolved in 0·9% NaCl saline, at a volume of 5 ml kg^{-1}.

Whole-brain GABA was assayed by microdansylation (Briel & Neuhoff 1972) using [^{14}C]GABA as internal standard (Snodgrass & Iversen 1973). 5-HT and 5-HIAA were measured by the method of Curzon & Green (1970). All statistical analysis was by Student's *t*-test.

Drugs: L-Tryptophan, *p*-chlorophenylalanine methyl ester and picrotoxin were obtained from Sigma, sodium n-dipropylacetate from Reckitt & Coleman, morphine sulphate from Macfarlane Smith and chlordiazepoxide from Roche.

Within 3 min of DPA administration (400 mg kg^{-1}) the general activity of the animals increased abruptly. Although some ataxia was apparent at this dose locomotor activity was markedly increased. Additional behavioural changes included episodes of vigorous grooming and the occurrence of whole-body shakes (WDS) which were occasionally of sufficient violence to throw the animal off balance. After 15–20 min, general activity began to subside and at 30 min the animals were sedated, exhibiting ptosis, piloerection and a hunched-back posture. The number of WDS were counted over the 30 min period and the effect of varying the dose of DPA is shown in Fig. 1. The effects of various drug pretreatments on the WDS behaviour elicited by DPA (400 mg kg^{-1}) are shown in Table 1. A lower dose of DPA (300 mg kg^{-1}) was used in the L-tryptophan

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