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Anthralin and its acetyl esters: spectroscopic properties and enzyme inhibitory activity

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Raab & Gmeiner (1975) have published experiments concerning a 'biochemically highly active compound' obtained from anthralin by irradiation. Raab's u.v.-spectra in dimethylformamide (DMF) are reproducible and indicate an anthracene species, whilst in methanol the spectrum is of the dihydroxyanthrone (benzophenone) type. The conversion of these two species is reversible and is brought about by various other aprotic dipolar solvents: mixtures of dimethylformamide/methanol of different percentages equimolar in anthralin show an isosbestic point at 367 nm. Infrared-spectra in dimethylsulphoxide show the carbonyl band at 1652 cm^{-1}, whilst in chloroform or in the solid state it is about 1630 cm^{-1}, indicating weakening of the intramolecular H-bridge. Anthralin mono- and diacetyl esters show similar u.v.-effects; C-10-dialkylated (Faro, Retzow & Wiegrebe, 1980) derivatives do not, indicating that the CH_2-group is involved. NMR-experiments, however, do not suggest a stable 1,8,9-trihydroxyanthracene: there is no aromatic proton seen at C-10, and the other aromatic protons resonate in the benzophenone position. Deuterium exchange experiments, however, indicate an anthrone-anthracene equilibrium, strongly shifted towards the anthrone side. This equilibrium is proved by a Diels-Alder-reaction with acetylene-dicarboxylic acid dimethylester: the product (Schultz & Frey, 1977) is formed in the absence of light, as well as in the presence of a triplet quencher, and is not prevented by traces of sulphuric acid (see below). A triplet would not be consistent with the stability of Raab's species, enabling it to operate as an enzyme inhibitor (Raab & Gmeiner, 1975). Moreover, a triplet is excluded by ESR-measurements. At the moment, our results are best interpreted by assuming photoionization (Kaupp, 1980) (Fig. 1), which results in the trapping of the anthracenetriol-tautomer out of the ground state equilibrium, leading to Raab's species which we consider to be the C-9-anion of 1,8,9-anthracenetriol (anthralin-1,8-diacetate shows similar effects). This assumption is supported by fluorescence spectra which have identical maxima at 465 nm in DMF and methanol (but of very low intensity in methanol). The dependence of light is underlined by taking the u.v.-spectra of anthralin solution in DMF/methanol, prepared in the dark: the first scan still shows a mixed spectrum of anthrone/anthracenetriol; further scans indicate the pure anthracene chromophore. This chromophore can also be detected in methanol, using NaOH as a (strong) base: the immediate u.v.-spectrum shows the anthracene species, but after a very short time the anthralin is degraded (spectrum 'anthralin red'). Adding traces of H_2SO_4 to a (fluorescent) solution of anthralin in DMF/methanol converts the spectrum to the anthrone type; this is understood as reprotonation followed by tautomerization to the more stable anthrone. Our working hypothesis is in accordance with findings for other phenols (Kaupp, 1980).

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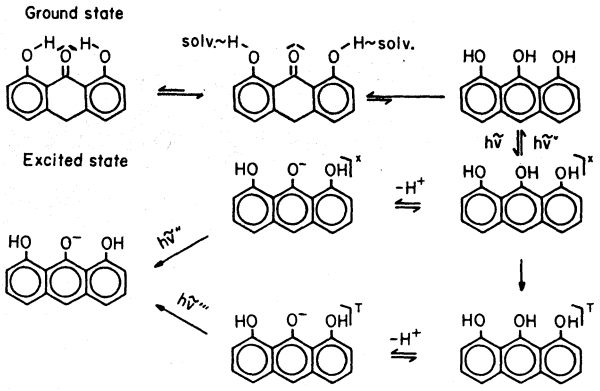

Compare β-naphthol: G. Kaupp, *Angew. Chem.* **92**, 252 (1980)

FIGURE 1. Photoionization of anthralin to its anion after ground state tautomerization.

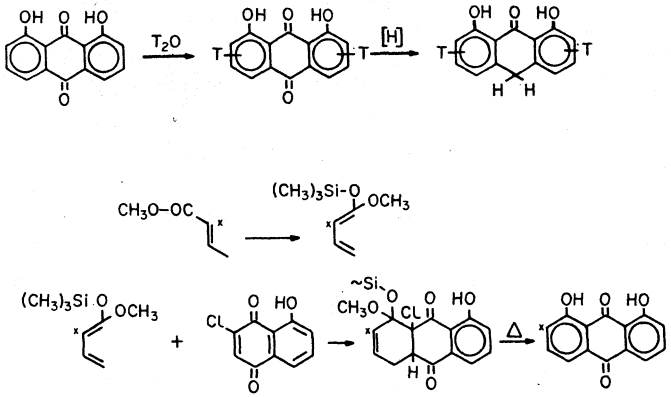

FIGURE 2. Syntheses of tritiated and ^{14}C-anthralin.

We have shown that an anthracene spectrum develops during daylight incubation of anthralin in buffered bovine serum albumin solution (pH 6·8), whilst in the dark, the molecule remains in the benzophenone state. This experiment points to the fact that the enzyme inhibiting species (Raab & Gmeiner, 1975) is the anthracenetriol anion already mentioned. We elaborated a technique for anthralin inhibition experiments with glucose-6-phosphate-dehydrogenase to avoid degradation of anthralin and diminish auto-degradation of the enzyme. This enabled us to demonstrate that quotations in the literature concerning unspecific or nonspecific inhibition by anthralin are incorrect, since they are based on the pre-supposition of a reversible process; plotting the velocity of the enzymatic process against the total amount of enzyme (Segel, 1975) in the presence of a constant quantity of anthralin, its di- or triacetate, indicate an irreversible inhibition. In this context, the partition coefficients of anthralin, 1,8-diacetoxy-9-anthrone and 1,8,9-triacetoxyanthracene are determined; they are about 1000, 196 and 72, respectively, calculated according to $k = C_A/C_B$ (A = n-octylalcohol; B = water).

In order to help biochemists to discover how anthralin binds to glucose-6-phosphate-dehydrogenase, we have synthesized tritium-labelled anthralin and tritium-^{14}C-double labelled anthralin triacetate

(Faro, Retzow & Wiegrebe, 1980). ^{14}C-Anthralin was synthesized by a Diels-Alder-reaction (Fig. 2) of chloro-juglone with silylated ^{14}C-crotonic acid methylester.

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