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THE PHOTO-OXIDATIVE HYDROXYLATION OF PHENAZINE-1-CARBOXYLIC ACID AND ITS QUATERNARY SALT

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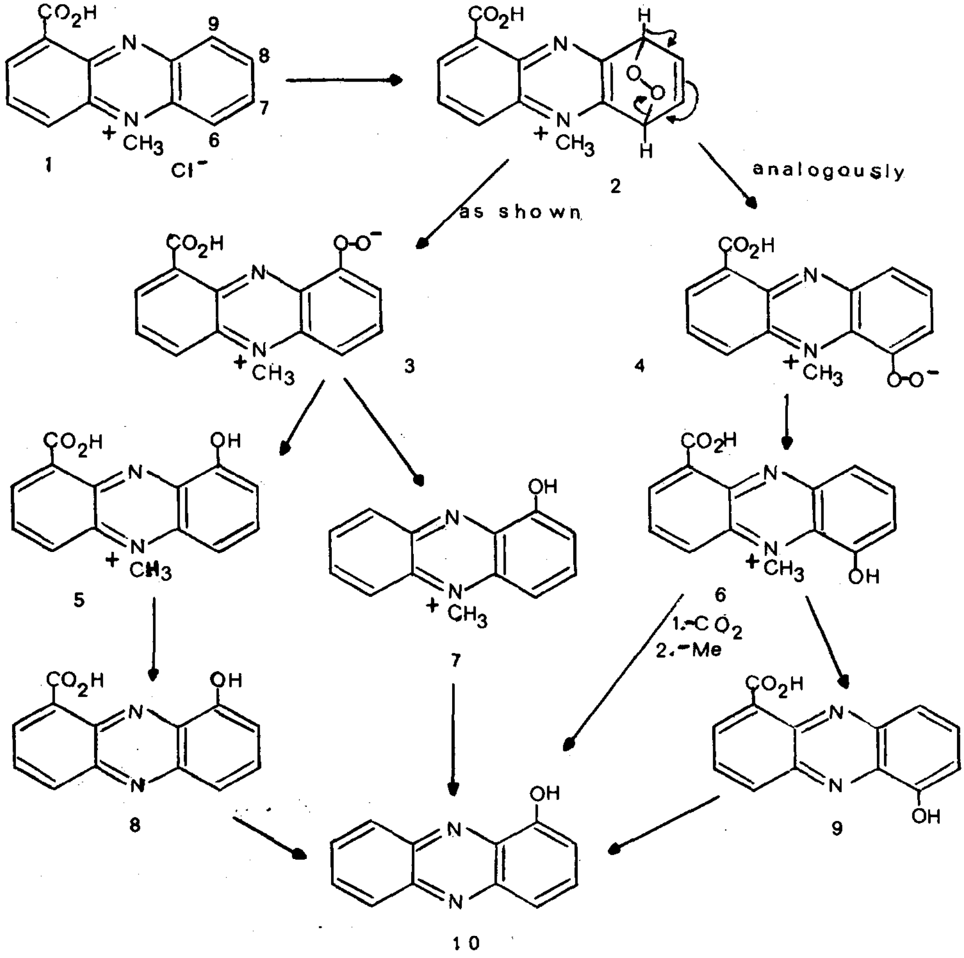
The recent report[1] that irradiation of phenazine in dilute phosphoric acid solution gives 1-hydroxyphenazine (10) prompts us to record our own observations on the photo-oxidation of phenazine and its derivatives by irradiation (Hanovia UVS 500A lamp) in the presence of air. We confirm the conversion of phenazine[1] to 1-hydroxyphenazine (10) and also that of the 5-methylphenazinium ion to pyocyanin (7),[2] in sufficiently high yield to make it the most useful method of preparation.[3]

More particularly, our experiments have been concerned with phenazine-1-carboxylic acid and its 5-methyl quaternary chloride (1), and were undertaken in connection with studies on the biosynthesis of microbial phenazine pigments.

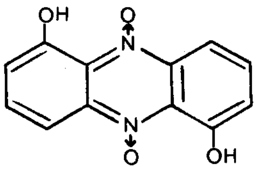
Photo-oxidation of the quaternary salt (1.05×10^{-3}M solution in 1N-hydrochloric acid) for 80 min. gave pyocyanin (7) in 5% yield, extracted into chloroform at pH 9 together with other minor products, including 1-hydroxyphenazine (10). The products remaining in the aqueous solution (which still showed colour changes characteristic of the 1-hydroxy-5-methylphenazinium system) were dequaternised by alkali. Chromatography of a chloroform extract of the neutralised solution showed the presence of not only the expected 9-hydroxyphenazine-1-carboxylic acid (8) but also of the 6-hydroxy isomer (9); appropriate procedures led to the isolation of similar amounts of 6- and 9-methoxyphenazine-1-carboxylic acids, identified by comparison with authentic samples.[4] By similar treatment, phenazine-1-carboxylic acid gave the same two methoxy acids.

That the pyocyanin arose by hydroxylation followed by decarboxylation, i.e. via the 1-carboxy-5-methyl-9-hydroxyphenazinium system, was shown by the photo-oxidation of 1-carboxy-5-methyl-6,7,8,9-tetradeuterophenazinium chloride (as 1)[5]: the isolated pyocyanin was demethylated to 1-hydroxyphenazine which was mainly the trideutero compound. Similarly, when the 7,9-dideutero salt (as 1)[5] was used, the hydroxyphenazine obtained from the pyocyanin was mainly monodeuterated as required by its genesis via hydroxylation in the 9-position; on the other hand, the 1-hydroxyphenazine isolated directly from the photolysed solution contained a much higher proportion of the dideutero compound, consistent with its origin by hydroxylation at either the 6- or the 9-position, followed by demethylation (to the hydroxy acids) and decarboxylation; alternatively decarboxylation may preceed demethylation.

Hydroxylation with similar facility at the 6- or the 9-position in (1) suggests that the mechanism is by 1,4-endoperoxide formation with singlet oxygen; the two positions are very distinct electronically and would be expected to differ markedly in susceptibility to radical attack. It could be that the sensitisation needed for singlet oxygen attack is provided by the phenazinium system itself. The endoperoxide would open to a 6- or 9-hydroperoxide which would decompose to the 6- or 9-hydroxy compounds. A possible scheme is then



Hydroxylation via 1,4-endoperoxide formation with singlet oxygen has already been suggested as a biogenetic model in connection with other systems,[6] and we suggest that the quaternary salt (1) might serve as a biosynthetic intermediate for a number of microbial metabolites based on phenazine; a number of hydroxyphenazine mono- and dicarboxylic acids have been recently isolated including (8),[7] and hydroxylated phenazines such as pyocyanin (7) and iodinin (11) could arise by decarboxylative processes. The phenazinium ion is also subject to nucleophilic attack[2]: indeed, (1) in dilute aqueous ammonia gives aeruginosin A (12)[8] another metabolite of the pyocyanin producing Pseudomonas aeruginosa.[9]



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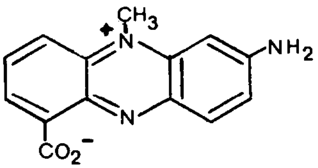

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