151. *Chemiluminescent Organic Compounds. Part VI. The Isolation of Peroxide Derivatives of Phthalaz-1 : 4-diones.*

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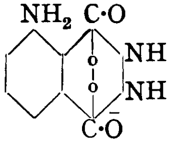
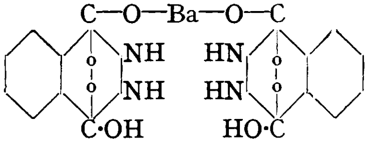
Crystalline compounds of the salts of several phthalaz-1 : 4-diones and hydrogen peroxide have been isolated. They have chemiluminescent properties and are probable intermediates in the luminescing reactions of the diones. The diones appear to act as vehicles, bringing hydrogen peroxide into a condition in which it can be oxidised with chemiluminescence. By the use of duroquinol, atmospheric oxygen may be employed in the chemiluminescent reactions of the diones.

A SOLUTION of the sodium salt of 5-aminophthalaz-1 : 4-dione in aqueous hydrogen peroxide gave, on precipitation with alcohol, a crystalline *peroxide*, which was shown by analysis to have the composition of a monohydrate of the sodium salt of the dione with a molecular proportion of hydrogen peroxide. The water molecule could be removed with little loss of hydrogen peroxide. When dissolved in water, the peroxide gave a bright luminescence with hæmoglobin or a copper salt as catalyst; the luminescence was enhanced by addition of more alkali. The aqueous solution of the peroxide gave the unchanged dione when precipitated with acetic acid; mineral acids likewise set free the hydrogen peroxide, and in water alone the peroxide appeared to be in equilibrium with its constituents.

Other considerations had already led us to suppose (J., 1937, 1843) that a peroxide of the above composition would be found as an intermediate in the chemiluminescent reactions of this series, and we had assigned to the ion the provisional formula (I). Since, however, amino-compounds sometimes form complexes in which hydrogen peroxide is apparently associated with the amino-group, attempt was made to see whether this might not be the case in the present instance. Phthalaz-1 : 4-dione, which contains no amino-group, was substituted for the former dione, and the peroxide of the *barium* salt was obtained in a fairly pure condition. This substance contained about two molecular proportions of hydrogen peroxide to one of the barium salt of the dione (II). It seems certain, therefore, that the hydrogen peroxide molecules are not associated with the phenyl groups of the diones, and they are thus probably in combination with the hydrazide rings. Although the structures must still be regarded as provisional, the alternatives are now few and none of them seems to offer so satisfactory an interpretation of the chemiluminescence.

(I.)



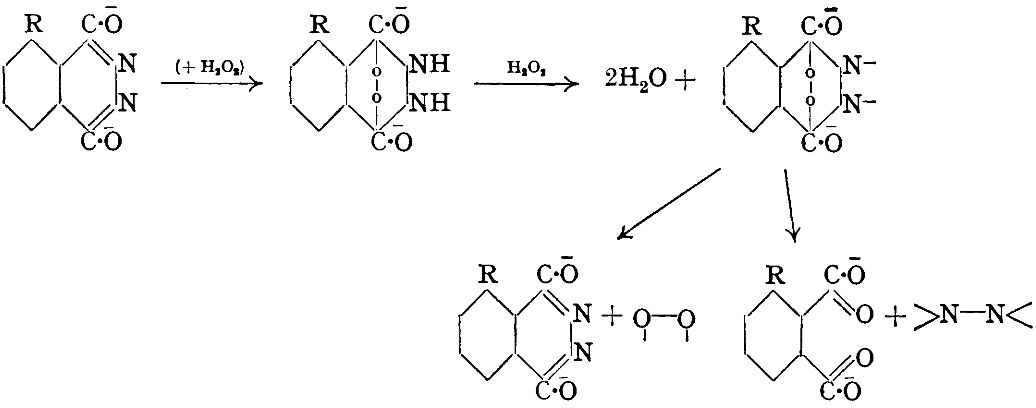


(II.)

Other salts of the foregoing diones, and also salts of other diones, have been shown to afford peroxide derivatives of the like character, although they have not yet been obtained pure.

When phthalaz-1 : 4-dione is oxidised with sodium hypochlorite, it gives benzophenone-

oo'-dicarboxylic acid (Zellner and Dougherty, *J. Amer. Chem. Soc.*, 1937, **59**, 2580). We confirmed this observation, but found that, when the oxidation with sodium hypochlorite was conducted in the presence of a constant excess of hydrogen peroxide, phthalic acid only was produced; 5-nitrophthalaz-1 : 4-dione similarly give 3-nitrophthalic acid. In the absence of hydrogen peroxide, phthalaz-1 : 4-dione gave the theoretical volume of nitrogen on oxidation with sodium hypochlorite, and 5-aminophthalaz-1 : 4-dione gave under the same conditions the whole of the nitrogen of the hydrazide ring and about two-fifths of that of the amino-group. When, however, an aqueous solution of the sodium salt of 5-aminophthalaz-1 : 4-dione (1 mol.), containing hydrogen peroxide (10 mols.), was treated slowly with sodium hypochlorite (2·5 mols.), with shaking, there was continuous chemiluminescence, but only about one-seventh of the hydrazide nitrogen was liberated. This, and other similar experiments, indicate that the dione is not necessarily oxidised in the chemiluminescent reaction, but rather enters into peroxide formation and is then released by the oxidation of the imino-hydrogen atoms of the peroxide. The dione thus may act as a vehicle for the oxidation of hydrogen peroxide by the same or by another oxidising agent (in this instance, the hypochlorite). Catalysts, such as hæmoglobin or copper salts, facilitate the latter oxidation, especially when hydrogen peroxide is the oxidising agent; but they do not influence the formation of the complex peroxide ion of the dione. The following sequence of reactions may thus be pictured during the chemiluminescent interaction between a dione and hydrogen peroxide :



Radiation of light will probably occur chiefly after the release of oxygen as shown on the left of the final stage, but the other scheme is not excluded. At the ordinary temperature and in the absence of catalysts, hydrogen peroxide is capable of oxidising the dione peroxide only very slowly, but other oxidising reagents are evidently more effective. Oxidising agents which produce a chemiluminescence with the diones in the absence of hydrogen peroxide may be regarded as generators of hydrogen peroxide from water molecules under alkaline conditions.

It has been shown in a number of cases of bioluminescence that atmospheric oxygen acts as the oxidising agent, in the presence of an enzyme functioning as a catalyst. We now find that atmospheric oxygen can be used also in the cases of several of the diones, if duroquinol (*e.g.*, in solution in alcohol) be added. An alkaline solution of 5-aminophthalaz-1 : 4-dione gives in this way a feeble glow when aërated in the absence of a catalyst, but a strong glow when hæmoglobin is added. James and Weissberger (*J. Amer. Chem. Soc.*, 1938, **60**, 98) have shown that duroquinol interacts wlth oxygen molecules at normal temperatures to give hydrogen peroxide and the quinone. The action of this and other quinols in promoting chemiluminescence, in conjunction with reducing mechanisms, is being studied.

Experimental.

Sodium Salt of 5-*Amino*-1 : 4-*dihydroxy*-2 : 3-*dihydrophthalazine Peroxide.*—The dione (1 g.) was dissolved in 5% aqueous sodium hydroxide (about 5 c.c.; 1 mol.), the solution cooled, and perhydrol (30% strength; 5 c.c.) added. After a time, the mixture was treated with about 20 c.c. of alcohol (96%), and cooled to 0°; small, very faintly yellow needles of the *sodium* salt of the *peroxide monohydrate* separated, and were filtered off, washed with alcohol and ether, and dried on porous tile in a vacuum over calcium chloride. After ½—4 hours, the product was analysed for peroxide oxygen and simultaneously by combustion for the remaining elements [Found: C, 38·3; H, 4·0; N, 17·1; Na, 9·2; O (by diff.), 31·4; H_2O_2 (observed), 11·4. $C_8H_8O_4N_3Na,H_2O$ requires C, 38·25; H, 4·0; N, 16·75; Na, 9·15; O, 31·85; H_2O_2, 13·55%]. The peroxide only slowly lost hydrogen peroxide in air; it was readily soluble in water, in which it was partly dissociated; in a vacuum over phosphoric oxide it lost the water molecule, without much loss of hydrogen peroxide, but at 120° the hydrogen peroxide also was lost. An aqueous solution of the peroxide gave the pure dione almost quantitatively when acidified with acetic acid (Found: dione, 69·6. Calc., 70·4%). The hydrogen peroxide estimation was carried out with potassium iodide and dilute sulphuric acid, in presence of ammonium molybdate as catalyst, according to a standard procedure; blank tests showed that 5-aminophthalaz-1 : 4-dione did not interfere with the estimation, but we are inclined to think that the amino-group of the peroxide was responsible for the low result obtained for the peroxide oxygen as compared with the result from the elementary analysis.

Peroxides of the potassium and barium salts of 5-aminophthalaz-1 : 4-dione have been obtained, but so far without the full content of hydrogen peroxide.

Barium Salt of 1 : 4-*Dihydroxy*-2 : 3-*dihydrophthalazine Peroxide*.—The sodium salt of the dione was treated, as described above, with perhydrol, the solution kept at 0° for 2 hours and filtered from a trace of precipitate, and the filtrate treated with an aqueous solution of barium chloride (1 mol.) containing the same proportion of hydrogen peroxide as the former solution: the precipitated *barium* salt was dried on a porous tile in air for a day and analysed as above [Found: C, 36·3; H, 3·05; Ba, 26·0; N (calc. on carbon), 10·5; O (by diff.), 24·15; H_2O_2 (observed), 8·6. $C_{16}H_{14}O_8N_4Ba$ requires C, 36·4; H, 2·65; Ba, 26·05; O, 24·3; N, 10·6; H_2O_2, 12·9%]. The peroxide is very sparingly soluble in water; it appears to be fairly stable in air.

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