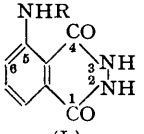
137. *Chemiluminescent Organic Compounds. Part VIII. Substitution in the Amino-group of "Luminol" and its Effect on the Luminescence.*

By B. E. Cross and H. D. K. Drew.

Substitution of one of the hydrogen atoms of the amino-group of luminol by several acyl groupings, and of both of them in the formation of a piperazone or a pyrazole ring, gave products of which the luminescence power indicated that the strong effect of the introduction of the amino-group in the unsubstituted *cyclo*hydrazide molecule may perhaps depend on the retention of at least one of the two hydrogen atoms of the amino-group. Substitution of one

of these hydrogen atoms by a group capable of producing a molecular proportion of hydrogen peroxide, *in situ*, in the presence of atmospheric oxygen and alkali, was achieved; but this did not lead to an enhancement of the luminescence.

" LUMINOL," 5-aminophthalaz-1 : 4-dione (I; R = H), the first discovered of the luminescent phthalic *cyclo*hydrazides, is still, in respect of this property, considerably the most powerful known member of the series, its nearest competitors being the 5-methylamino-, 5-hydroxy-, 5-hydrazino-, and 5 : 8-diacetamido-derivatives, in that order. Further substitutions of one or both of the hydrogen atoms of the 5-amino-group of luminol have now been made, in seeking both to improve the luminescence and to explain the dependence of this property on the nature and position of the substituents in the aromatic ring.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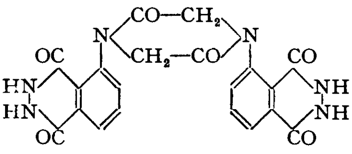
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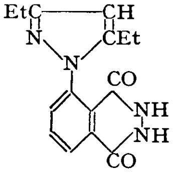
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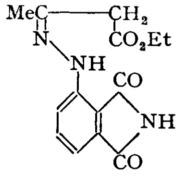
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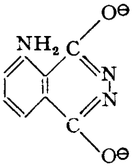
From luminol and chloroacetyl chloride, *5-chloroacetamidophthalaz-1 : 4-dione* (I; R = CO•CH$_2$•Cl) was obtained, which gave a strong bluish-white chemiluminescence (the luminescence, throughout, was tested with hydrogen peroxide and a trace of hæmoglobin, in presence of an aqueous solution of 12 molar proportions of sodium hydroxide). Treatment with ammonia converted the foregoing substance into *5-glycylamidophthalaz-1 : 4-dione* (I; R = CO•CH$_2$•NH$_2$), which gave a strong greenish-yellow chemiluminescence; its *ω-acetyl* derivative (I; R = CO•CH$_2$•NHAc) afforded a somewhat weaker but still strong luminescence of the same shade. Interaction of the above 5-chloroacetamido-derivative with hydrazine hydrate yielded *5-hydrazinoacetamidophthalaz-1 : 4-dione* (I; R = CO•CH$_2$•NH•NH$_2$), which likewise gave a strong greenish-yellow chemiluminescence.

Digestion of the 5-chloroacetamido-derivative with dilute aqueous potassium carbonate did not yield 5-hydroxyacetamidophthalaz-1 : 4-dione but gave as the sole product NN′-*bis*-(*5-phthalazyl-1 : 4-dione*)*piperazone* (II) (cf. Abenius, *J. pr. Chem.*, 1889, **40**, 426). This piperazone gave only a very weak chemiluminescence. 5-3′ : *5′-Diethyl-1′-pyrazolylphthalaz-1 : 4-dione* (III), obtained by condensing 3-hydrazinophthalimide with dipropionylmethane in acetic acid solution and then treating the product (the phthalimide corresponding to III) with hydrazine hydrate, was only weakly luminescent, though the glow was of long duration. Attempts were made to prepare an analogous pyrazolyl derivative of luminol, by condensing acetoacetic ester with 3-hydrazinophthalimide, but with acetic acid as solvent only *β-acetyl-3-hydrazinophthalimide* was produced, and, although by using ethanol as the solvent the *hydrazone* (IV) was obtained, we failed to effect ring-closure to the pyrazolyl compound.

The above experiments suggest, though tentatively, that the very strong enhancement of luminescence which accompanies the introduction of an amino-group into the aromatic nucleus of phthalaz-1 : 4-dione is little altered if one aminic hydrogen atom is substituted by an immobile atom or group, but is sensibly removed if both aminic hydrogens are so substituted. The preparation of a dimethylaminophthalaz-1 : 4-dione or other similar substance should decide the point.

Garwood and one of us (*J.*, 1938, 792; *Trans. Faraday Soc.*, 1939, 212) have suggested that luminescence in this series occurs after the ion (V) has taken up a hydrogen peroxide molecule, giving (VI), which is then further oxidised by hydrogen peroxide to the luminescent compound (VII). We thought that the proportion of molecules of the type (VI) might be increased if a grouping could be attached to the hydrazide residue which was capable of evolving a molecule of free hydrogen peroxide in the presence of alkali and atmospheric oxygen, for this molecule would be released in the vicinity of the hydrazide residue. Duroquinol would afford such a residue (James and Weissberger, *J. Amer. Chem. Soc.*, 1938, **60**, 98), but as this would be difficult to attach to the luminol molecule we employed dihydroxydurylic acid which was found to behave in the same way. Dinitrodurylic acid, prepared by known processes from *m*-xylene, was converted into the acid chloride, and this was condensed with 3-aminophthalimide at the amino-group to give 3-3′ : *6′-dinitroduryloylamidophthalimide*, which was reduced with zinc and acetic acid to the *diamino*-derivative. The latter was oxidised with ferric chloride in methanol to the corresponding *quinone*, which was then reduced with sulphur dioxide in methanol to 3-3′ : *6′-di-*

hydroxyduryloylamidophthalimide. Finally, digestion of this with hydrazine hydrate produced the required substance, 5-3′ : *6′-dihydroxyduryloylamidophthalaz*-1 : 4-*dione* (VIII). Most of these reactions were difficult to carry out owing to the rather intractable nature of the substances. The final product was, in fact, luminescent in presence of air and aqueous alkali, so that it did actually give rise to hydrogen peroxide : but even in the presence of added hydrogen peroxide and a trace of hæmoglobin it was not more luminescent than luminol.



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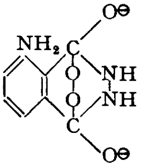

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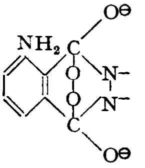

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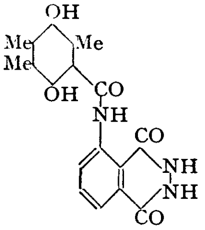

(VIII.)

EXPERIMENTAL.

(All m. p.s are uncorrected.)

5-*Chloroacetamidophthalaz*-1 : 4-*dione* (I, R = CO·CH$_2$Cl).—5-Aminophthalaz-1 : 4-dione (12 g.), chloroacetyl chloride (8 c.c.), and pyridine (6 c.c.) were covered with glacial acetic acid and heated under reflux for 30 minutes. When cold, the *chloroacetyl* compound was collected. It crystallised from glacial acetic acid (charcoal) with one molecule of solvent as small white needles (17 g.; 82%), m. p. 263° (decomp.) (Found : C, 45·8; H, 4·1. $C_{10}H_8O_3N_3Cl,C_2H_4O_2$ requires C, 45·9; H, 3·85%). The molecule of acetic acid was slowly lost at 140° in a vacuum (Found : C, 47·6; H, 3·35; N, 16·8; Cl, 13·7. $C_{10}H_8O_3N_3Cl$ requires C, 47·35; H, 3·2; N, 16·6; Cl, 14·0%).

5-*Glycylamidophthalaz*-1 : 4-*dione* (I, R = CO·CH$_2$·NH$_2$).—Finely powdered 5-chloroacetamidophthalaz-1 : 4-dione (2 g.) was covered with cold aqueous ammonia (*d* 0·880) and kept for 8 days in a closed vessel. The suspension was diluted with water, heated, and filtered, and the hot filtrate acidified with acetic acid. The white crystalline precipitate was twice recrystallised from dilute aqueous hydrazine as its hydrazine salt. The white crystalline *phthalazdione* was obtained by acidifying a hot aqueous solution of the salt with acetic acid. It was insoluble in all the usual solvents and had m. p. 244—245° (decomp.) (Found : C, 50·9; H, 4·6; N, 23·1. $C_{10}H_{10}O_3N_4$ requires C, 51·3; H, 4·3; N, 23·9%). Its *ω-acetyl* derivative formed white needles, m. p. 270° (decomp.), from aqueous alcohol (Found : C, 51·8; H, 4·75; N, 19·95. $C_{12}H_{12}O_4N_4$ requires C, 52·15; H, 4·4; N, 20·3%).

5-*Hydrazinoacetamidophthalaz*-1 : 4-*dione* (I, R = CO·CH$_2$·NH·NH$_2$).—A mixture of finely powdered 5-chloroacetamidophthalaz-1 : 4-dione (4 g.) with 100% hydrazine hydrate was allowed to stand for 3 days and then warmed until all the dione dissolved. The solution was diluted to 120 c.c. and filtered while hot. The filtrate slowly deposited a white solid, which was collected and twice crystallised from very dilute aqueous hydrazine as the hydrazine salt. Acidification of a hot aqueous solution of the salt precipitated the required *dione* as white microcrystals, blackening at 310°. It rapidly reduced cold Fehling's solution (Found : C, 48·45; H, 4·0; N, 28·4. $C_{10}H_{11}O_3N_5$ requires C, 48·2; H, 4·45; N, 28·1%).

NN′-*Bis*-(5-*phthalazyl*-1 : 4-*dione*)*piperazone* (II).—5-Chloroacetamidophthalaz-1 : 4-dione monoacetate (4 g.), anhydrous potassium carbonate (1·76 g.; 2 equivs.), and water (200 c.c.) were heated under reflux for 4 hours. The heavy, almost white product (2·6 g.; 90%) was separated from a small quantity of less dense greyish-black material by decantation. The *piperazone* formed small white crystals from a very large volume of glacial acetic acid, m. p. >325° (Found : C, 55·3; H, 3·55; N, 19·4. $C_{20}H_{14}O_6N_6$ requires C, 55·3; H, 3·25; N, 19·35%). It was insoluble in organic solvents, but readily soluble in cold 4% sodium hydroxide solution. It did not reduce Fehling's solution.

3-*Hydrazinophthalimide*.—A solution of 3-aminophthalimide (5·0 g.), sodium hydroxide (1·35 g.), and sodium nitrite (2·4 g.) in water (40 c.c.) was cooled to −5° and stirred into 18% hydrochloric acid (55 c.c.), the temperature being kept below 0°. The diazonium salt solution was stirred into a solution of sodium sulphite heptahydrate (23·5 g.) and anhydrous sodium carbonate (25 g.) in water (150 c.c.), the temperature not being allowed to rise above 0°. After standing at room temperature for 10—15 minutes, the mixture was acidified with concentrated hydrochloric acid (50 c.c.), and the clear yellow solution heated on the water-bath for 15 minutes. The pale yellow crystals of sodium phthalimide-3-hydrazine-β-sulphonate, which separated on cooling the solution to 0°, were collected and worked up as described by Drew and Pearman (*J.*, 1937, 588), giving 3-hydrazinophthalimide (4·0 g.; 73%), m. p. 208—210°.

3-3′ : 5′-*Diethyl*-1′-*pyrazolylphthalimide*.—Dipropionylmethane (2·8 g.) and 3-hydrazinophthalimide (3·75 g.) were heated under reflux in glacial acetic acid solution for 4 hours. Most of the acetic acid was removed by distillation, and the residual solution diluted with water. The precipitate was collected and crystallised from aqueous alcohol (charcoal) and then from benzene, giving silvery-white plates (occasionally needles) (3·9 g.; 66%), m. p. 207—208°, which did not reduce Fehling's solution (Found : C, 66·75; H, 5·45; N, 16·3. $C_{15}H_{17}O_3N_3$ requires C, 62·7; H, 5·9; N, 14·6. $C_{15}H_{15}O_2N_3$ requires C, 66·9; H, 5·6; N, 15·6%).

5-3′ : 5′-*Diethyl*-1′-*pyrazolylphthalaz*-1 : 4-*dione* (III).—The foregoing *imide* (1·7 g.), 50% hydrazine hydrate (2·6 c.c.), and water (15 c.c.) were heated under reflux for 3—4 hours. Acidification of the cold solution gave a greyish-white precipitate of the *dione*, which was twice crystallised from aqueous alcohol, giving small silvery-white plates (0·85 g.; 47%), m. p. 232—234° (Found: C, 63·6; H, 5·85; N, 19·9. $C_{15}H_{16}O_2N_4$ requires C, 63·4; H, 5·65; N, 19·7%). The *dione* was readily soluble in cold dilute sodium hydroxide solution; it did not reduce Fehling's solution.

Action of Acetoacetic Ester on Hydrazinophthalimide.—(a) A solution of 3-hydrazinophthalimide (2 g.) in glacial acetic acid was mixed with excess of acetoacetic ester (2 g.) and heated under reflux for 4 hours. The only product isolated from the red solution was *β-acetyl*-3-*hydrazinophthalimide* (2·0 g.; 80%), which crystallised from alcohol as cream-coloured needles, m. p. 283—285° (decomp.) (Found: C, 55·25; H, 4·3; N, 19·1. $C_{10}H_9O_3N_3$ requires C, 54·8; H, 4·1; N, 19·2%).

(b) A solution of 3-hydrazinophthalimide (8·7 g.) in aqueous alcohol was mixed with acetoacetic ester (7·0 g.) and heated under reflux for 6 hours. Concentration of the solution gave the almost pure *hydrazone* (IV) as yellow needles (12·3 g.; 86%). It crystallised from absolute alcohol or benzene as bright yellow needles, m. p. 160—162° (Found: C, 58·25; H, 5·3; N, 15·0. $C_{14}H_{15}O_4N_3$ requires C, 58·15; H, 5·2; N, 14·55. $C_{12}H_9O_3N_3$ requires C, 59·25; H, 3·7; N, 17·3%).

3 : 6-*Dinitrodurylic Acid.*—*ψ*-Cumene, prepared from *m*-xylene by the method of Maxwell and Adams (*J. Amer. Chem. Soc.*, 1930, **52**, 2962), was converted into acetyl-*ψ*-cumene as described by Wegler (*J. pr. Chem.*, 1937, **148**, 153). Oxidation of the ketone by a modification of Mills's method (*J.*, 1912, **101**, 2192) yielded durylic acid, which on nitration (Nef, *J.*, 1888, 432; Smith and Byrkit, *J. Amer. Chem. Soc.*, 1933, **55**, 4307) gave the required dinitro-acid.

3 : 6-*Dinitroduryloyl Chloride.*—A suspension of the dinitro-acid (40 g.) in dry benzene (130 c.c.) was heated under reflux with excess of thionyl chloride (23 c.c.) for 2 hours. The crystalline acid chloride, left after removal of the benzene and excess thionyl chloride under reduced pressure, recrystallised from light petroleum (b. p. 80—100°) as golden-brown bipyramids, m. p. 92—93° (39·2 g.; 92%) (Found: Cl, 12·9. Calc. for $C_{10}H_9N_2Cl$: Cl, 13·0%). Smith and Tenenbaum (*J. Amer. Chem. Soc.*, 1935, **57**, 1295) give m. p. 90—91°.

3-3′ : 6′-*Dinitroduryloylamidophthalimide.*—An intimate mixture of the dinitro-acid chloride (15 g.) and excess of 3-aminophthalimide (13·5 g.) was mixed with 100% acetic acid (50 c.c.) and heated under reflux in an oil-bath (bath temp. 125—130°) for 8 hours. When cold, the pale buff-coloured product was collected, pressed and drained, and washed with a little spirit. Crystallisation from glacial acetic acid (charcoal) gave small white needles, m. p. 305—310° (decomp.); yield, 7·15 g., 33%. Recrystallisation from acetic acid and from methanol gave the *duryloylamide* as felted white needles, m. p. 316—317° (decomp.) (Found: C, 53·85; H, 3·45; N, 14·45. $C_{18}H_{14}O_7N_4$ requires C, 54·25; H, 3·5; N, 14·05%).

The *diamino-duryloylamide* was prepared by adding zinc dust (8 g.) to a suspension of the foregoing dinitro-compound (4 g.) in a hot mixture of acetic acid (120 c.c.) and water (45 c.c.), followed by gentle heating under reflux for 45 minutes. The hot yellow solution was filtered from excess of zinc dust and reduced to small bulk under reduced pressure in an atmosphere of carbon dioxide. The residual solution was diluted, and the amine precipitated by the addition of ammonium chloride and ammonia. The greenish-yellow precipitate was collected and used in subsequent work without purification. Crystallisation from aqueous alcohol with addition of charcoal, followed by crystallisation from methanol–acetone, gave small white crystals, m. p. >305° (Found: C, 64·3; H, 5·7; N, 16·85. $C_{18}H_{18}O_3N_4$ requires C, 63·9; H, 5·35; N, 16·55%).

3-3′ : 6′-*Dihydroxyduryloylamidophthalimide.*—Finely powdered crude diaminoduryloylamidophthalimide (3 g.) was added to a solution of ferric chloride hexahydrate (15 g.) in a mixture of methanol (150 c.c.) and concentrated hydrochloric acid (6 c.c.) at 0—5°. After standing for 1 hour in ice, the dark red solution was left for 1 hour at room temperature and then filtered into cold water (350—400 c.c.). The mauve precipitate was collected, dried in a vacuum, powdered, and stirred into a saturated solution of sulphur dioxide in methanol (200 c.c.). After several hours' standing, charcoal was added, and the solution boiled and filtered. The required *quinol* separated from the filtrate, after concentration, as dark cream-coloured crystals, m. p. 218—219° (decomp.) (0·55—0·75 g.). Recrystallisation from alcohol, acetic acid, and finally methanol gave cream-coloured needles, m. p. *ca.* 235° (decomp.) (Found: C, 63·9; H, 5·3; N, 8·15. $C_{18}H_{16}O_5N_2$ requires C, 63·5; H, 4·75; N, 8·25%). A dilute alcoholic solution of the quinol gave a fairly strong luminescence on admixture with an alkaline solution of luminol plus hæmoglobin.

5-3′ : 6′-*Dihydroxyduryloylamidophthalaz*-1 : 4-*dione* (VIII).—The foregoing phthalimide (0·4 g.) was heated under reflux with excess of 100% hydrazine hydrate (2 c.c.) in an oil-bath (bath temp. 125°) for 20 minutes. It slowly dissolved (with evolution of ammonia) to a yellow solution from which the product was precipitated by cooling and dilution. The product slowly dissolved in boiling methanol, giving a yellow solution, which after filtration and concentration deposited a white solid. The *dione*, which slowly turned brown, was collected, washed, and dried in a vacuum, m. p. *ca.* 215° (decomp.) (Found: C, 60·4; H, 5·2; N, 12·3. $C_{18}H_{17}O_5N_3$ requires C, 60·85; H, 4·8; N, 11·85%).

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