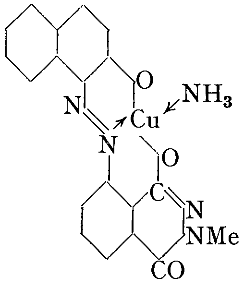
388. *Chemiluminescent Organic Compounds. Part V. The Methyl Derivatives of 5-Nitro- and 5-Amino-phthalaz-1 : 4-dione. Structural Features in Relation to Chemiluminescence.*

By H. D. K. Drew and R. F. Garwood.

5-Aminophthalaz-1 : 4-dione is probably the most powerfully chemiluminescent member of the *cyclo*hydrazide series. The object of the work was to substitute methyl for hydrogen in all possible positions of the hydrazide ring of this substance, to determine the structures of the methyl derivatives, and then to trace the effect of the structural changes on the luminescence property. In this way we hoped to get a clue to the nature of the change concerned in the luminescence, even although the luminescing molecules represent only a small fraction of 1% of the *cyclo*hydrazide molecules present. Seven out of the eight possible methyl derivatives have now been prepared and examined. The crucial step in settling the orientation of the methyl groups was taken by preparing the copper lakes of the azo-β-naphthol derivatives of the mono-*N*-methyl compounds and determining their structures. Further methylation then gave the structures of the dimethylated derivatives. The chief conclusion from the work is that chemiluminescence is dependent on the ion derived from the dilactim form of the *cyclo*hydrazide. This in all probability forms a peroxide, directly responsible for the reaction which initiates the radiation. The peroxide has not yet been characterised.

The orientation of the methyl group in the α- and the β-form of 5-amino-*N*-methylphthalaz-1 : 4-dione, and therefore also in the 5-nitro-compounds from which the amino-compounds were obtained by reduction (Drew, Hatt, and Hobart, this vol., p. 33), has now been ascertained by determination of the structures of the cupric derivatives of the *azo*-compounds formed by coupling the respective diazotised amines with β-naphthol. The azo-compound of the α-form, when treated with aqueous cupric chloride and ammonia, gave the *amminocupric* derivative (I), which did not combine with more copper when boiled with aqueous cupric chloride, showing that a hydroxyl group was present in the 4-position of the hydrazide ring; whereas the azo-compound of the β-form gave, under the same conditions, the *ammonium* salt of the *hydroxycupric* derivative (II), which with aqueous cupric chloride became the *cupric* salt of the hydroxycupric complex [*i.e.*, $Cu_{\frac{1}{2}}$ for NH_4 in (II)], showing that a carbonyl group was present in the 4-position. These copper lakes appear to be of novel type. Analysis of the corresponding derivatives with pyridine in place of ammonia confirmed the composition of the complexes, although, owing to a tendency towards loss of pyridine as vapour, the pyridine compounds were not obtained pure. For comparison, the *cupric* derivative (III) of the azo-compound from 5-amino-2 : 3-dimethylphthalaz-1 : 4-dione was prepared in presence of pyridine; it proved to be similar in type to (II), but, as was to be expected, was incapable of adding pyridine. α-5-Amino-*N*-methylphthalaz-1 : 4-dione is therefore *5-amino-2-methylphthalaz-1 : 4-dione* (IV; R = NH_2) and the β-compound is the *3-methyl* isomeride.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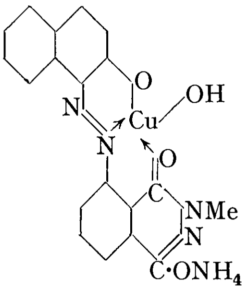
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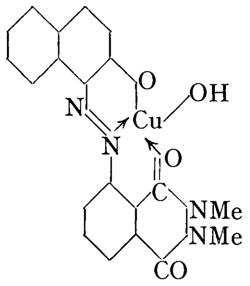
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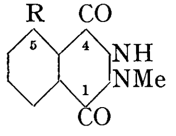
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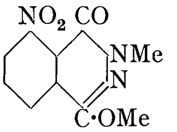
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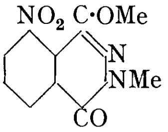
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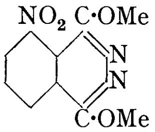
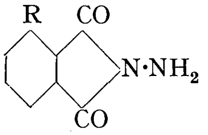

(VII.)

There are four possible dimethyl derivatives of 5-nitrophthalaz-1 : 4-dione with the methyl groups substituted in the hydrazide ring, one of which, the 2 : 3-dimethyl compound is already known (*loc. cit.*). The remainder have now been obtained. With methyl sulphate, 5-nitrophthalaz-1 : 4-dione became methylated initially in the 3-position, the product being a mixture of 5-nitro-3-methylphthalaz-1 : 4-dione, which is evidently the hitherto unidentified substance (m. p. 264°) obtained by Mihailescu and Protopopescu (*Bul. Soc. Chim. România*, 1930, **12**, 95), together with its *O*-methyl derivative, *5-nitro-1-methoxy-3-methylphthalaz-4-one* (V). The last substance was obtained also in two other ways: (i) methylation of the silver salt of 5-nitrophthalaz-1 : 4-dione gave an *O*-methyl derivative, which on further methylation gave (V); the *O*-methyl derivative was therefore *5-nitro-1-methoxyphthalaz-4-one*; and (ii) methylation of the silver salt of 5-nitro-3-methylphthalaz-1 : 4-dione. Methylation of the silver salt of 5-nitro-1-methoxyphthalaz-4-one gave *5-nitro-1 : 4-dimethoxyphthalazine* (VII). Finally, methylation of the silver salt of 5-nitro-2-methylphthalaz-1 : 4-dione (IV; R = NO_2) gave 5-nitro-4-methoxy-2-methylphthalaz-1-one (VI). The *amino*-compounds corresponding with (V), (VI), and (VII) also were prepared. Of the four possible monomethyl derivatives of 5-nitrophthalaz-1 : 4-dione with the methyl group in the hydrazide ring, only 5-nitro-4-methoxyphthalaz-1-one now remains unknown; the same holds for the corresponding 5-amino-compounds.

The action of methylhydrazine upon the following substances gave rise in each case to a mixture of the α- and the β-form of the corresponding *cyclo*hydrazides: 3-nitro-2-

carbethoxybenzoic acid, 3-nitro-2-carbethoxybenzoyl chloride, 6-nitro-2-carbethoxybenzoic acid, 3-acetamidophthalic anhydride, and 3-aminophthalimide. The reactions with the last two afforded an independent means of synthesising the α- and the β-forms of 5-amino- and of 5-acetamido-methylphthalaz-1 : 4-dione, previously obtained only by reduction of the nitro-compounds. The physical properties of the amino- and the nitro-methylphthalaz-1 : 4-diones prepared during the present work confirm the data previously given (this vol., p. 33, footnote).



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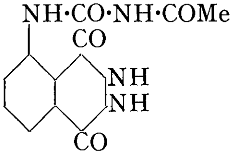

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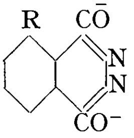

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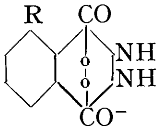

(XI.)

3-*Nitro*-N-*aminophthalimide* (VIII; R = NO_2) and 3-*acetamido*-N-*aminophthalimide* were prepared; they are readily transformed into the corresponding *cyclo*-hydrazides. 5-*Acetcarbamidophthalaz*-1 : 4-*dione* (IX) was strongly chemiluminescent on oxidation in presence of alkali.

It has already been shown (*loc. cit.*) that the substitution of methyl for both of the enolisable hydrogen atoms of the highly luminescent amino-*cyclo*hydrazides caused complete loss of chemiluminescence, and the substitution for only one of them caused such a profound diminution in this property as to suggest that the residual activity was due to traces of unmethylated product. We have now shown that this is so in the case of 5-amino-2-methylphthalaz-1 : 4-dione, prepared by the action of methylhydrazine on 3-aminophthalimide: eight crystallisations of its acetyl derivative from acetic anhydride gave a specimen (m. p. 190°), which on hydrolysis of the acetyl group gave the amine (m. p. 309°); the latter showed no trace of luminescence on oxidation with hydrogen peroxide in alkaline solution in presence of hæmoglobin, whereas the uncrystallised substance showed the usual feeble chemiluminescence. It may therefore be concluded that *the presence of both of the imino-hydrogen atoms of the* cyclo*hydrazides is essential for chemiluminescence.*

Since the luminescence appears only in alkaline solution and since more than one equivalent of alkali is necessary for its highest development (compare Drew and Pearman, this vol., p. 588), it is strongly indicated that the luminescent agent is the di-enolised (lactim) form of the molecule. This is supported by the fact that neither of the amino-*cyclo*hydrazides corresponding with (V) and (VI) was chemiluminescent. The matter can be carried a step further, since we observed that 5-amino-1 : 4-dimethoxyphthalazine (as VII), which has the dilactim form, is also non-luminescent, leading to the conclusion that the ion (X) is the essential structure for luminescence.

We have obtained some evidence, though at present inconclusive, that a peroxide, which might have the form (XI), is produced by the addition of hydrogen peroxide to (X). As a working hypothesis, it seems possible that the chemiluminescence follows the oxidation of the imino-hydrogen atoms of the peroxide and the consequent elimination of an oxygen molecule with regeneration of the original *cyclo*hydrazide ion. Attempts to characterise the peroxide are in progress.

EXPERIMENTAL.

5-*Acetcarbamidophthalaz*-1 : 4-*dione* (IX).—3-Acetcarbamidophthalic anhydride (Scott and Cohen, J., 1921, **119**, 667) (2 g.) was heated under reflux (1 hour) in glacial acetic (20 c.c.) containing hydrazine hydrate (0·4 c.c.). The cyclo*hydrazide* separated on cooling; it was purified by precipitation from a solution in 5% aqueous sodium hydroxide with acetic acid and was obtained as a yellowish powder, m.p. 320° (decomp.), which was strongly chemiluminescent (greenish-yellow) (Found: C, 50·3; H, 4·15. $C_{11}H_{10}O_4N_4$ requires C, 50·4; H, 3·8%).

3-*Acetamido*-N-*aminophthalimide* (VIII; R = NH·COMe).—3-Acetamidophthalimide (2 g.) was added to alcohol (30 c.c.), and hydrazine hydrate (0·6 c.c.) run in. The mixture was shaken for 5 minutes, boiled for 5 minutes, and poured into water (300 c.c.); the required *substance* then crystallised. From its yellow solutions in alcohol or water it formed colourless needles

(Found: C, 54·5; H, 4·3. $C_{10}H_9O_3N_3$ requires C, 54·8; H, 4·1%), m.p. 163° (with transformation into the *cyclo*hydrazide). It was soluble in organic solvents and strong acids but not in alkali, and it reduced warm Fehling's solution; it was not chemiluminescent, but became so when boiled with alkali, owing to transformation into the *cyclo*hydrazide. The *benzylidene* derivative was obtained in long white needles, m. p. 214°, from alcohol (Found: C, 66·65; H, 4·85. $C_{17}H_{13}O_3N_3$ requires C, 66·45; H, 4·25%). The *p*-anisylidene derivative formed colourless fibrous needles, m. p. 186°.

*3-Amino-*N*-anilinophthalimide.*—This was prepared by heating under reflux (3 hours) 3-aminophthalimide (1 g.) with phenylhydrazine (0·7 c.c.) in water (50 c.c.); yellow crystals separated and were washed with aqueous ammonia and recrystallised from dilute alcohol (yield, 1·4 g.). The substance had m. p. 222° (Found: C, 66·2; H, 4·6. Calc. for $C_{14}H_{11}O_2N_3$: C, 66·45; H, 4·35%), was insoluble in alkalis but soluble in acids, and reduced Fehling's solution; on acetylation it gave 3-acetamido-*N*-anilinophthalimide, m. p. 179°, identical with that obtained from 3-acetamidophthalic anhydride and phenylhydrazine (this vol., p. 30). Since this work was done, Gleu and Pfanstiehl have described the substance (*J. pr. Chem.*, 1936, **146**, 137).

Condensation of Methylhydrazine and 3-Aminophthalimide.—When a mixture of methylhydrazine sulphate (3·5 g.; 2 mols.) in water (20 c.c.), 5% aqueous sodium hydroxide (40 c.c.), and 3-aminophthalimide (2 g.) was heated under reflux (2 hours), ammonia was evolved. The clear solution gave on acidification with acetic acid crystals of a mixture (2·2 g.; m. p. 260—270°) of α- and β-5-amino-*N*-methylphthalaz-1 : 4-diones; two crystallisations from glacial acetic acid gave the pure α-form, m. p. 308° (compare this vol., p. 35).

Condensation of Methylhydrazine and 2-Carbethoxy-3 or -6-nitrobenzoic Acid.—In each case a mixture of the benzoic acid derivative (1 g.), methylhydrazine sulphate (0·6 g.; 1 mol.), and hydrated sodium acetate (1·1 g.) was dissolved in alcohol, evaporated to dryness, and heated gradually to 150°. After cooling, the product was dissolved in 5% aqueous sodium hydroxide, and dilute acetic acid added: the crystals were in each case a mixture of the α- and the β-form of 5-nitro-*N*-methylphthalaz-1 : 4-dione, which were separated by the methods previously described (this vol., pp. 35, 36). A similar result was obtained on condensing methylhydrazine with 3-nitro-2-carbethoxylbenzoyl chloride in acetic acid.

Methylation of 5-Nitrophthalaz-1 : 4-dione.—Methylation of 1 g. with methyl sulphate (0·7 c.c.) at 120—130° (8 hours) gave a mixture of substances, from which the following were isolated: (*a*) β-5-nitro-*N*-methylphthalaz-1 : 4-dione, m. p. 275°, which was separated through its solubility in sodium hydroxide; (*b*) a dimethyl derivative (see below), colourless needles, m. p. 139°, from water, which was later shown to be the *O*-methyl ether of β-5-nitro-*N*-methylphthalaz-1 : 4-dione.

5-Nitro-1-methoxy-3-methylphthalaz-4-one (V).—The silver salt (1 g.) of 5-nitro-3-methylphthalaz-1 : 4-dione prepared from the ammonium salt and aqueous silver nitrate (yellow plates, insoluble in water) was refluxed with methyl iodide (1 c.c.) in methyl alcohol (20 c.c.), the silver iodide removed, and the residue from the filtrate crystallised from water: the colourless needles, m. p. 139°, were identical with those mentioned at (*b*) above (Found: C, 51·2; H, 4·15. $C_{10}H_9O_4N_3$ requires C, 51·1; H, 3·8%). Zellner and Dougherty have since described this substance (*J. Amer. Chem. Soc.*, 1936, **58**, 1811), but have not determined the structure.

5-Nitro-4-methoxy-2-methylphthalaz-1-one (VI).—The *silver* salt of α-5-nitro-*N*-methylphthalaz-1 : 4-dione [yellow; slightly soluble in hot water (Found: Ag, 32·9. $C_9H_6O_4N_3Ag$ requires Ag, 32·9%)] gave, with methyl-alcoholic methyl iodide as before, the required *substance*, which formed plates, m. p. 199—200°, from dilute alcohol (Found: C, 51·1; H, 4·15. $C_{10}H_9O_4N_3$ requires C, 51·1; H, 3·85%). Zellner and Dougherty (*loc. cit.*) give m. p. 207°.

5-Amino-4-methoxy-2-methylphthalaz-1-one (as VI), prepared by reduction of the nitro-compound with tin and hydrochloric acid, formed white needles, m. p. 136°, from water (Found: C, 58·5; H, 5·8. $C_{10}H_{11}O_2N_3$ requires C, 58·5; H, 5·35%). Its 5-N-*acetyl* derivative formed colourless needles, m. p. 187°, from alcohol (Found: C, 58·6; H, 5·5. $C_{12}H_{13}O_3N_3$ requires C, 58·4; H, 5·25%).

5-Amino-1-methoxy-3-methylphthalaz-4-one (as V), similarly prepared, crystallised in white needles, m. p. 222°, from water (Found: C, 58·3; H, 5·5%). Its 5-N-*acetyl* derivative had m. p. 220° (Found: C, 57·9; H, 5·4%).

2-Methylphthalaz-1 : 4-dione-5-azo-β-naphthol was prepared in the usual manner from α-5-amino-*N*-methylphthalaz-1 : 4-dione as a brick-red powder, m. p. 334° (decomp.); on crystallisation from pyridine it gave red needles having the same m. p. (Found: C, 65·7; H, 4·4. $C_{19}H_{14}O_3N_4$ requires C, 65·9; H, 4·05%).

3-*Methylphthalaz*-1 : 4-*dione*-5-*azo*-β-*naphthol* was similarly obtained from β-5-amino-*N*-methylphthalaz-1 : 4-dione; it separated as a brick-red powder, m. p. 326° (decomp.), from pyridine (Found : C, 65·4; H, 4·3%).

Copper Salts.—(a) *From* 2-*methylphthalaz*-1 : 4-*dione*-5-*azo*-β-*naphthol.* (i) *Amminocupric salt* (I). A solution of hydrated cupric chloride (0·2 g.) in water (10 c.c.) and concentrated aqueous ammonia (10 c.c.) was shaken with the azo-compound (0·2 g.) for 1 hour; the *copper* salt was filtered off, washed with aqueous ammonia, dried over calcium chloride, and freed from impurities by extraction with chloroform (Soxhlet); the residue consisted of violet crystals with greenish-gold reflex, soluble in alcohol (Found : C, 53·1; H, 4·4; Cu, 15·1. $C_{19}H_{15}O_3N_5Cu$ requires C, 53·6; H, 3·55; Cu, 14·9%). When this salt was boiled with aqueous cupric chloride, it did not absorb more copper, but appeared to lose the molecule of ammonia.

(ii) *Pyridinocupric salt.* The azo-compound (1 mol.), dissolved in hot pyridine, was treated with cupric chloride (1 mol.) in a little water. The deep purple solution was boiled for a few minutes, cooled, filtered, and diluted with water (4 vols.); purple needles with greenish reflex slowly separated and were washed with water and dried over calcium chloride. The *compound* was soluble in organic solvents, forming deep purple solutions, and evolved pyridine when boiled with water or alkali. The same salt was formed when 2 mols. of cupric chloride were used (Found : C, 56·6; H, 4·2; N, 13·6; Cu, 12·7. $C_{24}H_{17}O_3N_5Cu,H_2O$ requires C, 57·1; H, 3·8; N, 13·85; Cu, 12·6%).

(b) *From* 3-*methylphthalaz*-1 : 4-*dione*-5-*azo*-β-*naphthol.* (i) *Ammonium cupric salt* (II). This was obtained as above as a violet crystalline powder, soluble in organic solvents; it lost ammonia when boiled with alkali (Found : C, 50·9; H, 4·2; Cu, 14·9. $C_{19}H_{17}O_4N_5Cu$ requires C, 51·5; H, 3·85; Cu, 14·4%). When the salt was boiled with aqueous cupric chloride, it was converted into the *dicupric* salt (II, with $Cu_{\frac{1}{2}}$ instead of NH_4) (Found : Cu, 20·1. $C_{38}H_{26}O_8N_8Cu_3,2H_2O$ requires Cu, 20·1%).

(ii) *Pyridinocupric salt.* This was obtained as in the former case, but was gelatinous when first precipitated; dried over calcium chloride, it became a purplish-black powder, insoluble in water but soluble in organic solvents (Found : C, 53·4; H, 4·0; N, 13·4; Cu, 12·1. $C_{24}H_{19}O_4N_5Cu$ requires C, 57·1; H, 3·8; N, 13·85; Cu, 12·6%. $C_{24}H_{19}O_4N_5Cu,H_2O$ requires C, 55·1; H, 4·0; N, 13·4; Cu, 12·2%). The same substance was produced when 2 mols. of cupric chloride or acetate were used.

(c) *From* 2 : 3-*dimethylphthalaz*-1 : 4-*dione*-5-*azo*-β-*naphthol.* The *cupric* salt (III), prepared in pyridine with cupric chloride and obtained by evaporation, was washed with water and purified by extraction with alcohol; the evaporated alcoholic extract left a purplish-red powder with greenish reflex (Found : C, 55·2; H, 4·4; Cu, 15·2. $C_{20}H_{16}O_4N_4Cu$ requires C, 54·6; H, 3·65; Cu, 14·5%).

5-*Nitro*-1-*methoxyphthalaz*-4-*one* was prepared by heating under reflux (2 hours) the orange-yellow silver salt of 5-nitrophthalaz-1 : 4-dione (10 g.) with methyl iodide (6 c.c.) and methyl alcohol (200 c.c.); the silver iodide was removed, the filtrate evaporated, and the residue fractionated from methyl alcohol. After seven crystallisations, the required *O*-methyl ether was obtained in colourless plates, m. p. 269°, slightly soluble in hot water and soluble in alkali; its solubility in organic solvents was much higher than that of the *N*-methyl ether (Found : C, 48·9; H, 4·0. $C_9H_7O_4N_3$ requires C, 48·9; H, 3·15%). Its *acetyl* derivative formed colourless prisms, m. p. 188—190° (Found : C, 50·0; H, 3·6. $C_{11}H_9O_5N_3$ requires C, 50·2; H, 3·45%). Its sodium salt formed colourless nacreous plates, soluble in water to a yellow solution. Methylation of the *O*-methyl ether in dilute caustic soda solution with methyl sulphate gave 5-nitro-1-methoxy-3-methylphthalaz-4-one (above), m. p. and mixed m. p. 139° : this verifies the structure.

5-*Amino*-1-*methoxyphthalaz*-4-*one* was prepared by reducing the foregoing nitro-compound with tin and hydrochloric acid; the rhomboidal tablets of the stannichloride of the amine were decomposed with calcium carbonate, and the amine obtained in colourless felted needles, m. p. 234° (Found : C, 56·2; H, 5·0. $C_9H_9O_2N_3$ requires C, 56·5; H, 4·7%). It was moderately soluble in boiling water, readily in hot alcohol, and soluble in aqueous caustic soda.

1 : 4-*Dimethoxyphthalazine.*—The silver salt of 1-methoxyphthalaz-4-one was prepared as a greyish-white mass which contained silver oxide but was sufficiently pure to undergo methylation. This was carried out with boiling methyl-alcoholic methyl iodide (2 hours), the *dimethyl* ether being obtained from the filtered solution in colourless hexagonal plates; boiling with 5% aqueous sodium hydroxide removed a little of the monomethyl ether. The substance melted at 121°, but sintered from 100° (Found : N, 15·1. $C_{10}H_{10}O_2N_2$ requires N, 14·75%); evaporation with 40% hydrobromic acid demethylated it completely, giving the *cyclo*hydrazide.

5-*Nitro*-1 : 4-*dimethoxyphthalazine* (VII) was prepared by methylating the crude silver salt (greyish-yellow) of 5-nitro-1-methoxyphthalaz-4-one (above) with methyl iodide in methyl alcohol. It formed colourless needles, m. p. 212—214°, from dilute ethyl alcohol, and was insoluble in alkali and only very sparingly soluble in boiling water (Found : C, 51·3; H, 4·0. $C_{10}H_9O_4N_3$ requires C, 51·1; H, 3·8%). It was not chemiluminescent.

5-*Amino*-1 : 4-*dimethoxyphthalazine* was obtained by reducing the above nitro-ether with tin and hydrochloric acid at 60°; it formed faintly yellow prisms, m. p. 172—174°, from aqueous alcohol (Found : C, 58·5; H, 5·45. $C_{10}H_{11}O_2N_3$ requires C, 58·5; H, 5·35%), and was soluble in acids but not in alkalis.

Action of Methylhydrazine on 3-*Acetamidophthalic Anhydride*.—Condensation in boiling acetic acid (1 hour) gave a mixture of α- and β-5-acetamido-*N*-methylphthalaz-1 : 4-diones (m. p. 264°). After four crystallisations from glacial acetic acid, the α-form, 5-*acetamido*-2-*methylphthalaz*-1 : 4-*dione*, was obtained pure in white needles, m. p. 291° (Found : C, 57·0; H, 5·1. $C_{11}H_{11}O_3N_3$ requires C, 56·7; H, 4·7%). Hydrolysis of the acetyl group gave the pure α-amino-*cyclo*hydrazide, m. p. and mixed m. p. 308°.

The following preparations were made with the help of Dr. H. H. Hatt and Mr. S. C. Goddard, B.Sc., to whom we are indebted.

3-*Nitro*-N-*aminophthalimide*.—3-Nitrophthalimide (3 g.) in 20 c.c. of cold water was treated with hydrazine hydrate (0·78 g.). From the filtered liquid, some 5-nitrophthalaz-1 : 4-dione separated after 1 day and was removed; after 2 days more, orange needles of the required substance separated (0·5 g.), m. p. 192° (after two crystallisations from alcohol), with resolidification owing to change into the *cyclo*hydrazide. 3-*Nitro*-N-*aminophthalimide* was slightly soluble in water; on being boiled with water, it tended to change into 5-nitrophthalaz-1 : 4-dione. It formed a benzylidene derivative and reduced ammoniacal silver nitrate, Fehling's solution, and Nessler's reagent (Found : C, 46·3; H, 2·6; N, 20·7. $C_8H_5O_4N_3$ requires C, 46·4; H, 2·4; N, 20·3%). When it was dissolved in hot glacial acetic acid and sodium nitrite added, 3-nitrophthalimide separated on cooling (m. p. and mixed m. p. 215—216°). Further, when it was condensed with phthalic anhydride in glacial acetic acid, 3-nitro-*N*-aminophthalimide gave *N*-3′-nitrophthalimidophthalimide (m. p. and mixed m. p. 246°) (Drew and Hatt, this vol., p. 22). The last two reactions verify the structure.

3 : 3′-*Dinitro*-N-*phthalimidophthalimide* was prepared : (i) by refluxing 3-nitro-*N*-aminophthalimide (0·25 g.) and 3-nitrophthalic anhydride (0·5 g.) in glacial acetic acid (½ hour); addition of water precipitated the required substance, which was crystallised from alcohol; (2) by adding hydrazine hydrate (2 g.) in glacial acetic acid (40 c.c.) slowly to 3-nitrophthalic anhydride (15·6 g.) in the same solvent (40 c.c.) and heating the mixture under reflux (2 hours); crystals (6 g.) of the required substance separated and were freed from admixed *cyclo*hydrazide by keeping for some hours under very dilute aqueous ammonia (400 c.c.). The substance (Found : C, 49·8; H, 1·8; N, 15·3. $C_{16}H_6O_8N_4$ requires C, 50·2; H, 1·6; N, 14·7%) formed pale yellow micro-crystals, m. p. 321°, and was insoluble in water and most organic solvents; it was sparingly soluble in glacial acetic acid. It was hydrolysed by cold sodium hydroxide solution (5%), giving 3-nitrophthalic acid and the *cyclo*hydrazide, together with an unidentified product (yellow needles, m. p. 315°, soluble in water). The action of hydrazine hydrate in acetic acid also gave the *cyclo*hydrazide. Mineral acids gave 3-nitrophthalic acid and other products.

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