939. *The Structure of Nidulin, a Metabolite of* Aspergillus nidulans.

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Nidulin is allocated structure (IIa): the structures of nornidulin (" ustin ") and dechloronornidulin, compounds produced along with nidulin by a strain of *Aspergillus nidulans*, follow from this. A 1-methylpropenyl substituent in ring B has not been encountered before amongst depsidones and is rare in other fields: its chemistry is greatly modified by the neighbouring chlorine atom.

A PARTIAL structure (I) for nidulin, the chief of the related metabolites of a non-ascosporic strain of *Aspergillus nidulans*, was suggested [1] in 1954. Evidence is now presented for the complete structure (IIa), in which ring B is of a type novel in the depsidone field.

It was reported earlier that *O*-methylnidulin could not be hydrogenated, did not add osmium tetroxide or halogens or give an epoxide with perbenzoic acid, and did not undergo ozonolysis, but that nitric acid converted it into an unreactive olefin which differed from the parent in that it gave an epoxide when attacked by perbenzoic acid or by ozone. This olefin was called *O*-methyldehydronidulin because it was thought that nitric acid effected dehydrogenation. Sufficient results have now been amassed to show that nitric acid effects isomerisation and not dehydrogenation so the product is now renamed *O*-methylisonidulin. In particular, *O*-methylnidulin has been found to yield an epoxide when treated, under the appropriate conditions, with ozone (though not with perbenzoic acid); and both *O*-methylnidulin and *O*-methylisonidulin rapidly add dinitrogen tetroxide to give the same adduct, $C_{21}H_{19}Cl_3O_5(NO_2)_2$, which, as it has an infrared spectrum appropriate to an aliphatic nitro-compound,[2] but not to an aromatic nitro-compound or to a nitrite, is formulated with the grouping $O_2N-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-NO_2$ and designated dihydro-*O*-methyldinitronidulin.

Although earlier work had shown that methanolysis converted *O*-methylnidulin into the phenolic methyl *O*-methylnidulinate (III) which afforded methyl dichloroeverninate (IV) when degraded by nitric acid, no fragment corresponding to ring B was isolated, and all the evidence for such a ring was indirect.[1] Fission of methyl *O*-methylnidulinate by nitric acid has now been found to give very small quantities of a chloroquinone clearly derived from ring B, and a study of oxidising agents other than nitric acid showed that periodate, first found by Adler and Magnusson [3] to split phenoxyphenols, afforded low but useful yields of the same chloroquinone. This yellow compound, $C_{11}H_{11}ClO_3$, had chemical and spectroscopic properties indicative of a hydroxyquinone of partial structure (V), making it clear that ring B in nidulin (IIa) is derived from hydroxquinol as is usual in the depsidone series.

Treatment of nidulin with hydriodic acid followed by aerial oxidation of an alkaline solution of the product supplied a red compound, $C_{11}H_{12}O_4$, which behaved as a 2,5-dihydroxyquinone (see Fig. 1); and as the second hydroxyl group must have replaced the chlorine atom in the chloroquinone, partial structures (VI; R = OH) and (VI; R = Cl) can be written for these compounds.

C-Methyl determinations on nidulin and its methyl ether gave values ranging unaccountably from 0·92 to 2·05. In marked contrast, the chloroquinone (VI; R = Cl) gave values of 2·75 and 2·77, and the dihydroxyquinone (VI; R = OH) gave a value of 2·93, showing that the carbon atoms of the C_5 residue must be distributed so as to include

[1] Dean, Roberts, and Robertson, *J.*, 1954, 1432.
[2] Brown, *J. Amer. Chem. Soc.*, 1955, **77**, 6341.
[3] Adler and Magnusson, *Acta Chem. Scand.*, 1959, **13**, 505.

three *C*-methyl groups, the intact nidulin molecule containing four such groups. As neither a C_5 system nor a $C_2 + C_3$ system can be arranged so as to give rise to three moles of acetic acid, but a $C_1 + C_4$ system can, it follows that the partial structure (VI; R = Cl)

FIG. 1. *Ultraviolet absorption spectra (in ethanol) of: A, 2,5-dihydroxy-3-methyl-6-1′-methylpropenyl-1,4-benzoquinone; B, 2,5-dihydroxy-3-methyl-1,4-benzoquinone; C, 2-chloro-5-hydroxy-6-methyl-3-1′-methylpropenyl-1,4-benzoquinone* (VII).

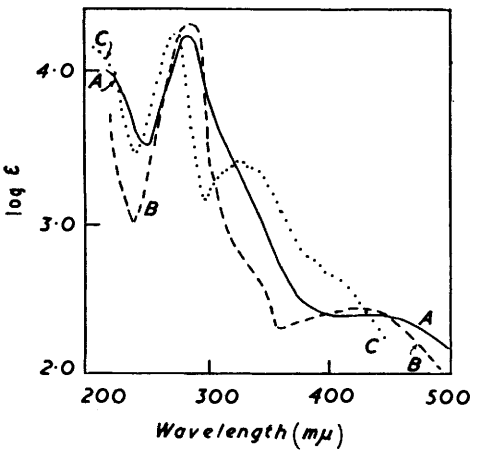
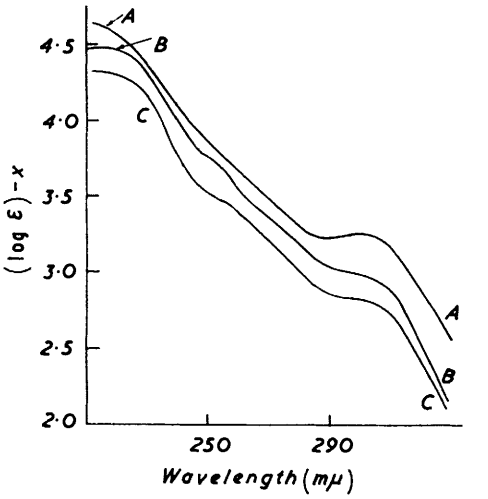
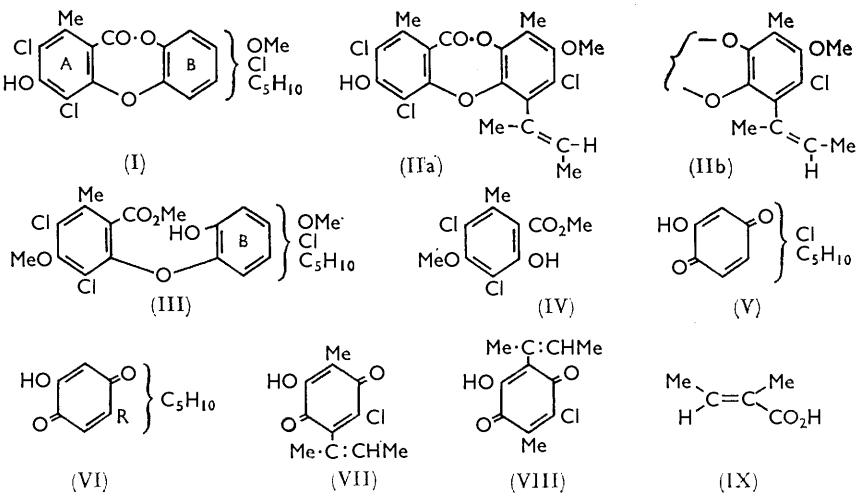


FIG. 2. *Ultraviolet absorption spectra (in ethanol) of: A, Dihydrodinitro-O-methylnidulin (x = 0); B, O-methylnidulin (x = 0·2); C, O-methylisonidulin (x = 0·4).*



for the chloroquinone can be expanded to either (VII) or (VIII). To confirm the existence of a 1-methylpropenyl side-chain, the chloroquinone was oxidised by alkaline hydrogen peroxide to tiglic acid (IX): and this is why the side-chain in nidulin (IIa) is given the *cis*-methyl configuration. Had angelic acid, the geometrical isomer of tiglic acid, been



formed, it might have isomerised to tiglic acid during the oxidation and isolation as the latter is the more stable of the two,[4] but the nuclear magnetic resonance spectrum * of

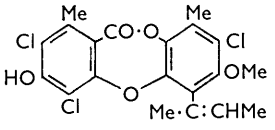
* Details of this and of model compounds connected with this investigation will form the subject of a separate communication.

[4] Buckles, Mock, and Locatell, *Chem. Rev.*, 1955, **55**, 659.

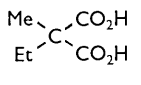
O-methylnidulin independently demonstrated that the methyl groups in the side-chain are *cis*-related.

The structures (VII) and (VIII) for the chloroquinone can each be combined in two ways with the everninate nucleus (IV), so that there are four possible structures for nidulin, two of type (IIa) and two of type (X). In all four, ring B is fully substituted, which explains the facts that methyl *O*-methylnidulinate (III) does not couple with diazonium salts or with Gibbs's reagent, that its allyl ether (III; CH_2:CH·CH_2·O for OH) is thermally stable, and that *O*-methylnidulin does not suffer aromatic nitration or halogenation. That methyl *O*-methylnidulinate is not methylated by diazomethane supports the view that the phenolic hydroxyl group has an *ortho*-alkyl substituent: when there is no such substituent, or when the substituent is chlorine, alkylation is normal.[5] Finally, the full substitution of ring B accounts for our inability to convert nidulin into a xanthone comparable with those obtained from gangaleoidin [6] and other depsidones.[7]

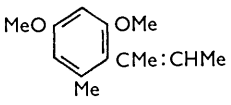
In marked contrast to nidulin itself, the amorphous product obtained by heating nidulin or *O*-methylisonidulin with hydriodic acid was oxidised by chromic acid in sulphuric acid to ethylmethylmalonic acid (XI). This compound contains one more carbon atom than tiglic acid or any other acid which can be accounted for solely in terms of rings B as in (IIa) or (X). Moreover, the model compound (XII) failed to yield ethylmethylmalonic



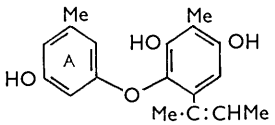
(X)



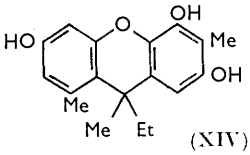
(XI)



(XII)



(XIII)



(XIV)

(XV)

acid when treated similarly, so it can be inferred that the extra carbon atom originated in ring A. To account for this and the part played by hydriodic acid we regard the expected functions of hydriodic acid, *i.e.*, demethylation, dechlorination, and decarboxylation, as leading to a diphenyl ether (XIII) in which an acid-catalysed internal Friedel–Crafts alkylation at a vacant site in ring A is effected by the 1-methylpropenyl side-chain. This would afford a xanthen such as (XIV), which has not been isolated but would suffer oxidation to ethylmethylmalonic acid. Thus structures (IIa) and (X) are both acceptable for nidulin, but their isomers with the methyl and the methylpropenyl substituents interchanged in ring B are not.

The resistance of the side-chain in *O*-methylnidulin and *O*-methylisonidulin to double-bond reagents being significant, the model compound (XII) was studied. This olefin was readily cleaved to acetaldehyde by ozone, added osmium tetroxide rapidly, and could be hydrogenated. As rings A in structures (IIa) and (X) are too remote to influence the methylpropenyl side-chain, it now follows that it is the chlorine atom of ring B which renders the double bond inert. This effect is probably not merely steric in origin, since chlorine and methyl are held to be of similar size,[8] but is probably electronic. In structure (IIa) for nidulin, but not in structure (X), the chlorine atom is well placed to exert

[5] Murphy and Nolan, *Sci. Proc. Roy. Dublin. Soc.*, 1941, **22**, 315, and earlier papers.
[6] Keane and Nolan, *Sci. Proc. Roy. Dublin Soc.*, 1940, **22**, 199.
[7] Hayashi, *J. Pharm. Soc. Japan*, 1937, **57**, 112; Asahina and Shibata, *Ber.*, 1939, **72**, 1388.
[8] de la Mare, in " Progress in Stereochemistry," ed. Klyne, Butterworths Scientific Publications, London, Vol. I, 1954.

electronic effects both directly through space and indirectly through the aromatic ring, so we prefer the former structure. Moreover, only structure (IIa) possesses methoxyl placed in ring B as in other depsidones and at a position which conforms with the positive colour tests for resorcinol nuclei given by nornidulin.

The isomerisation of *O*-methylnidulin by nitric acid can now be interpreted as geometrical isomerisation in a structure (IIa), giving (IIb), which we regard as the structure of *O*-methylisonidulin. This transformation is radical-catalysed, for it cannot be induced by hydrochloric acid or by nitric acid when urea is present to destroy oxides of nitrogen. That both *O*-methylnidulin and *O*-methylisonidulin give the same adduct with dinitrogen tetroxide is doubtless due to the same phenomenon, and so is the formation of small amounts of *O*-methylisonidulin when *O*-methylnidulin is treated with *N*-bromosuccinimide or with limited quantities of bromine. With an excess of bromine, *O*-methylnidulin gives what is considered to be bromo-*O*-methylisonidulin because it has infrared absorption bands characteristic of the spectrum of *O*-methylisonidulin and because it can also be obtained by brominating the last compound. With sulphuryl chloride, *O*-methylnidulin was converted into an alkyl halide which reacted promptly with silver ions and on hydrogenation regenerated *O*-methylnidulin in low yield: the halide is not a single substance, however, for the nuclear magnetic resonance spectrum shows it to be an equimolecular mixture of chloro-*O*-methylnidulin and chloro-*O*-methylisonidulin, geometrical isomers with the side-chain (XV).

The bromo-compound should probably be formulated similarly but has not been closely examined.

Allylic activity in highly hindered olefins is, of course, well-known in other fields (notably in steroids and triterpenes) and enables the low and erratic *C*-methyl values given by nidulin to be attributed to the ease with which the methyl groups of the methylpropenyl side-chain are oxidised. This view is supported by the higher values (2·3 and 3·3 respectively) obtained from methyl *O*-methylnidulinate (III) and decarbonidulin;[1] here ring B is an unprotected phenolic nucleus and therefore more susceptible to oxidation and thus gives values nearer to the theoretical.

Scale models show that in a structure (IIa) the 1-methylpropenyl side-chain is almost rigidly held in a plane at right-angles to that of ring B, so there can be little conjugation between the two and it is not surprising that there is great similarity in the ultraviolet spectra (Fig. 2) of *O*-methylnidulin, *O*-methylisonidulin, and their adduct with dinitrogen tetroxide.

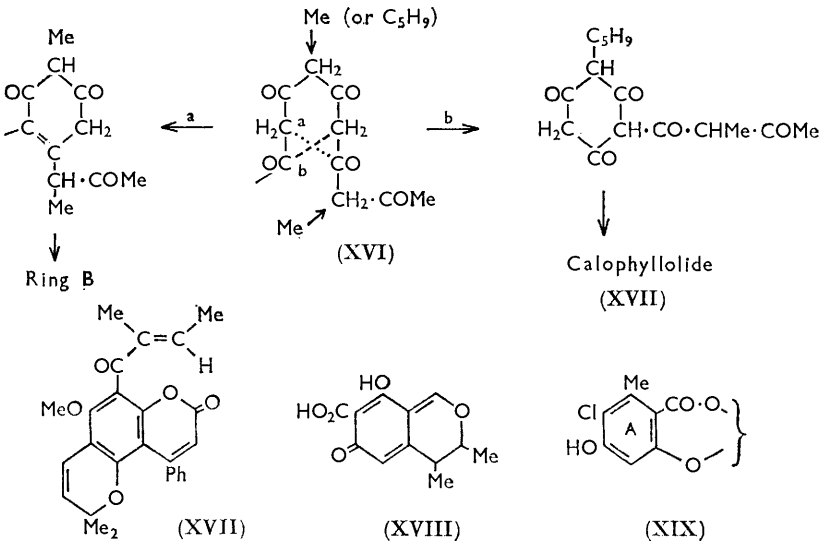
There is, however, a striking difference between the infrared spectra of methyl *O*-methylnidulinate and *O*-methylisonidulinate. Both these compounds (III) behave as phenols ($\nu_{max.}$ 3570 cm.$^{-1}$) and as methyl benzoates ($\nu_{max.}$ 1736 cm.$^{-1}$), yet the isonidulinate has additional, but weaker, broader bands at 2390 and 1712 cm.$^{-1}$. These bands persist in the solid state and in dilute solution in carbon tetrachloride. In the same solvent, both *O*-methylnidulin and *O*-methylisonidulin show only a single band, at 1754 cm.$^{-1}$, appropriate to phenyl benzoates. These observations suggest that in the isonidulinate, but not in the nidulinate, there is some intramolecular hydrogen-bonding between the hydroxyl and the ester groups, and that this is connected in some way with the geometrical configuration of the C_4 side-chain. The most probable connection is through restriction of rotation of ring A with respect to ring B in the ether (III), and this provides another reason for locating the C_4 side-chain at position 6 in (III).

Ring B in nidulin (II) is of a type new to depsidones, but it conforms to the acetate rule[9] as indicated in (XVI). Viewed in this light, ring B is less novel since calophyllolide[10] (XVII) can be regarded as a close relative, one in which the aldol condensation (a) leading to ring B is replaced by an acylation (b) leading to the phloroglucinol system. In calophyllolide, however, one of the "extra" methyl groups of nidulin is replaced by an

[9] Birch, Ryan, and Smith, *J.*, 1958, 4773, and other papers in this series.
[10] Polonsky, *Compt. rend.*, 1956, **242**, 2961.

isoprenoid substituent. There are also general similarities to the fungal metabolite citrinin [11] (XVIII).

Nornidulin [1] can now be formulated as (IIa; OH for OMe), and dechloronornidulin [12]



as the analogue with (XIX) for ring A. It is possible to isomerise the dimethyl ether of dechloronornidulin with nitric acid, so this compound also has the *cis*-configuration in its C_4 side-chain.

Experimental

O-*Methylnidulin Oxide*.—The reaction between perbenzoic acid and *O*-methylnidulin in chloroform was so slow that no accurate estimation of the oxygen uptake could be made, and no pure material could be isolated after 14 days.

Oxygen, containing ~3% of ozone, was slowly bubbled through *O*-methylnidulin (0·5 g.) in methylene dichloride (25 ml.) at −78°. The blue solution was kept at −78° for some hours, and after the solvent had been removed at room temperature and under reduced pressure a gum remained which crystallised on the addition of methanol. Repeated recrystallisation from aqueous methanol finally supplied O-*methylnidulin oxide* in rods (61 mg.), m. p. 144—145° (Found: C, 53·0; H, 4·2; Cl, 22·2. $C_{21}H_{19}Cl_3O_6$ requires C, 53·2; H, 4·0; Cl, 22·5%).

O-*Methylisonidulin* (IIb).—As described earlier,[1] the reaction between nitric acid and *O*-methylnidulin gave material melting at 168°. Fractional crystallisation of this from acetic acid afforded a little *O*-methylnidulin and much O-*methylisonidulin*, which formed prisms, m. p. 174° [Found: C, 55·0, 55·1; H, 4·0, 4·2; Cl, 23·1; OMe, 13·3. $C_{19}H_{13}Cl_3O_3(OMe)_2$ requires C, 55·1; H, 4·2; Cl, 23·3; OMe, 13·6%]. The infrared spectrum of a sample of m. p. 174° was but slightly different from that of a sample of m. p. 168°. When *O*-methylnidulin (100 mg.) in acetic acid (5 ml.) containing concentrated hydrochloric acid (1 ml.) was kept at 100° for ½ hr. and the hot solution diluted with water until crystallisation started, *O*-methylnidulin (40 mg.), m. p. and mixed m. p. 144°, separated. Dilution of the mother-liquors afforded a gum which failed to crystallise and was completely soluble in aqueous sodium hydrogen carbonate. Search failed to reveal any *O*-methylisonidulin.

O-*Methylisonidulinic Acid*.—A solution of *O*-methylisonidulin (0·1 g.) in dioxan (10 ml.) and 2N-aqueous sodium hydroxide was kept at 100° until dilution with water no longer produced a precipitate. Liberated by the addition of 2N-sulphuric acid, O-*methylisonidulinic acid*

[11] Birch, Fitton, Pride, Ryan, Smith, and Whalley, *J.*, 1958, 4576.
[12] Dean, Erni, and Robertson, *J.*, 1956, 3545.

separated from benzene–light petroleum (b. p. 60—80°) in plates, m. p. 138° [Found: C, 52·7; H, 4·6; Cl, 22·5; OMe, 12·5. $C_{19}H_{15}Cl_3O_4(OMe)_2$ requires C, 53·0; H, 4·4; Cl, 22·4; OMe, 13·0%].

O-Methylisonidulin (0·23 g.) was added to a solution from sodium (0·012 g.) in methanol (20 ml.) and kept at the b. p. for 2 hr. The base was neutralised by a stream of carbon dioxide and the solvent was removed under reduced pressure leaving a solid which, when purified from aqueous methanol, yielded *methyl* O-*methylisonidulinate* in prisms, m. p. 142° (Found: C, 53·8; H, 4·5; Cl, 21·9. $C_{22}H_{23}Cl_3O_6$ requires C, 53·9; H, 4·7; Cl, 21·8%).

Dihydro-O-*methyldinitronidulin*.—(*a*) To *O*-methylisonidulin (220 mg.) in ethyl acetate (8 ml.) was added an ethereal solution of dinitrogen tetroxide until a permanent orange tint appeared. After ½ hr., the solvent and the excess of reagent were removed *in vacuo* and the residue was treated with methanol to induce crystallisation. Recrystallised from acetic acid, this product afforded *dihydro*-O-*methyldinitronidulin* in prisms (109 mg.), m. p. ~198° (decomp.), $\nu_{max.}$ 3070 (CH in $>$CH·NO$_2$), 1739 (lactone), and 1558 cm.$^{-1}$ ($\geqslant$C·NO$_2$) [Found: C, 46·0; H, 3·4; N, 4·8; OMe, 11·7. $C_{19}H_{13}Cl_3N_2O_7(OMe)_2$ requires C, 45·8; H, 3·5; N, 5·1; OMe, 11·3%]. At its m. p., this compound evolved oxides of nitrogen. (*b*) Dinitrogen tetroxide (1 ml.) was added to *O*-methylnidulin (0·25 g.) in acetic acid (10 ml.). ½ Hr. later, water was added, giving a solid which was thoroughly washed with water, dried in air, and shaken with ether. The insoluble portion was purified from acetic acid, giving dihydro-*O*-methyldinitronidulin in prisms (93 mg.), m. p. 198° (decomp.), further identified with a specimen prepared as in (*a*) by means of its infrared spectrum.

The ether-soluble portion was recovered, taken up in benzene, and chromatographed on a silica column (1 × 15 cm.). The first two bands eluted contained no identifiable material. The third contained what appeared to be a *mononitro-compound*. This separated from methanol in needles (33 mg.), m. p. 162°, $\nu_{max.}$ 1736, 1553 cm.$^{-1}$ [Found: C, 50·1; H, 3·7; Cl, 21·1; N, 2·8; OMe, 12·5. $C_{19}H_{12}Cl_3NO_5(OMe)_2$ requires C, 50·1; H, 3·6; Cl, 21·2; N, 2·8; OMe, 12·3%]. The ultraviolet spectrum was almost identical with that of the dinitro-compound.

The fourth band supplied *O*-methylisonidulin, m. p. and mixed m. p. 173°. The fifth band supplied *O*-methylisonidulin oxide, m. p. and mixed m. p. 200°. Finally, a little dihydro-*O*-methyldinitronidulin was eluted.

Halogenation.—(a) *Sulphuryl chloride*. To *O*-methylnidulin (0·5 g.) in acetic acid (15 ml.) was added freshly distilled sulphuryl chloride (1 ml.) and a trace of benzoyl peroxide. Next day, dilution with water precipitated a solid which was dissolved in ether, washed with aqueous sodium hydrogen carbonate, then with water, dried (Na_2SO_4), and recovered by evaporation. The residue was recrystallised several times from methanol, giving *chloro*-O-*methylnidulin* (as a mixture of geometrical isomers) forming needles (0·21 g.), m. p. 132—133°, $\nu_{max.}$ 1754 cm.$^{-1}$ (mull or CCl_4 solution) (Found: C, 50·9; H, 3·6; O, 16·1; Cl, 28·5. $C_{21}H_{18}Cl_4O_5$ requires C, 51·2; H, 3·7; O, 16·3; Cl, 28·8%). This compound rapidly afforded silver chloride when treated with silver nitrate in acetic acid, and gave chloride ion when treated with aqueous sodium hydroxide.

Chloro-*O*-methylnidulin (0·3 g.) in methanol (20 ml.) was shaken under hydrogen with 5% palladium–calcium carbonate (0·1 g.) until absorption ceased. The catalyst was removed by filtration and the filtrate was concentrated to a small bulk. The material which separated was recrystallised from methanol and then acetic acid, giving *O*-methylnidulin in needles (0·11 g.), m. p. and mixed m. p. 146°.

(b) *Bromine*. Interaction of *O*-methylnidulin (1 g.) in acetic acid (20 ml.) with an excess of bromine (1 ml.) in acetic acid (5 ml.) in sunlight was stopped after 5 min. by dilution with water. Repeatedly crystallised from methanol, the gum produced afforded *bromo*-O-*methylisonidulin* in long needles (0·25 g.), m. p. 162° (Found: C, 46·7; H, 3·6; Hal, 28·2. $C_{21}H_{18}BrCl_3O_5$ requires C, 47·0; H, 3·4; Hal, 28·6%). This compound was not affected by piperidine at 100° for 5 hr., but reacted at once with silver nitrate in acetic acid to give silver bromide. The same bromo-compound, m. p. and mixed m. p. 162°, resulted when *O*-methylnidulin was replaced by *O*-methylisonidulin.

Interaction of *O*-methylnidulin (1 g.) in acetic acid with a limited amount (0·05 ml.) of bromine also led to a gum. This gum, in benzene, was chromatographed on a silica column, (2 × 20 cm.), and the eluate was collected in 5 ml. fractions. The first fractions contained *O*-methylnidulin, but later ones contained *O*-methylisonidulin which separated from acetic acid in prisms (32 mg.), m. p. and mixed m. p. 173°.

(c) N-*Bromosuccinimide*. *O*-Methylisonidulin was unaffected when heated with *N*-bromosuccinimide in carbon tetrachloride for 4 hr. After a week in these conditions *O*-methylisonidulin was the only product recoverable.

O-Methylnidulin (0·29 g.) and *N*-bromosuccinimide (0·12 g.) were heated together in boiling carbon tetrachloride (20 ml.) for 4 hr. The succinimide which separated was removed and the filtrate evaporated to dryness. The gummy residue, in benzene, was chromatographed on a silica column, but the only crystalline fractions consisted of *O*-methylisonidulin (16 mg.), m. p. and mixed m. p. 173°.

Alkylation Studies on Methyl O-*Methylnidulinate*.—(a) *Allyl bromide*. Interaction of methyl *O*-methylnidulinate (0·5 g.), allyl bromide (0·2 ml.), and potassium carbonate (2 g.) in boiling acetone was complete in 4·5 hr. The filtered solution was evaporated, and the residue, in ether, was washed with 2N-sodium hydroxide and then with water, dried (Na_2SO_4), and regained by evaporation of the solvent. The oil produced crystallised in contact with light petroleum (b. p. 40—60°) and when recrystallised from this solvent gave *methyl* O-*allyl*-O-*methylnidulinate* in massive prisms (0·23 g.), m. p. 93—95° (Found: C, 56·9; H, 5·3. $C_{25}H_{27}Cl_3O_6$ requires C, 56·7; H, 5·1%). This ether (0·15 g.) was kept in dimethylaniline (10 ml.) and acetic anhydride (1 ml.) at 170° under nitrogen for 5 hr. but most (0·09 g.) of it was recovered unchanged.

(b) *Diazomethane*. To methyl *O*-methylnidulinate (or methyl nornidulinate) (0·1 g.) in ether (30 ml.) was added a large excess of ethereal diazomethane. After 4 days at 0°, volatile material was removed *in vacuo* and the crystalline residue (0·1 g.) was purified from methanol, giving methyl *O*-methylnidulinate in needles, m. p. and mixed m. p. 161°. No non-phenolic material could be detected in the crude product. Treated similarly, nornidulin (0·1 g.) afforded *O*-methylnidulin (0·083 g.), which, when crystallised from alcohol, formed rods, m. p. and mixed m. p. 145°; and decarbonidulin (0·15 g.) afforded *O*-methyldecarbonidulin as prisms (0·12 g.), m. p. and mixed m. p. 134°, after recrystallisation from light petroleum (b. p. 60—80°).

Attempted Conversion of Nidulin Derivatives into Xanthones.—(a) *Aluminium chloride*. Powdered aluminium chloride (2·8 g.) was added to a solution of nidulin (2·2 g.) in benzene (40 ml.) at the b. p. After 10 min. a red-brown complex began to separate: after 6·5 hr. it was collected and decomposed with ice-cold dilute hydrochloric acid. The product, in benzene, was extracted into 2N-sodium hydrogen carbonate (4 × 25 ml.) and recovered therefrom by acidification, forming a cream-coloured powder (2·2 g.). This powder did not crystallise, so it was dissolved in ether (250 ml.) and kept with diazomethane for one day at 0°. Obtained by evaporation of the solvent, the oily product was chromatographed from benzene on alumina. One fraction afforded a gummy solid which, when purified by recrystallisation from methanol, gave a *substance* as lemon-yellow rhombs (20 mg.), m. p. 165—167° [Found: C, 54·0; H, 5·3; OMe, 38·0%; *M* (Rast) 470], soluble in 2N-sodium hydroxide but not in aqueous sodium hydrogen carbonate, and giving no ferric reaction. Its infrared spectrum ($\nu_{max.}$ 1732, 1672, 1612, and 1586 cm.$^{-1}$) could not be reconciled with a xanthone-type formulation, and no colour test for xanthones or hydroxyxanthones was positive.

(b) *Oxalyl chloride*. *O*-Methylnidulinic acid (0·12 g.) was heated for 1 hr. on the steam-bath with oxalyl chloride (1 ml.) in chloroform (10 ml.). Isolated by means of aqueous sodium hydrogen carbonate, the acidic fraction yielded *O*-methylnidulinic acid, m. p. and mixed m. p. 185°. The neutral fraction crystallised from methanol, to give *O*-methylnidulin in needles, m. p. and mixed m. p. 148°. No other product was detected. Similarly, nidulinic acid afforded a mixture of nidulinic acid and nidulin.

Oxidative Degradation of Methyl O-*Methylnidulinate*.—(a) *Nitric acid*. Nitric acid (*d* 1·4; 0·68 ml.) was added to methyl *O*-methylnidulinate (1·8 g.) in propionic acid (140 ml.) at 0° ± 1°. After 20 min., the cherry-red solution was diluted with water (700 ml.) and extracted with ether (3 × 300 ml.). The combined ethereal solutions were washed with water and aqueous sodium hydrogen carbonate, and then extracted with 2N-sodium hydroxide until the alkaline layer became purple. Acidification of the alkaline extract then yielded methyl 3,5-dichloroeverninate (IV) which separated from 90% methanol in feathery needles (0·8 g.), m. p. and mixed m. p. 82—83°. The ethereal solution was dried (Na_2SO_4) and evaporated to a red oil (0·8 g.) which, when kept at 100°/17 mm., gave an orange sublimate (70 mg.). Resublimation followed by recrystallisation from light petroleum (b. p. 40—60°) furnished 2-*chloro*-5-*hydroxy*-6-*methyl*-3-1′-*methylpropenyl*-1,4-*benzoquinone* (VII) in yellow, pyramid-tipped prisms (42 mg.), m. p. 141—142° (Found: C, 58·4; H, 5·0; Cl, 15·4; OMe, 0. $C_{11}H_{11}ClO_3$ requires C, 58·3;

H, 4·9; Cl, 15·7%). This compound had $\lambda_{max.}$ (in hexane) 274, 280, 330, 404 (infl.) mμ (log ε 4·22, 4·25, 3·42, 2·53), and $\nu_{max.}$ (KCl) 3390 (OH), 1650 (C:O), 1600 (C:C), or (in CCl_4) 3390, 1650, 1586 cm.$^{-1}$. It gave an intensely violet solution in dilute aqueous sodium hydroxide, but only a yellow solution in concentrated sulphuric acid. An alcoholic solution became cherry-red when ferric chloride was added, and magenta slowly becoming ultramarine when diethyl malonate and ammonia were added. Attempts to methylate the compound gave intractable gums.

(b) *Lead tetra-acetate.* To methyl *O*-methylnidulinate (1·8 g.) in acetic acid (140 ml.) was added lead tetra-acetate (1·5 g.). The mixture rapidly reddened, and after 24 hr. at 0° tests for quadrivalent lead were negative. Ether (150 ml.) and water (100 ml.) were added, and the organic layer was washed with water, dried (Na_2SO_4), and evaporated to a gum. When this was chromatographed on silica (2 × 40 cm.) from benzene–light petroleum (b. p. 40—60°) (1 : 1), methyl dichloroeverninate (0·7 g.), m. p. and mixed m. p. 82—83°, was eluted first. Only traces of the chloroquinone were detected in later eluates.

(c) *Periodic acid.* To methyl *O*-methylnidulinate (1·0 g.) in acetic acid (120 ml.) was added sodium metaperiodate (1 g.) in 60% acetic acid (120 ml.). When kept in the dark, the mixture became red and 12 hr. later was diluted with much water and extracted with ether. The ethereal solution was washed with water (6 × 200 ml.) and then shaken with portions of aqueous sodium hydrogen carbonate until the extracts were no longer coloured. The combined purple extracts were acidified with 2N-hydrochloric acid, and the semi-crystalline product was collected into ether, washed with water, dried ($MgSO_4$), and recovered by evaporation. The residue was chromatographed from benzene–light petroleum (1 : 1) on silica. The eluate contained methyl dichloroeverninate (0·34 g.), m. p. and mixed m. p. 82—83°. The column was then developed with benzene–light petroleum (2 : 1), whereupon a yellow band appeared. The corresponding eluate, when evaporated, left a yellow gum which, when purified from light petroleum (b. p. 40—60°), afforded the chloroquinone in prisms (0·16 g.), m. p. and mixed m. p. 141—142°, further identified with a specimen prepared as in (a) by its infrared absorption spectrum.

Degradation of the Chloroquinone to Tiglic Acid (with J. G. Underwood).—The chloroquinone (0·1 g.) was treated in N-sodium hydroxide (4·4 ml.) with 2·6% aqueous hydrogen peroxide (17·3 ml.). The deep purple colour of the mixture faded to straw-yellow in 20 min. 2 Days later, acidification with concentrated hydrochloric acid (0·7 ml.) followed by extraction with ether (25 + 2 × 20 ml.) removed organic material. The combined extracts, having been washed with water (5 ml.), dried (Na_2SO_4), and evaporated, gave a residue which was kept *in vacuo* over potassium hydroxide for 2 hr. The remaining gum (*ca.* 20 mg.) at 60°/0·4 mm. afforded an acidic sublimate (*ca.* 3 mg.) consisting of stout prisms. Resublimed (55°/0·3 mm.), this product yielded prisms (1·5 mg.), m. p. 61·5—63·5° (Kofler block) or m. p. 62—64° after admixture with authentic tiglic acid of m. p. 63—64°, and having $\lambda_{max.}$ (in H_2O) 218 mμ (ε 10,860) [lit.,[13] $\lambda_{max.}$ 216—217 mμ (ε 10,700)]. In a second run the chloroquinone (0·5 g.) was oxidised by alkaline 6% hydrogen peroxide for 4 days: the tiglic acid produced (4·5 mg.) was converted into the 4-bromophenacyl ester, m. p. 67°, not depressed on admixture with an authentic specimen.

Degradation of Nidulin to the Dihydroxyquinone (VII; OH for Cl).—Nidulin (0·9 g.), acetic acid (15 ml.), and hydriodic acid (b. p. 126°; 9 ml.) were heated under reflux, in the dark, for 5 hr. Water (9 ml.) was added, and the total volume was reduced to 6 ml. by evaporation *in vacuo*. Dilution with water then precipitated a brown gum which was dissolved in ether, washed with aqueous sodium hydrogen sulphite and then with water, dried (Na_2SO_4), and recovered by evaporation. A slow stream of air (free from carbon dioxide) was passed through a solution of 0·25 g. of the resulting crude resin in 2·5% methanolic potassium hydroxide (20 ml.) at the reflux temperature. After 4 hr. the methanol was removed under reduced pressure. Addition of water (5 ml.) and acidification with 2N-hydrochloric acid afforded a solid which was collected at the pump, washed with water, dried in air, and subjected to fractional sublimation at 120°/0·05 mm. The main fraction formed orange crystals (7 mg.) which, when purified from light petroleum (b. p. 60—80°), gave *2,5-dihydroxy-3-methyl-6-1′-methylpropenyl-1,4-benzoquinone* in dull-red, slender prisms softening at 182° and melting at 198° (rapid heating) (Found: C, 63·5, 63·2; H, 6·2, 5·9; Cl, 0. $C_{11}H_{12}O_4$ requires C, 63·5; H, 5·8%). This compound gave a blue-violet solution in dilute aqueous alkali and a red-brown ferric reaction in

[13] Bueding, *J. Biol. Chem.*, 1953, **202**, 510.

alcohol, and had $\lambda_{max.}$ (in EtOH) 287 and 436 mμ (ε 17,300, 249). 2,5-Dihydroxy-3-methyl-1,4-benzoquinone [14] has $\lambda_{max.}$ (in EtOH) 288 and 410 mμ (ε 20,600, 279).

The same quinone was obtained when nidulin was attacked by hydrogen bromide and red phosphorus in acetic acid before being subjected to aerial oxidation in alkaline solution.

Degradation of Nidulin and O-*Methylisonidulin to Ethylmethylmalonic Acid.*—Treatment of nidulin (0·9 g.) with hydriodic acid gave a yellow resin as described for the foregoing experiment. This resin was powdered, suspended in 64% sulphuric acid (60 ml.), and oxidised by being vigorously shaken at <20° (water-cooling) with a solution of sodium dichromate dihydrate (4·5 g.) in water (15 ml.), added portionwise. After 16 days with occasional shaking, the mixture no longer contained chromic acid and was filtered through glass wool and partially neutralised by the careful addition of 14% aqueous sodium hydroxide (210 ml.), the temperature being kept below 20°. A brown oil could then be isolated with ether, and when freed from acetic acid by being kept over potassium hydroxide in an evacuated vessel it formed a wax which was partially purified by being pressed on filter-paper to remove oil. When then crystallised from benzene–light petroleum (b. p. 60—80°) the product formed a colourless powder, m. p. 102—106°. Sublimation at 87°/0·1 mm. afforded prisms, m. p. 118—120°, which were washed with benzene and then with light petroleum (b. p. 60—80°). Resublimed, these gave ethylmethylmalonic acid in prisms (12 mg.), m. p. 122—123° [Found: C, 49·1; H, 6·8%; equiv. (titration), 75·1. Calc. for $C_4H_8(CO_2H)_2$: C, 49·3; H, 6·9%; equiv. 73·1], which showed chromatographic behaviour [15] identical with that of authentic material; R_F on Whatman No. 1 paper were (*a*) 0·26 in alcohol (80)–water (16)–ammonia (*d* 0·88, 4), (*b*) 0·61 in alcohol (80)–water (8)–pyridine (12), and (*c*) 0·53 in alcohol (80)–water (16)–morpholine (4).

Similarly, *O*-methylisonidulin (0·2 g.) afforded ethylmethylmalonic acid (1·5 mg.), m. p. and mixed m. p. 121—122°. When the treatment with hydriodic acid was omitted, ethylmethylmalonic acid could not be detected amongst the oxidation products from either *O*-methylisonidulin or nidulinic acid.

*Dechlorodi-*O-*methylnorisonidulin.*—Dechlorodi-*O*-methylnornidulin (0·2 g.) was heated at 80° in acetic acid (3 ml.) containing nitric acid (*d* 1·4; 0·2 ml.) for 4 min. Precipitated by the addition of water, the product was crystallised twice from methanol and then from 80% acetic acid, giving *dechlorodi-*O-*methylnorisonidulin* in thin prisms (93 mg.), m. p. 139·5—140·5°, $\lambda_{max.}$ 223, 260 mμ (log ε 4·63, 4·00) [Found: C, 59·3; H, 4·9; OMe, 15·0. $C_{19}H_{14}Cl_2O_3(OMe)_2$ requires C, 59·6; H, 4·7; OMe, 14·7%].

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