# *The Chemistry of Fungi. Part XXII.* Nidulin and Nornidulin ("Ustin"): Chlorine-containing Metabolic Products of* Aspergillus nidulans.

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The isolation of nidulin and nornidulin ("ustin") from a strain of *Aspergillus nidulans* is described and the empirical formula previously allocated to nornidulin has been confirmed. Nidulin has been shown to be a methyl ether of nornidulin, and the compounds are considered to be chlorinated depsidones. Partial structural formulæ for nidulin and nornidulin are proposed.

FROM the metabolic liquor of a mould, which had been previously isolated and examined by Kurung (*Science*, 1945, **102**, 11) who considered it to be a strain of *Aspergillus ustus* (Bain.) Thom and Church, Hogeboom and Craig (*J. Biol. Chem.*, 1946, **162**, 363) isolated, by a counter-current technique, two crystalline products named compound I, m. p. 185—187°, and compound II, m. p. 214—216°, which contained covalent chlorine and were formulated as $C_{21}H_{17}O_6Cl_3$ and $C_{21}H_{18}O_6Cl_2$ respectively. By a simpler method Doering, Dubos, and Noyce (*J. Amer. Chem. Soc.*, 1946, **68**, 725) obtained compound I from the mycelium and metabolic liquors of the same mould and, concluding that this was the major constituent, gave it the name "ustin" and the empirical formula $C_{19}H_{15}O_5Cl_3$; the compound was characterised by the formation of a mono-, m. p. 174°, and a di-methyl ether, m. p. 147°.

It has been reported already that this mould is, in fact, a non-ascosporic strain of *A. nidulans*, and that, when grown under the same or somewhat different conditions from those used by previous workers, the main metabolic product is a compound, m. p. 180°, for which the name nidulin was proposed (Dean, Robertson, Roberts, and Raper, *Nature*, 1953, **172**, 344). Further, nidulin was found to be an *O*-methyl ether of "ustin" and it was suggested that "ustin" should be re-named nornidulin.† In the present paper partial structures for nidulin and nornidulin are developed.

Cultivation of *A. nidulans* at a relatively low temperature for 4—5 weeks yielded a mycelium, which occasionally contained appreciable quantities of nidulin, and a metabolic liquor, from which a small amount of nornidulin was isolated by the method of Doering *et al.* (*loc. cit.*). Thus obtained, nidulin is a colourless, optically inactive compound, m. p. 180°, $C_{20}H_{17}O_5Cl_3$ containing a methoxyl and a hydroxyl group; it readily yields a monoacetate and a monomethyl derivative. The compound, which has a negative ferric reaction, does not contain a reducing or a carbonyl group. With nidulin the Kuhn–Roth determination of *C*-methyl groups was invalidated by formation of hydrogen chloride on distillation with the acetic acid.

Crystalline nidulin was insoluble in aqueous sodium hydroxide, apparently owing to a surface effect since the amorphous or finely divided compound (formed by pouring an ethanolic solution of the compound into water) was readily soluble in aqueous sodium hydroxide or in aqueous sodium hydrogen carbonate. In cold aqueous alcohol, nidulin behaved on titration as a monobasic acid, a property attributed to the presence of an acidic hydroxyl group rather than to a carboxyl group because, under the same conditions, *O*-acetylnidulin was neutral. The behaviour of nidulin as a dibasic acid on more vigorous treatment with alkali, and the hydrolysis of nidulin to nidulinic acid ($C_{20}H_{19}O_6Cl_3$) without loss of carbon, disclosed a lactone system, the presence of which was confirmed by (*a*) the formation of a hydroxy-ester, methyl nidulinate ($C_{21}H_{21}O_6Cl_3$), on treatment of nidulin with methanolic

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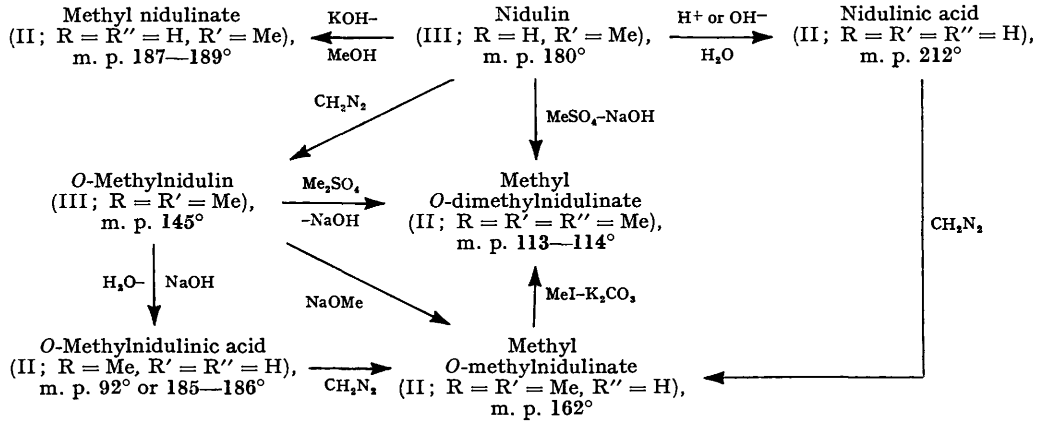
* Part XXI, *J.*, 1953, 2434.

† The name "ustin" has priority; but in view of Dr. Raper's revised classification (personal communication and Dean *et al.*, *loc. cit.*) of the mould producing this compound [not produced by authentic *Aspergillus ustus* (Bain.) Thom and Church, unpublished work in this laboratory] and to avoid further confusion we regard the proposed changes as essential.

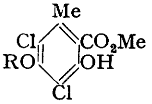
potassium hydroxide, and (*b*) the production of nidulinic acid and decarbonidulin ($C_{19}H_{19}O_4Cl_3$), a dihydric phenol, when the compound is boiled with excess of sodium hydroxide in aqueous dioxan. Nidulin also behaved as a lactone in reacting with hydroxylamine to form a compound $C_{20}H_{20}O_6NCl_3$, which did not give the expected ferric reaction typical of hydroxamic acids. However, it may well be that the bulk of the rest of the molecule prevents the formation of the hexacovalent iron complex necessary for the production of the colour.

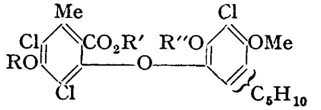
The relations between nidulin, nidulinic acid, and various methylated derivatives are summarised in the annexed scheme.



The chlorine atoms of nidulin are stable to hydrolysis with aqueous or alcoholic sodium hydroxide, to hydrogenolysis over palladium or platinum catalysts, and to the action of Raney nickel alloy and hot alkali according to the method of Schwenk, Papa, and Ginsberg (*J. Org. Chem.*, 1944, **9**, 1). Of the five oxygen atoms in the nidulin molecule, the presence of a lactone, a methoxyl, and a hydroxyl group accounts for four. Further, it seems reasonable to assume that the inert fifth oxygen atom is present in an ether system. Accordingly, from its composition and properties in conjunction with the occurrence of known chlorine-containing depsidones in lichens it seemed probable that nidulin was a depsidone. This assumption is strongly supported by the isolation of methyl 4 : 6-dichloro-*o*-orsellinate (I; R = H) and of methyl 4 : 6-dichloroeverninate (I; R = Me) as degradation products respectively of methyl nidulinate (II; R = R″ = H; R′ = Me) and methyl *O*-methylnidulinate (II; R = R′ = Me; R″ = H). Both degradations were carried out by a sequence of chlorination, reduction, and methanolysis, devised by Nolan, McCann, Manahan, and Nolan (*Sci. Proc. Roy. Dublin Soc.*, 1948, **24**, 319; cf. Zincke and Schurmann, *Annalen*, 1918, **417**, 236) for the fission of diploicin (IV). Further, the remarkably easy fission of methyl *O*-methylnidulinate by nitric acid to give a high yield of methyl dichloroeverninate (I; R = Me) also supported this hypothesis and served to establish the nature and orientation of the substituents of ring A of nidulin (III; R = H; R′ = Me).

The proposed depsidone structure implies the presence of a second aromatic ring system (ring B) and, although we have not been able to effect a scission of the compound to give the ring-B fragment, the following observations are most simply explained on the view that residue B contains an aromatic system. (*a*) All three chlorine atoms are remarkably stable and, since two have been shown to be in ring A, the third must be attached, presumably, to a second aromatic ring. (*b*) *O*-Methylnidulin (III; R = R′ = Me) reacted smoothly with methanolic sodium methoxide, to give methyl *O*-methylnidulinate (II; R = R′ = Me, R″ = H) which appeared to be phenolic since it was insoluble in sodium hydrogen carbonate solution but dissolved readily in cold aqueous sodium hydroxide. (*c*) In the absence of an aromatic ring (other than ring A), nidulin would be highly unsaturated, whereas it resisted attempts at hydrogenation with Adams catalyst or Raney nickel and hydrogen at 60 lb./in.$^2$ (*d*) In the ultra-violet region nidulin has an inflexion at 323 mμ and a peak at 267 mμ which disappears on decarboxylation and is therefore associated with ring A. If the phenolic hydroxyl of ring B is freed by fission of the lactone ring, the inflexion is augmented to a peak at 288 mμ consistent with the presence of an aromatic system.


(I)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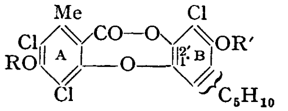
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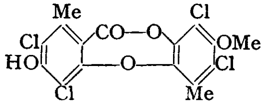
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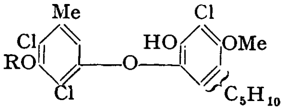
(V)

There remain to be allocated one methoxyl group, one chlorine atom, and a $C_5$ residue. The methoxyl group was placed in the 4′-position by analogy with other depsidones, an orientation confirmed by the positive bleaching-powder reaction of nornidulin (see below). Numerous attempts to isolate the $C_5$ residue as carbonyl compounds or as simple fatty acids by oxidation of nidulin were unsuccessful, although permanganate oxidation gave a minute quantity of an acid, m. p. 85°, which had a positive fluorescein reaction. None of the possible succinic acids has m. p. 85°, and a number of adipic acids and α-hydroxy-acids which have a similar m. p. were examined but were not identical with the degradation acid (see below). Therefore, in spite of the fact that analogy with known depsidones suggested *n*-pentyl at the 6′-position, we believe that the $C_5$ residue is present as a ring system attached at the 5′- and 6′-positions of ring B. The third chlorine atom is therefore placed at position 3′, with the resulting formula (III; R = H, R′ = Me) for nidulin.

In attempts to clarify the nature of the $C_5$ residue, it was found that nidulin is rapidly attacked by ozone but that *O*-methylnidulin is hardly affected by this reagent; in neither case could any significant product be identified. *O*-Methylnidulin is inert to lead tetra-acetate at 100°, indicating the absence of an active methylene group, whilst on treatment with concentrated nitric acid in acetic acid it affords, not a nitro-compound, but *O*-methyl-dehydronidulin by loss of two hydrogen atoms. Although the dehydro-derivative could not be hydrogenated and does not react with osmium tetroxide and pyridine, it forms an oxide with ozone or perbenzoic acid, suggesting the presence of a tetrasubstituted double bond, and hence the isoprene system $\cdot CHMe\cdot CHMe\cdot CH_2\cdot$ for the $C_5$ residue. This residue has two methine groups which would facilitate oxidation or nitration (cf. Hass and Riley, *Chem. Reviews*, 1943, **32**, 373) to give intermediates which could yield an olefin, thus accounting for the unusual reaction with nitric acid. The system also has two asymmetric carbon atoms; thus nidulin would be a racemate since it is optically inactive. It is not yet known whether nidulin or any derivative of it can be resolved.

Formula (III; R = H, R′ = Me) for nidulin leads to structure (V; R = H) for decarbonidulin which formed a neutral di-*p*-nitrobenzoate and possessed an acidic hydroxyl group responsible for its solubility in aqueous sodium hydrogen carbonate and for its rapid methylation by diazomethane to the phenol *O*-methyldecarbonidulin. A study of the increase in the dissociation constant of phenol caused by chlorine substituents led Tiessens (*Rec. Trav. chim.*, 1929, **48**, 1066) to state that " it is obvious that the presence of two chlorine atoms in *ortho*-positions to the hydroxyl has a predominating effect over all else." Accordingly, *O*-methyldecarbonidulin has been given formula (V; R = Me).

In agreement with Doering *et al.* (*loc. cit.*), we find that nornidulin has the formula $C_{19}H_{15}O_5Cl_3$. Complete methylation of this compound gives a dimethyl ether, m. p. 145° (Doering *et al.* give m. p. 147°), identical with *O*-methylnidulin, and hence the partial structure (III; R = R′ = H) for nornidulin (" ustin ") is proposed. Formed by hydration of nornidulin, nornidulinic acid gave a positive bleaching-powder reaction for a resorcinol derivative, thus confirming the position of the free hydroxyl of ring B of nornidulin with respect to the lactonic system. The monomethyl ether, m. p. 174°, mentioned by Doering *et al.*, is isomeric with nidulin and therefore has structure (III; R = Me, R′ = H).

It appears that nidulin is the first recorded depsidone to be identified as a metabolic product of a fungus grown on a synthetic medium, although compounds of this type are well known as constituents of lichens, and at least three (diploicin, gangaleoidin, and pannarin) contain chlorine (Nolan *et al., loc. cit.*; Davidson, Keane, and Nolan, *Sci. Proc. Roy. Dublin Soc.*, 1943, **23**, 143; Yosioka, *J. Pharm. Soc. Japan*, 1941, **61**, 332). The present discovery is of interest in considerations of phytochemical relations between fungi and lichens.

Succinic acid and mannitol have also been identified as metabolic products of *A. nidulans* (cf. Birkenshaw, Charles, Hetherington, and Raistrick, *Trans. Roy. Soc.*, 1931, *B*, **220**, 153).

The antibiotic properties of nidulin have already been reported elsewhere (Dean *et al., loc. cit.*; cf. Hogeboom and Craig, *loc. cit.*).

## EXPERIMENTAL

*Isolation of Nidulin.*—Stock cultures of *Aspergillus nidulans* (NRRL, No. 2006) (supplied by Dr. Raper) were kept on slopes of Czapek–Dox agar. Fifty flasks each containing 400 ml. of an aqueous solution of Czapek–Dox salts with glucose (4%) and " Marmite " (0·1%), at an initial pH 5·2, were inoculated with a suspension of mould spores in water obtained from a culture at least 15 days old. During 32 days in diffuse daylight at about 13° a rather thin mycelial felt developed, the top of which was green with numerous white or pale-brown spots, whilst the reverse was greyish-green. The substrate was brownish-green with a pH of about 8. Because yields were very variable the method of isolation had to be varied somewhat with different batches. The following account describes the isolation of nidulin from a mycelium which gave a comparatively good yield.

The washed, dried, and powdered mycelial felts (126 g.) from 49 flasks were extracted in Soxhlet apparatus with (*a*) light petroleum (b. p. 40—60°) for 25 hr., (*b*) ether (41 hr.), and (*c*) acetone (34 hr.). In 6 days at room temperature, the light petroleum extract deposited crude, semi-crystalline nidulin (7·3 g.). The brown residue (6·6 g.) left on evaporation of the ethereal extract partly crystallised during 14 days. The combined crystalline products from the two extracts were triturated with cold light petroleum (b. p. 40—60°) and then crystallised from 95% alcohol, giving impure nidulin (6·3 g.), m. p. 174—178°. Repetition of this process followed by crystallisation from light petroleum (b. p. 60—70°; 225 ml. for 1 g. solid) gave nidulin, m. p. 180°, unchanged on recrystallisation from 90% alcohol.

The light petroleum extract, from which crude nidulin had separated, contained fat (2·9 g.) and an intractable, acidic, brown gum (3·8 g.) which yielded positive Liebermann–Burchard and Salkowski reactions and a purple ferric reaction. In addition to nidulin, the ether contained fat (0·2 g.) and an amorphous acidic substance (2·3 g.) devoid of chlorine. On being cooled, the acetone extract yielded mannitol (1·5 g.) which, after being washed with light petroleum (b. p. 40—60°) and ether, crystallised from 90% alcohol (charcoal) in prisms, m. p. 164—168°, forming an acetate, m. p. and mixed m. p. 123—124°.

*Isolation of Nornidulin.*—The mould was grown on Czapek–Dox medium (25 l.) containing malt extract (0·2%) for 15 days at 28—30°. The filtered culture medium was exhaustively extracted with ether, and the extract was dried and evaporated, leaving a yellow gum (3 g.) which was dissolved in ether (200 ml.), washed with aqueous sodium hydrogen carbonate (3 × 50 ml.), and extracted with 2N-sodium carbonate (3 × 50 ml.). Liberated from this extract by 2N-sulphuric acid (200 ml.), the gummy product was washed with water and ground with a little 2N-sodium carbonate, giving a solid which was dissolved in 50% alcohol (20 ml.). Treatment of this with 2N-sulphuric acid (2 ml.) furnished crystalline nornidulin which separated from aqueous alcohol and then benzene–light petroleum (b. p. 60—80°) in hexagonal plates or prisms (0·6 g.), m. p. 185—186°, $\lambda_{max.}$ 266 mμ (ε 8120), inflection at 323 mμ (ε 746) (Found: C, 53·4; 53·2; H, 3·7, 3·7; Cl, 23·7; MeO, 0. Calc. for $C_{19}H_{15}O_5Cl_3$: C, 53·1; H, 3·5; Cl, 24·8%).

After being extracted with ether the culture liquor was strongly acidified with sulphuric acid and again extracted with ether. When concentrated, this extract deposited crystals which were isolated by decantation and recrystallised from ethyl acetate, giving succinic acid in prisms (0·85 g.), m. p. and mixed m. p. 190—191°, having a positive fluorescein reaction (Found: equiv., 59·3 by titration against 0·1N-sodium hydroxide. Calc. for $C_4H_6O_4$: equiv., 59·0).

*Nidulin.*—Nidulin crystallises from light petroleum (b. p. 60—70°) in colourless, slender, shining rods, and from alcohol (90%) in rhombs, m. p. 180°, $\lambda_{max.}$ 267 mμ (ε 9010), inflection at 323 mμ (ε 1200), having a negative ferric reaction [Found: C, 54·3, 54·0; H, 4·0, 4·0; Cl, 23·7, 23·4; OMe, 6·9%; *M* (micro-Rast), 463. $C_{19}H_{14}O_4Cl_3{\cdot}OMe$ requires C, 54·1; H, 3·9; Cl, 24·0; OMe, 7·0%; *M*, 443·7]. It is insoluble in water, easily soluble in chloroform, sparingly soluble in 95% alcohol, or benzene, and very sparingly soluble in hot light petroleum (b. p. 60—70°). A 4% solution of the compound in chloroform is optically inactive. Nidulin does not reduce Fehling's solution and does not react with aqueous-methanolic 2 : 4-dinitrophenylhydrazine sulphate.

Addition of water to a solution of nidulin in one equivalent of aqueous sodium hydroxide and cold 95% alcohol did not give a precipitate. Subsequent treatment of this with dilute mineral acid yielded unchanged nidulin, m. p. and mixed m. p. 180° after purification from alcohol.

A solution of nidulin (0·100 g.) in 95% alcohol was neutralised with 0·1N-sodium hydroxide (2·32 ml.) (phenolphthalein as an external indicator) (Calc. for 0·100 g. of a compound $C_{20}H_{17}O_5Cl_3$: 2·25 ml.). To this a further quantity of alkali (2·70 ml.) was added and the solution kept at room temperature overnight, heated under reflux on the steam-bath for 1 hr., cooled, diluted with alcohol (4 ml.), and titrated with standard acid to pH 8·3, with "fractional pH" paper as external indicator because the solution had become pink. The titration indicated the uptake of a further 2·28 ml. of 0·1N-alkali (Calc. for one ester or lactone group: 2·25 ml.). The reaction mixture did not contain chloride ions.

O-*Acetylnidulin* (III; R = Ac, R′ = Me).—A solution of nidulin (0·5 g.) in acetic anhydride (5 ml.) and pyridine (2·5 c.c.) was kept at room temperature for 12 days and poured on crushed ice (100 g.). Two hours later the precipitate (0·53 g.) was collected, washed with water, dried, and crystallised from 95% alcohol, giving O-*acetylnidulin* in plates or slender rods (0·46 g.), m. p. 167—168° (Found: C, 54·4; H, 3·8; Cl, 21·8. $C_{22}H_{19}O_6Cl_3$ requires C, 54·4; H, 3·9; Cl, 21·9%). This derivative was also prepared by acetylation with boiling acetic anhydride containing sodium acetate for 1·5 hr.

Powdered acetylnidulin (produced by pouring an alcoholic solution into water) is insoluble in aqueous sodium hydrogen carbonate but dissolves slowly in a boiling solution of sodium hydroxide.

O-*Methylnidulin* (III; R = R′ = Me).—(*a*) This *ether* was prepared almost quantitatively by treatment of nidulin with an excess of ethereal diazomethane; it separated from 95% methanol in shining, long rods, m. p. 145°, inflection at 298 mμ (ε 1725) [Found, C, 55·4; H, 4·0; Cl, 23·1; OMe, 13·1. Calc. for $C_{19}H_{13}O_3Cl_3(OMe)_2$: C, 55·1; H, 4·2; Cl, 23·3; OMe, 13·6%].

(*b*) A mixture of nidulin (0·44 g.), methyl iodide (1 c.c.), acetone (50 c.c.), and anhydrous potassium carbonate (2·0 g.) was heated under reflux for 6 hr. with the addition of more methyl iodide (0·5 c.c.) after 3 hr. The *O*-methylnidulin (0·2 g.) had m. p. and mixed m. p. 144—145°.

(*c*) By method (*b*), nornidulin (0·4 g.) furnished *O*-methylnidulin (0·35 g.), m. p. and mixed m. p. 144—145° (Found: C, 55·1; H, 4·2; Cl, 23·9; OMe, 13·8%) (cf. Doering *et al., loc. cit.*, who give m. p. 147°).

*O*-Methylnidulin was not appreciably soluble in boiling sodium hydroxide solution during 3 min.

*Reaction of Nidulin with Hydroxylamine.*—The pH of a solution of nidulin (1·0 g.), hydroxylamine hydrochloride (2·0 g.), and anhydrous sodium acetate (2·3 g.) in 95% alcohol (50 ml.) and water (25 ml.) was adjusted to 4·8—5·1 with 2N-sodium hydroxide (*ca.* 1·2 ml.). After being heated under reflux on the steam-bath for 4 hr., this mixture was cooled and poured into water (250 ml.), giving a precipitate which was washed free from chloride, dried, and freed from unchanged nidulin by extraction with ice-cold chloroform (6 ml.). Crystallised from aqueous alcohol, the grey, amorphous residue gave a *substance* in slender, colourless prisms (0·20 g.), m. p. 236° (decomp.), which had a negative ferric reaction in alcohol (Found: C, 50·4; H, 4·3; N, 3·1; Cl, 22·9. $C_{20}H_{20}O_6NCl_3$ requires C, 50·4; H, 4·2; N, 2·9; Cl, 22·3%). On being warmed a solution of this compound in acetone reduced Fehling's solution within 5 min.; a control experiment with nidulin did not show reduction.

*Nidulinic Acid* (II; R = R′ = R″ = H).—A mixture of nidulin (1·5 g.), alcohol (95%; 120 ml.), and 2N-sulphuric acid (180 ml.) was heated under reflux for 17 hr. After part of the alcohol (70 ml.) had been distilled an oil began to separate and the mixture was then kept at −2° overnight. The solid was collected, washed free from acid with water, dried, and crystallised from benzene, giving *nidulinic acid* in diamond-shaped plates (0·9 g.), m. p. 212° (decomp.), unchanged after a further purification from aqueous alcohol (Found: C, 52·1; H, 4·1; Cl, 22·8; OMe, 5·2. $C_{19}H_{16}O_5Cl_3{\cdot}OMe$ requires C, 51·9; H, 4·1; Cl, 23·0; OMe, 6·7%). Direct titration of this acid gave erratic results, possibly because of partial lactonisation, but the following method was satisfactory. 0·1N-Aqueous sodium hydroxide (6·12 ml.) was added to nidulinic acid (0·1122 g.) in alcohol (10 ml.) and water (10 ml.) and, after being kept in a stoppered flask overnight, the mixture required 0·1N-sulphuric acid (1·26 ml.) for neutralisation to pH 8·3 with pH paper as external indicator (Found: equiv., 231. $C_{20}H_{19}O_6Cl_3$ as a dibasic acid requires equiv., 230·9).

Nidulinic acid is soluble in sodium hydrogen carbonate solution (with slight effervescence), and in alcohol gives a pale-green ferric reaction which is transiently intensified by the addition of water, fading completely in 30 sec.

*Methyl Nidulinate* (II; R = R″ = H, R′ = Me).—A solution of nidulin (0·80 g.) in methanol (50 ml.) at 40° was treated with methanolic potassium hydroxide (5%; 15 ml.) and then boiled on the steam-bath for 1 min., cooled, diluted with water, and acidified with dilute hydrochloric acid. The copious white precipitate was well washed with water, dried, and crystallised from aqueous methanol, giving *methyl nidulinate* in stout prisms (0·68 g.), m. p. 187—189° (Found: C, 52·9; H, 4·5; Cl, 22·2. $C_{21}H_{21}O_6Cl_3$ requires C, 53·0; H, 4·5; Cl, 22·4%). Powdered methyl nidulinate is soluble in aqueous sodium hydrogen carbonate but does not give a ferric reaction in alcohol.

*Methyl* O-*Methylnidulinate* (II; R = R′ = Me, R″ = H).—Nidulinic acid (0·8 g.) and an excess of ethereal diazomethane reacted vigorously and 3 hr. later the solution was decanted from a small flocculent precipitate. Evaporation of the ether with air left a residue which on crystallisation from 95% methanol gave *methyl* O-*methylnidulinate* in thick hexagonal plates (0·58 g.), m. p. 161°, having a negative ferric reaction $\lambda_{max.}$ 289 mμ (ε 3900) [Found: C, 53·7; H, 5·1; Cl, 22·4; OMe, 17·7. $C_{19}H_{14}O_3Cl_3(OMe)_3$ requires C, 53·9; H, 4·7; Cl, 21·7; OMe, 19·0%].

The same ester was formed by the interaction of *O*-methylnidulin (0·23 g.) and sodium methoxide (from 0·012 g. of sodium) in boiling methanol for 2 hr. and was isolated by neutralisation of the solution with carbon dioxide and evaporation of the solvent. The ester formed pyramid-tipped prisms, m. p. and mixed m. p. 162°, from aqueous methanol.

Powdered methyl *O*-methylnidulinate is soluble in aqueous sodium hydroxide and insoluble in aqueous sodium hydrogen carbonate.

O-*Methylnidulinic Acid* (II; R = Me, R′ = R″ = H).—A mixture of *O*-methylnidulin (0·1 g.), 2N-aqueous sodium hydroxide (10 ml.), and dioxan (10 ml.) was heated on the steam-bath until the solution did not give a precipitate on dilution with water (*ca.* 3 hr.), and after the addition of water (50 ml.) the resulting O-*methylnidulinic acid* was precipitated with 2N-sulphuric acid (20 ml.) and purified from benzene–light petroleum (b. p. 60—80°), forming plates, m. p. 185—186° [Found: C, 53·2; H, 4·7; Cl, 22·0; OMe, 13·0. $C_{19}H_{15}O_4Cl_3(OMe)_2$ requires C, 53·0; H, 4·5; Cl, 22·4; OMe, 13·0%]. In some experiments this acid separated from aqueous dioxan in shining thick parallelograms, m. p. 92° (no sign of decomposition), which solidified at a higher temperature, and then melted at 185°. Recrystallised from benzene, the form of m. p. 92° reverted to plates, m. p. 185°. Both forms were readily soluble in aqueous sodium hydrogen carbonate and on methylation with ethereal diazomethane (or by attempted recrystallisation from hot methanol) gave methyl *O*-methylnidulinate, m. p. and mixed m. p. 162°.

*Methyl* OO-*Dimethylnidulinate* (II; R = R′ = R″ = Me).—A mixture of nidulin (0·5 g.) or *O*-methylnidulin (0·5 g.), 2N-aqueous sodium hydroxide (20 ml.), and methyl sulphate (1·8 ml.) was heated under reflux for 2 hr., with the addition of more hydroxide (10 ml.) and sulphate (1·8 ml.) after 1 hr. The product was isolated with ether, distilled (240°/0·005 mm.), and crystallised from hot methanol, giving *methyl* OO-*dimethylnidulinate* in prisms (0·21 g.), m. p. 113—114°, insoluble in sodium hydroxide solution [Found: C, 55·0; H, 5·1; Cl, 21·0; OMe, 23·7. $C_{19}H_{13}O_2Cl_3(OMe)_4$ requires C, 54·8; H, 5·0; Cl, 21·1; OMe, 24·6%].

When methyl *O*-methylnidulinate was (0·05 g.) treated with anhydrous potassium carbonate (0·2 g.) and methyl iodide (0·2 ml.) in boiling acetone for 2 hr. the same ester was obtained, having m. p. and mixed m. p. 113—114°.

*Decarbonidulin* (V; R = H).—A solution of nidulin (1·0 g.) in dioxan (10 ml.) and 2N-aqueous sodium hydroxide (20 ml.) was boiled for 1 hr., cooled, and acidified with dilute sulphuric acid. An ethereal solution of the sticky product was washed (as rapidly as possible) with aqueous sodium hydrogen carbonate solution, to remove nidulinic acid which was regained by acidification, forming prisms (0·2 g.) of m. p. and mixed m. p. 210—212° from benzene–light petroleum (b. p. 60—80°). Spontaneous evaporation of the ether then left *decarbonidulin* as a semi-solid which on purification from methanol formed prisms (0·2 g.), m. p. 148—149°, $\lambda_{max.}$ 284 mμ (ε 2850), slowly soluble in aqueous sodium hydrogen carbonate (Found, in specimen dried in a vacuum at 80° for 2 hr. : C, 54·8, 54·5; H, 4·3, 4·4; OMe, 7·6. $C_{18}H_{16}O_3Cl_3\cdot OMe$ requires C, 54·6; H, 4·6; OMe, 7·4%). Occasionally this compound separated from aqueous dioxan in plates, m. p. 117—118°. The two forms could be quantitatively interconverted by recrystallisation from the appropriate solvents or by seeding. Prepared by the usual method, the *di-*p-*nitrobenzoate* of decarbonidulin separated from benzene in cream squat prisms, m. p. 195°, insoluble in aqueous sodium hydroxide (Found : N, 3·8; OMe, 4·0. $C_{32}H_{22}O_9N_2Cl_3\cdot OMe$ requires N, 3·9; OMe, 4·3%).

O-*Methyldecarbonidulin* (V; R = Me).—When kept with ethereal diazomethane for 3 hr. decarbonidulin (0·2 g. of either form) gave a viscous oil which was dissolved in methanol. Slow evaporation of the solvent left O-*methyldecarbonidulin* in thick prisms (0·11 g.), m. p. 133—135° [Found : C, 55·9; H, 4·9; OMe, 14·0. $C_{18}H_{15}O_2Cl_3(OMe)_2$ requires C, 55·6; H, 4·9; OMe, 14·4%]. This ether was insoluble in aqueous sodium hydrogen carbonate but was soluble in aqueous sodium hydroxide.

*Degradation of Methyl Nidulinate to Methyl* 4 : 6-*Dichloro-*o-*orsellinate* (I; R = H).—A solution of methyl nidulinate (0·2 g.) in chloroform (7 ml.) was treated with chlorine (0·63 g.) in cold carbon tetrachloride (7 ml.), and the mixture kept at room temperature, in the dark, for 2 days. The solvents and excess of chlorine were removed at 40—50° by a current of dry air, and the residue in acetic acid (12 ml.) was treated with a solution of crystalline stannous chloride (0·7 g.) in a mixture of concentrated hydrochloric acid (10 ml.) and water (8 ml.) at 40°, added in small portions with constant agitation. A pale yellow gum separated and, next day, water (40 ml.) was added and the resulting emulsion extracted with chloroform. The brown oil left on evaporation of the well-washed and dried extracts was heated under reflux with 10% methanolic potassium hydroxide (15 ml.) in hydrogen on the steam-bath for 1 hr. After dilution with water (50 ml.) the cooled solution was acidified with 2N-sulphuric acid (15 ml.), and the sticky product isolated with ether. The ethereal solution, which had been washed with water, was extracted with N-aqueous sodium carbonate and the extracts acidified with dilute sulphuric acid. Crystallised from aqueous methanol (charcoal) and then from 70% methanol, the buff precipitate (62 mg.) gave methyl 4 : 6-dichloro-*o*-orsellinate as a colourless micro-crystalline powder (13 mg.), m. p. 115°, undepressed on admixture with an authentic specimen (Found : C, 40·4; H, 4·0. Calc. for $C_9H_8O_4Cl_2,H_2O$ : C, 40·2; H, 3·7%). This compound had the characteristic violet ferric reaction in alcohol and on rubbing became highly electrified (cf. Nolan and Murphy, *Sci. Proc. Roy. Dublin Soc.*, 1941, **22**, 317, and Nolan, Algar, McCann, Manahan, and Nolan, *ibid.*, 1948, **24**, 326).

*Degradation of Methyl* O-*Methylnidulinate to Methyl* 4 : 6-*Dichloroeverninate* (I; R = Me).—(*a*) Under the foregoing conditions except that the chlorination was continued for 4 days, methyl *O*-methylnidulinate (0·3 g.) gave a crude product, a solution of which in ether (45 ml.) was washed with water and then with 3% aqueous sodium hydrogen carbonate (20 + 10 ml.), and extracted with 1% aqueous sodium hydroxide (20 + 10 + 10 ml.). From the acidified extracts methyl 4 : 6-dichloroeverninate (80 mg.) was isolated and, on crystallisation from aqueous methanol, formed colourless, feathery needles (20 mg.), m. p. 80°, undepressed on admixture with an authentic specimen and having a violet ferric reaction in alcohol [Found : C, 45·7; H, 3·8; Cl, 26·2; OMe, 22·2. Calc. for $C_8H_4O_2Cl_2(OMe)_2$ : C, 45·3; H, 3·8; Cl, 26·8; OMe, 23·4%] (cf. Nolan and Murphy, *loc. cit.*, p. 319, who gave m. p. 79—80°).

(*b*) A solution of methyl *O*-methylnidulinate (0·45 g.) in acetic acid (50 ml.) was treated with nitric acid (0·17 ml., *d* 1·4) at room temperature. 15 Minutes later the mixture was diluted with water and extracted with ether. From the combined ethereal extracts which had been washed with aqueous sodium hydrogen carbonate, methyl dichloroeverninate was isolated with the aid of 2N-sodium hydroxide (2 × 25 ml.) and purified from dilute methanol, forming needles (0·2 g.), m. p. and mixed m. p. 82—83°, and having a violet ferric reaction in alcohol.

*Oxidation of Nidulin with Potassium Permanganate.*—A solution of nidulin (4 g.) in acetone (1 l.) at room temperature was treated with powdered potassium permanganate (10 g.), followed next day with 2N-aqueous sodium carbonate (100 ml.). The filtered mixture was evaporated and the residual aqueous liquor extracted with ether to remove a brown wax from which unchanged nidulin (0·1 g.) was isolated with boiling light petroleum (b. p. 60—80°). After acidification with 2N-sulphuric acid, the parent solution was repeatedly extracted with ether, giving a brown oil, more of which was obtained from the oxides of manganese by clarification in water with sulphur dioxide and extraction with ether. This oil did not distil smoothly but on being kept at 130°/20 mm. gave a sublimate in colourless plates which on resublimation had m. p. 85° (yield, 2 mg.). This product reacted vigorously with aqueous sodium hydrogen carbonate solution and, with resorcinol and sulphuric acid, gave a positive fluorescein reaction which was destroyed by excess of alkali. The acid, m. p. 85°, depressed the m. p. of the following: (±)-, (+)-, and (−)-β-methyladipic, α-hydroxy*iso*butyric, α-hydroxy*iso*valeric, and (±)-, (+)-, and (−)-α-hydroxy-αβ-dimethylbutyric acid (see below).

O-*Methyldehydronidulin.*—A mixture of *O*-methylnidulin (0·5 g.), acetic acid (6 ml.), and nitric acid (1 ml.; *d* 1·4) was heated on the steam-bath for 5 min., and the product precipitated with water and then crystallised from acetic acid, giving O-*methyldehydronidulin* in long thin prisms (0·4 g.), m. p. 168°, inflection at 298 mμ (ε 1750) [Found: C, 54·6, 54·8; H, 3·6, 3·9; N, nil; Cl, 24·7, 23·1; OMe, 12·8. $C_{19}H_{11}O_3Cl_3(OMe)_2$ requires C, 55·3; H, 3·8; Cl, 23·3; OMe, 13·6%]. This substance could not be hydrogenated with hydrogen and 5% palladium–charcoal at atmospheric pressure.

O-*Methyldehydronidulin Oxide.*—(*a*) A stream of ozone and oxygen was led into a solution of *O*-methyldehydronidulin (0·5 g.) in ethyl acetate (25 ml.) for 15 min. at room temperature. Triturated with water, the white gum left on removal of the solvent in a vacuum gradually solidified and on purification from alcohol gave O-*methyldehydronidulin oxide* in needles and then in colourless prisms (0·1 g.), m. p. 200° [Found: C, 53·3; H, 3·7; Cl, 22·3; OMe, 15·3. $C_{19}H_{11}O_4Cl_3(OMe)_2$ requires C, 53·5; H, 3·6; Cl, 22·6; OMe, 13·2%]. This compound did not react with carbonyl reagents and was only very slowly soluble in boiling aqueous sodium hydroxide. The aqueous liquor from the crude ozonolysis product did not react with 2 : 4-dinitrophenylhydrazine.

(*b*) A solution of *O*-methyldehydronidulin (0·459 g.) in chloroform was mixed with 0·57N-chloroformic perbenzoic acid (20·0 ml.), the volume was made up to 100 ml. with the same solvent, and the mixture was kept at room temperature for 10 days: titration of a sample then indicated the absorption of one atom of oxygen. The mixture was washed successively with 10% aqueous potassium iodide, sodium thiosulphate solution, 2N-aqueous sodium carbonate, and water, dried, and evaporated, giving a pale yellow gum which slowly crystallised. After being washed with ether the oxide separated from alcohol in needles and then in prisms, m. p. and mixed m. p. 200° (Found: C, 53·1; H, 4·1; Cl, 22·4; OMe, 14·8%).

*Resolution of* (±)-*α-Hydroxy-αβ-dimethylbutyric Acid.*—The acid (3·3 g.) (Perkin, *J.*, 1896, **69**, 1457) was added to (−)-brucine (9 g.) dissolved in dry ethyl acetate (200 ml.). On being kept, this deposited (−)-*brucine* (±)-*α-hydroxy-αβ-dimethylbutyrate* in prisms (10·7 g.), m. p. 194—197° [Found: N, 5·3; OMe, 12·3. $C_{28}H_{32}O_5N_2(OMe)_2$ requires N, 5·2; OMe, 11·6%]. Fractional crystallisation of this salt from ethyl acetate gave as the less soluble component (−)-*brucine* (+)-*α-hydroxy-αβ-dimethylbutyrate*, m. p. 208° (decomp.), $[\alpha]_D^{20}$ −29·65° (*c*, 5 in $CHCl_3$). Liberated from the salt with dilute sulphuric acid and isolated with ether, the (+)-*acid* was distilled [130° (bath)/20 mm.] and then crystallised from light petroleum (b. p. 60—80°), forming plates, m. p. 69—71°, $[\alpha]_D^{20}$ 4·57° (*c*, 5 in $CHCl_3$) (Found: C, 55·0; H, 9·4%; equiv., 128. $C_6H_{12}O_3$ requires C, 54·5; H, 9·2%; equiv., 132). The more soluble salt could not be obtained optically pure but on decomposition furnished the (−)-*acid* which separated from light petroleum (b. p. 60—80°) in plates, m. p. 67—69°, $[\alpha]_D^{20}$ −3·34° (*c*, 5 in $CHCl_3$) (Found: C, 54·5; H, 9·3%; equiv., 132).

*Bleaching-powder Tests* (cf. Brown, Thesis, Liverpool, 1947).—A filtered, saturated, aqueous solution (0·5 ml.) of bleaching-powder was added to the test substance (5 mg.) after hydrolysis by a mixture of alcohol (0·5 ml.), water (1 ml.), and 2N-aqueous sodium hydroxide (four drops). The following results were obtained: resorcinol, purple (fading rapidly); nidulin and nidulinic acid, very faint pink (fading rapidly); nornidulin, purple (fading rapidly); methyl dichloro-everninate, no colour; methyl dichloro-orsellinate, intense red.

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