

685. The Chemistry of Fungi. Part XXVI.* Dechloronornidulin.

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The isolation of dechloronornidulin $C_{19}H_{16}O_5Cl_2$ from a strain of *Aspergillus nidulans* is reported. The new compound belongs to the same series as nidulin and may be identical with the substance previously called "ustin II." A partial structural formula is advanced.

FURTHER examination of the mycelium of the non-ascosporic strain of *Aspergillus nidulans* which produced nidulin¹ (I; R' = Me, R'' = H) and nornidulin ("ustin") (I; R' = R'' = H) has disclosed the presence of a third metabolite of the same general type. The new member of the series has m. p. 212—214°, a molecular formula $C_{19}H_{16}O_5Cl_2$, and an ultraviolet spectrum closely similar to that of nidulin, and may, therefore, be identical with Compound II ("ustin II") isolated by Hogeboom and Craig² for which these authors give m. p. 214—216° and suggest a molecular formula $C_{21}H_{18}O_6Cl_2$. The name dechloronornidulin is now proposed for the new substance.

Like nornidulin, dechloronornidulin is an acidic dihydric phenol devoid of methoxyl groups, forming a dimethyl ether with the same characteristic lactonic-carbonyl absorption at 1733 cm^{-1} (Nujol). Disruption of the lactonic system by sodium methoxide gave a phenolic ester, methyl dechloro-*OO*-dimethylnornidulinate, and, on fission by the nitric acid method used originally to isolate the ring A moiety of methyl *O*-methylnidulinate, this compound gave two monochlorinated products of which one appeared to be a nitro-derivative of the other. By synthesis, these degradation products were shown to be methyl 6-chloro- (II; R = H) and 6-chloro-4-nitro-everninate † (II; R = NO₂). Because in the nitric acid fission methyl *O*-methylnidulinate gave methyl dichloreverninate, the present result could not be attributed to the extrusion of a chlorine atom from a dichlorinated ring A in dechloronornidulin and hence it was inferred that ring A contains but one chlorine atom.

Accordingly the partial formulæ (III; R = H) and (IV) may be allocated to dechloronornidulin and to methyl dechloro-*OO*-dimethylnornidulinate respectively. The residue B of dechloronornidulin (III; R = H) appears to be identical with the ring B of nornidulin (I; R' = R'' = H); thus neither compound couples with diazonium salts or

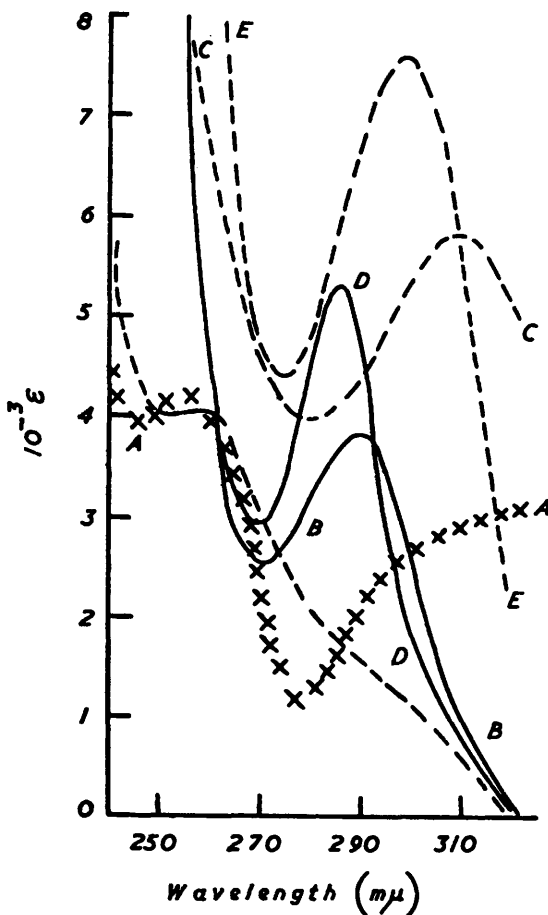
* Part XXV, *J.*, 1955, 1432.

† Me is numbered 1 in these trivial names.

¹ Dean, Roberts, and Robertson, *J.*, 1954, 1432.

² Hogeboom and Craig, *J. Biol. Chem.*, 1946, 162, 363.

Gibb's reagent, and methyl dechloro-*OO*-dimethylnornidulinate (IV), like methyl *O*-methylnidulinate, behaves as a phenol towards alkalis and in alcohol has a pronounced peak in the ultraviolet spectrum at about 282 (ϵ 9810) (see Figure) similar to those of several polyhydric phenols and in a region in which both methyl 6-chloro- and 4:6-dichloroeverninate have minima. This allocation is confirmed by the bathochromic shifts shown by methyl *O*-methylnidulinate and by methyl *OO*-dimethylnidulinate when alkali is added to their alcoholic solutions, a behaviour which is characteristic of phenols and cannot be due to ring A, even though this ring undoubtedly exerts some effect on the ring-B absorption by reason of the poor insulating properties of the diphenyl oxygen atom.

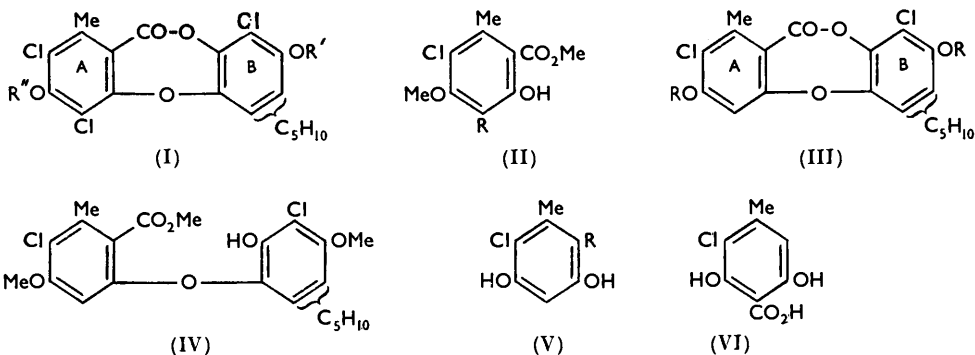


Absorption spectra (10^{-4} M-solutions in EtOH) of:
 A, methyl 4:6-dichloroeverninate; B, methyl *O*-methylnidulinate; C, solution B with added alkali; D, methyl dechloro-*OO*-dimethylnornidulinate; and E, solution D with added alkali.

Typical spectra are given in the Figure. Further evidence relating the rings B of nidulin and dechloronornidulin will form part of a later communication dealing with the C_5 residue.

By Gattermann's method 4-chloro-orsinol was converted into 3-chloro-4:6-dihydroxy-2-methylbenzaldehyde (V; $R = \text{CHO}$), the di(methoxycarbonyl) derivative of which on oxidation with potassium permanganate and subsequent hydrolysis furnished 6-chloro-orsellinic acid (V; $R = \text{CO}_2\text{H}$). To exclude the possibility that the aldehyde group had been introduced into the 4-position of the orcinol ring ($\text{Me} = 1$), the isomeric 3-chloro-*p*-orsellinic acid (VI) was prepared by monochlorination of methyl *p*-orsellinate followed by hydrolysis of the resulting ester and was clearly different from the acid (V; $R = \text{CO}_2\text{H}$). Selective methylation of 6-chloro-orsellinic acid by diazomethane afforded methyl 6-chloroeverninate (II; $R = \text{H}$) having the properties of an *o*-hydroxy-ester and being identical with the non-nitrogenous degradation product. Although nitration of this ester supplied methyl 6-chloro-4-nitroeverninate (II; $R = \text{NO}_2$) identical with the second degradation

product, the reaction appeared to be more sluggish than that occurring during the degradation. This result can hardly be due to a comparatively rapid substitution of the intact diphenyl ether, followed by fission, because dechloro-*OO*-dimethylnornidulin cannot be



nitrate even at 80°. Therefore it is reasonable to attribute the increased rate to the well-established catalysis of nitration of phenols and their derivatives by nitrous acid, here produced by oxidation of the ring B fragment.

EXPERIMENTAL

Dechloronornidulin (III; R = H).—*Aspergillus nidulans* (NRRL, No. 2006) was cultivated by the method described in Part XXII¹ and the dried and powdered mycelial felt from 50 l. of culture medium was exhaustively extracted with light petroleum (b. p. 40–60°). The semisolid extract left on evaporation of the solvent was dissolved in ether (500 ml.), and shaken with 10% aqueous sodium carbonate, forming three layers. On acidification the small dark middle layer yielded crude nidulin, whilst the lowest layer gave, on some occasions, a mixture of nidulin and nornidulin, and on others, a mixture of nidulin and dechloronornidulin. Some increase in the proportion of dechloronornidulin seemed to result from the use of culture media with a somewhat reduced chloride content, but unfortunately the production of this compound was too erratic to allow a satisfactory assessment. *Dechloronornidulin* was freed from nidulin by repetition of the sodium carbonate extraction and on crystallisation from methanol formed needles (0.8 g.), m. p. 212–214°, λ_{max} (in EtOH) 264 μ (ϵ 9810), having a negative ferric reaction [Found: C, 57.7; H, 4.2; Cl, 18.2%; OMe, negative; *M* (micro-Rast), 356 (decomp.). $\text{C}_{19}\text{H}_{16}\text{O}_5\text{Cl}_2$ requires C, 57.7; H, 4.1; Cl, 17.9%; *M*, 395]. This compound was readily soluble in aqueous sodium hydrogen carbonate containing a few drops of alcohol.

Dechloro-OO-dimethylnornidulin (III; R = Me).—In refluxing acetone (20 ml.), containing potassium carbonate (0.5 g.), dechloronornidulin (0.2 g.) reacted with methyl iodide (5 ml.) during 3 hr. to give the *dimethyl ether* which on isolation formed rhombs (0.18 g.), m. p. 156–156° from methanol, λ_{max} 223, 260 μ (ϵ 17,600, 4130), insoluble in cold aqueous 2*N*-sodium hydroxide [Found: C, 59.3; H, 4.7; OMe, 15.0%; *M* (Micro-Rast), 406. $\text{C}_{19}\text{H}_{14}\text{O}_3\text{Cl}_2(\text{OMe})_2$ requires C, 59.6; H, 4.7; OMe, 14.7%; *M*, 423].

Methyl Dechloro-OO-dimethylnornidulinate (IV).—A solution of the aforementioned ether (0.2 g.) in methanol (40 ml.), containing sodium methoxide (from 0.011 g. of sodium), was gently boiled for 6 hr. and the solvent removed with a current of air. A solution of the residue in ether was washed with dilute sulphuric acid followed by water, dried, and evaporated, leaving a yellow gum which solidified in contact with light petroleum (b. p. 60–80°). Crystallised from aqueous methanol, this gave *methyl dechloro-OO-dimethylnornidulinate* in squat prisms (0.12 g.), m. p. 125–126°, with a negative ferric reaction in alcohol [Found: C, 57.6; H, 5.3; OMe, 20.4. $\text{C}_{19}\text{H}_{15}\text{O}_3\text{Cl}_2(\text{OMe})_2$ requires C, 58.0; H, 5.3; OMe, 20.4%]. This ester, which was insoluble in aqueous sodium hydrogen carbonate solution, dissolved in 2*N*-aqueous sodium hydroxide but did not yield dyes with diazotised sulphanilic acid or with Gibb's reagent. In neutral alcohol it had λ_{max} 284 μ (ϵ 5460) which changed on addition of one drop of 1% alcoholic potassium hydroxide to λ_{max} 300 μ (ϵ 7600). Under the same conditions methyl *O*-methylnidulinate had λ_{max} 290 μ (ϵ 3800) in neutral alcohol, changing to λ_{max} 310 μ (ϵ 5750) on addition of a drop of 1% alcoholic potassium hydroxide.

6-Chloro-*o*-orcylaldehyde (V; R = CHO).—A mixture of anhydrous zinc cyanide (1 g.), 4-chloro-*o*-rcinol (4 g.), and dry ether (40 ml.) at 0° was saturated with hydrogen chloride during 2 hr. and next day the precipitated aldimine complex was washed with ether and hydrolysed with water (60 ml.) on the steam-bath. The resulting 6-chloro-*o*-orcylaldehyde separated from dilute alcohol in prisms (3.9 g.), m. p. 171—173°, soluble in 2*N*-aqueous sodium hydroxide and having a purple ferric reaction (Found: C, 51.5; H, 4.0; Cl, 19.5. C₈H₇O₃Cl requires C, 51.5; H, 3.8; Cl, 19.0%). Acetylation of this compound (1 g.) by the standard methods gave 6-chloro-*o*-orcylidene tetra-acetate which crystallised from alcohol in needles (1.2 g.), m. p. 115—116°, with a negative ferric reaction [Found: C, 51.4; H, 4.8; OAc, 51.5. C₈H₅Cl(OAc)₄ requires C, 51.6; H, 4.6; OAc, 46.2%].

6-Chloro-*o*-orsellinic Acid (V; R = CO₂H).—2*N*-Aqueous sodium hydroxide (26.8 ml.) was added dropwise to a well-stirred solution of 6-chloro-*o*-orcylaldehyde (4 g.) and methyl chloroformate (6 g.) in acetone (40 ml.) at below 0° and on dilution with water (80 ml.) $\frac{1}{2}$ hr. later the mixture deposited 6-chloro-3:5-dimethoxycarbonyl-*o*-orcylaldehyde, forming long needles (5.3 g.), m. p. 138°, from light petroleum (b. p. 60—80°), with a negative ferric reaction (Found: C, 47.9; H, 4.0; Cl, 12.4. C₁₂H₁₁O₇Cl requires C, 47.6; H, 3.7; Cl, 11.7%). This derivative (3 g.) was oxidised in acetone (50 ml.) at 50—55° with a solution of potassium permanganate (4.7 g.) in water (75 ml.), added in portions during 30 min. Clarification of the mixture at 5° with sulphur dioxide and removal of the solvent left an oil which slowly solidified and was then hydrolysed by dissolution in *N*-aqueous sodium hydroxide (25 ml.) for 30 min. Precipitated by concentrated hydrochloric acid, the resulting 6-chloro-*o*-orsellinic acid was purified by means of aqueous sodium hydrogen carbonate and then crystallised from aqueous acetone, forming cream needles (0.7 g.), m. p. 194° (decomp.), with a purple alcoholic ferric reaction (Found: C, 47.5; H, 3.8; Cl, 17.6. C₈H₇O₄Cl requires C, 47.4; H, 3.5; Cl, 17.5%).

Methyl 6-Chloroeverninate (II; R = H).—With an excess of ethereal diazomethane at 0° for 20 min. 6-chloro-*o*-orsellinic acid (0.5 g.) gave methyl 6-chloroeverninate which was freed from acidic impurities with sodium hydrogen carbonate and then crystallised from methanol, forming long needles (0.45 g.), m. p. 143—144°, with a violet ferric reaction [Found: C, 52.1; H, 4.8; Cl, 15.9; OMe, 26.2. C₈H₉O₂Cl(OMe)₂ requires C, 52.1; H, 4.8; Cl, 15.4; OMe, 26.9%].

Methyl 6-Chloro-4-nitroeverninate (II; R = NO₂).—Nitric acid (*d* 1.4; 0.08 ml.) was added to methyl 6-chloroeverninate (0.1 g.) in acetic acid (10 ml.). After being kept for 50 min. at room temperature the yellow solution was diluted with water (250 ml.), and the precipitate fractionally crystallised from light petroleum (b. p. 40—60°). The less soluble fractions gave methyl 6-chloro-4-nitroeverninate in pale yellow prisms (10 mg.), m. p. 86°, λ_{\max} . (in EtOH) 317 m μ (ϵ 2390), having a red ferric reaction (Found: C, 44.0; H, 3.5; N, 5.2. C₁₀H₁₀O₆NCl requires C, 43.6; H, 3.7; N, 5.1%).

3-Chloro-*p*-orsellinic Acid (VI).—A mixture of methyl *p*-orsellinate (1 g.) and ether (30 ml.) at 0° was treated with sulphuryl chloride (0.75 g.) and next day the solvent was evaporated and the residue twice crystallised from methanol (charcoal), giving methyl 3-chloro-*p*-orsellinate in rhombs (0.75 g.), m. p. 120—121°, with an olive green ferric reaction in water (Found: C, 49.5; H, 4.2; OMe, 14.3. C₈H₈O₃Cl·OMe requires C, 49.8; H, 4.2; OMe, 14.3%). This ester (0.2 g.) was hydrolysed with 0.5*N*-aqueous sodium hydroxide (3 ml.) in alcohol (3 ml.) on the steam-bath for 1.5 hr. After the removal of the alcohol in a vacuum, 3-chloro-*p*-orsellinic acid was precipitated by 2*N*-sulphuric acid and crystallised from water (charcoal), forming slender needles (0.15 g.), m. p. 198—199° (decomp.), with a deep blue ferric reaction (Found: C, 47.2; H, 3.7; Cl, 17.4. C₈H₇O₄Cl requires C, 47.4; H, 3.5; Cl, 17.5%). A mixture of this acid and 6-chloro-*o*-orsellinic acid had m. p. 170—180° (decomp.).

Nitric Acid Degradation of Methyl Dechloro-*OO*-dimethylnormidulinate.—The addition of nitric acid (*d* 1.47; 0.5 ml.) to methyl dechloro-*OO*-dimethylnormidulinate (0.5 g.) in acetic acid (6 ml.) at the room temperature caused a colour change through yellow to red, complete in 10 min., whereupon the solution was poured into water (100 ml.). Collected in ether, the products were washed with water (5 \times 20 ml.) and extracted with aqueous sodium hydrogen carbonate (4 \times 10 ml.). Acidification of the dark extract with concentrated hydrochloric acid gave a yellow gum which was isolated with ether and fractionally crystallised from methanol. The first fractions contained the chief fission product, methyl 6-chloro-4-nitroeverninate, which formed yellow prisms (100 mg.), m. p. and mixed m. p. 86—87° having the same ultraviolet absorption spectra and ferric reaction as an authentic specimen (Found: N, 5.0; OMe, 22.4%). Later fractions furnished a very small quantity of methyl 6-chloroeverninate, forming prisms, m. p. and mixed m. p. 143—145°, from methanol.