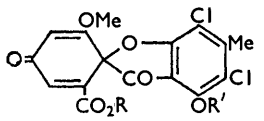
568. *The Constitution of Geodoxin, a Metabolic Product of* Aspergillus terreus *Thom.*

By C. H. Hassall and T. C. McMorris.

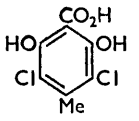
Geodoxin, $C_{17}H_{12}O_8Cl_2$, has been isolated from the culture fluid of *Aspergillus terreus* Thom. Degradation experiments lead to the constitution (IX). The relation of this structure to those of geodin and erdin is discussed.

The metabolic products of *Aspergillus terreus* Thom (Ac.100) were first investigated by Raistrick and his collaborators [1a] in connection with the ability of certain fungi to assimilate chlorine from culture solutions containing chloride ion. The two chlorinated metabolites geodin and erdin, which were isolated from the culture fluids of this species and *A. terreus* Thom (Ac.45), have been the subject of an interesting series of investigations.[1a-d,2] As a result, the structures (Ia) and (II) have been assigned to geodin and erdin respectively. We have found that a culture of the strain Ac.100 lost the capacity to synthesise geodin but acquired the ability to produce a new chlorinated metabolite. This was, presumably, the result of spontaneous mutation favoured by the conditions of subculturing. The new compound, which we name geodoxin, has been studied with the expectation that elucidation of its structure would contribute to our understanding of the catabolism or biogenesis of geodin and erdin.

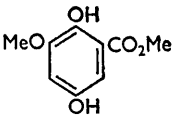
Geodoxin, $C_{17}H_{12}O_8Cl_2$, is not optically active. It has phenolic properties which include insolubility in sodium hydrogen carbonate solution but solubility in dilute, aqueous sodium hydroxide. Analytical evidence indicates one active hydrogen atom and one *C*-methyl and two methoxyl groups. Pyrolysis readily affords 2,4-dichloro-orcinol (4,6-dichloro-5-methylresorcinol); dichloro-*p*-orsellinic acid (III) is produced by oxidation with hydrogen peroxide in alkali.



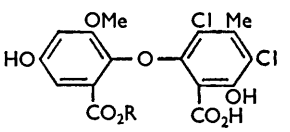
(Ia) R = Me, R′ = H
(Ib) R = R′ = Me
(II) R = R′ = H



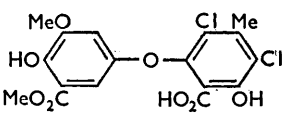
(III)



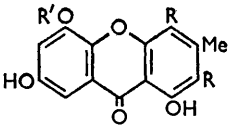
(IV)



(V) R = H
(VI) R = Me



(VII)



(VIIIa) R = Cl, R′ = Me
(VIIIb) R = R′ = H

In the presence of palladium–charcoal, geodoxin absorbs two atoms of hydrogen very rapidly. The product, dihydrogeodoxin, dissolves in sodium hydrogen carbonate solution and can be titrated as a monobasic acid. It gives an intense violet colour with ferric chloride, contains two methoxyl groups, and with diazomethane affords a neutral compound containing five methoxyl groups. Dihydrogeodoxin is hydrolysed by alkali to a dibasic acid, $C_{16}H_{12}O_8Cl_2$, containing a single methoxyl group. These properties of dihydrogeodoxin, and its infrared and ultraviolet absorption spectra, require one methoxycarbonyl,

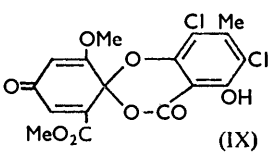
[1] (*a*) Raistrick and Smith, *Biochem. J.*, 1936, **30**, 1315; (*b*) Clutterbuck, Koerber, and Raistrick, *ibid.*, 1937, **31**, 1089; (*c*) Calam, Clutterbuck, Oxford, and Raistrick, *ibid.*, 1939, **33**, 579; (*d*) *Idem*, *ibid.*, 1947, **41**, 458.

[2] Barton and Scott, *J.*, 1958, 1767.

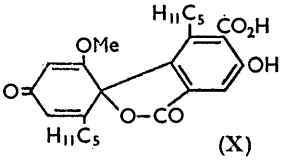
one carboxyl, one methoxyl, and two phenolic hydroxyl groups. The remaining oxygen atom may be attributed to a bridge in a substituted diphenyl ether. To test this possibility, dihydrogeodoxin was treated with 80% sulphuric acid: dichloro-orcinol, a high-melting compound, $C_{15}H_{10}O_5Cl_2$, and methyl 2,5-dihydroxy-3-methoxybenzoate (IV) were isolated from the reaction mixture. This result, taken with the evidence of a dichloro-*p*-orsellinic acid unit in geodoxin, indicates structure (VI) or (VII) for dihydrogeodoxin.

The structures (V) and (VI) correspond to those assigned by Barton and Scott [2] to erdin and geodin hydrates, compounds obtained by mild treatment of geodin and erdin with 80% sulphuric acid. The structure (VII) for dihydrogeodoxin has been excluded by showing that erdin hydrate and the dibasic acid obtained by hydrolysis of dihydrogeodoxin are identical. The high-melting by-product, $C_{15}H_{10}O_5Cl_2$, contained one methoxyl group and showed ultraviolet and infrared characteristics indicative of a xanthone. It must be formulated as (VIIIa), being produced unambiguously from the sequence: ester hydrolysis, decarboxylation, and ring closure. This structure is related to that proposed [2] for norgeodin A (VIIIb) which is formed when geodin (Ia) is treated with hydriodic acid.

The structure of dihydrogeodoxin, the fact that this acid is formed very readily by hydrogenolysis of geodoxin, a compound with phenolic properties but with no free carboxyl group, and the evidence of a single active hydrogen atom in geodoxin leads to the structure (IX) for this compound. There is spectroscopic evidence in support of this formulation. The carbonyl frequencies in the infrared absorption spectrum are readily explained. In particular, a band near 1670 cm.$^{-1}$, which also occurs in the spectra of geodin, erdin, and picrolichenic acid,[3] is attributed to a doubly conjugated carbonyl group in a six-membered ring. Since the aromatic and the cyclohexadienone ring are not in conjugation, the sum of the contributions of these individual chromophores should account for the absorption spectrum of geodoxin. A curve obtained by subtracting the spectrum of dichloro-*p*-orsellinic acid (III) from that of geodoxin has $\lambda_{max.}$ 230, 275 mμ (log ε 4·35,

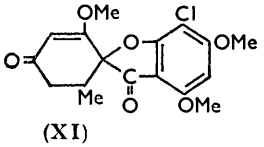


(IX)

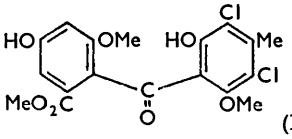


(X)

3·9). There is a remarkable similarity to the curve with $\lambda_{max.}$ 235, 280 mμ (log ε 4·4, 3·8) obtained by subtracting the absorption spectrum of orsellinic acid from that of picrolichenic acid (X). The curves show a similarity to that of a 5-methoxycyclohexa-2,5-dienone derivative prepared by Jeger and his co-workers.[4]



(XI)



(XII)

Recent studies support the suggestion that the biogenesis of griseofulvin (XI) involves the linkage of " acetate units " to form a benzophenone derivative which may, in turn, be converted by oxidative coupling into a spiran.[5,6] There is no direct evidence on the biogenesis of the related compounds geodin and erdin although Scott [6] has shown that geodin methyl ether (Ib) is formed from the related 2,4′-dihydroxybenzophenone derivative (XII) by oxidative coupling *in vitro*. Comparison of the structures of geodin and geodoxin suggests that the latter compound may be synthesised in Nature from geodin either directly

[3] Wachtmeister, *Acta Chem. Scand.*, 1958, **12**, 147.
[4] Jeger, Ruegg, and Ruzicka, *Helv. Chim. Acta*, 1947, **30**, 1294.
[5] Birch, Blance, and Smith, *J.*, 1958, 4582.
[6] Scott, *Proc. Chem. Soc.*, 1958, 195.

by a Baeyer–Villiger-type oxidation,[7] such as has been indicated in other metabolic sequences,[8] or indirectly by formation of geodin hydrate followed by oxidative coupling. We are seeking further evidence on the mode of biogenesis of geodoxin.

Experimental

M. p.s were determined by means of a Kofler block. Ultraviolet spectra were determined in ethanol on a Beckman spectrophotometer, model D.U. Infrared spectra, for which we are grateful to Dr. S. M. Nagy, Massachusetts Institute of Technology, and Dr. H. E. Hallam and Mr. D. Jones, University College of Swansea, were measured for potassium bromide discs unless otherwise stated. Microanalyses were carried out by Dr. F. Pascher, Bonn, Germany.

Isolation of Geodoxin.—Czapek–Dox agar slopes of *Aspergillus terreus* Thom (Ac.100) which had been growing for 14 days were used to inoculate sterilised Czapek–Dox medium (Raistrick and Smith [1α] modification) in flat-sided bottles of approximately 1 l. capacity. One slope was used to inoculate four bottles. The bottles were kept at 28—30°. After five weeks the dark brown culture fluid was separated from mycelium. The fluid (10 l. batch) was adjusted to pH 6·6 and stirred with activated charcoal (100 g.) for 1 hr. The charcoal was separated by filtration, dried, and extracted with methanol (3 × 1·5 l.). The methanol extract was concentrated in vacuo at 35—40° to 500 c.c. and left in a refrigerator. During two days *geodoxin* (225 mg.) separated as yellow crystals. It crystallises from chloroform–ether or ethyl acetate–light petroleum (b. p. 60—80°) as yellow needles, m. p. 216—217° (decomp.) [Found: C, 49·3; H, 3·00; O, 31·0; Cl, 16·1; *C*-Me, 3·7; OMe, 15·5; active H, 0·31%; *M* (Rast), 375. $C_{17}H_{12}O_8Cl_2$ requires C, 49·2; H, 2·9; O, 30·8; Cl, 17·1; 1*C*-Me, 3·6; 2OMe, 14·9; active H, 0·24%; *M*, 415], $[\alpha]_D$ 0° (*c* 1·0 in chloroform), $\lambda_{max.}$ 270, 345 mμ (log ε 3·95, 3·73). The subtraction curve geodoxin — dichloro-*p*-orsellinic acid [$\lambda_{inflex.}$ 250, $\lambda_{min.}$ 277 mμ (log ε 3·85, 2·3)] gave $\lambda_{max.}$ 230, 275 mμ (log ε 4·35, 3·9). The infrared spectrum in chloroform showed bands at 3250 (OH), 1735 (lactone and CO_2Me), and 1672 cm.$^{-1}$ (conjugated CO).

Degradation of Geodoxin.—(a) *Pyrolysis.* Geodoxin (40 mg.) was heated to 200°/1 atm. until no more sublimate was formed. This product was resublimed (60°/0·1 mm.), to yield needles, m. p. and mixed m. p. with 2,4-dichloro-orcinol, 166—168°.

(b) *By alkaline hydrogen peroxide.* Geodoxin (105 mg.) in N-sodium hydroxide (2 c.c.) was treated with 30% hydrogen peroxide (2·5 c.c.). After 2 hr. further sodium hydroxide (4 c.c.) and hydrogen peroxide (5 c.c.) were added. The mixture was set aside for 2 days during which the solution became colourless. Acidification gave a precipitate (20 mg.) which was purified by sublimation at 100° in a high vacuum, to give crystals, m. p. and mixed m. p. with dichloro-*p*-orsellinic acid, 217° (Found: C, 41·0; H, 2·6; O, 26·6; Cl, 30·0. Calc. for $C_8H_6O_4Cl_2$: C, 40·5; H, 2·5; O, 27·0; Cl, 30·0%).

The dichloro-*p*-orsellinic acid which was used for comparison was prepared by the following unambiguous route. 2,6-Dihydroxy-4-methylbenzoic acid was converted into its methyl ester by refluxing it with dimethyl sulphate, sodium hydrogen carbonate, and acetone, followed by working up in the usual way. A saturated solution of chlorine in carbon tetrachloride was added to the ester at 0° until the yellow colour persisted. Next morning the excess of chlorine and carbon tetrachloride were removed. The product, m. p. 166—167° (from ethanol), was converted into *dichloro*-p-*orsellinic acid* (*3,5-dichloro-2,6-dihydroxy-4-methylbenzoic acid*) by refluxing it for 3 hr. in 0·5N-sodium hydroxide to which ethanol (50% by volume) had been added. Working up in the usual way and recrystallisation from acetic acid gave the acid, m. p. 217° (decomp.) (Found: C, 40·5; H, 2·3; Cl, 29·6. $C_8H_6O_4Cl_2$ requires C, 40·2; H, 2·5; Cl, 29·7%).

Dihydrogeodoxin.—Geodoxin (300 mg.), in ethanol, was shaken with hydrogen in the presence of palladium–charcoal (0·9 g.). Uptake of hydrogen (0·98 mol.) was complete in 30 sec. The product crystallised from ethyl acetate–light petroleum (b. p. 60—80°) as white prisms, m. p. 203—206° (decomp.) (Found: C, 49·4; H, 3·4; O, 30·3; Cl, 16·8; OMe, 15·2%; equiv., 402. $C_{17}H_{14}O_8Cl_2$ requires C, 49·0; H, 3·4; O, 30·7; Cl, 17·0; 2OMe, 15·9%; equiv. for a monobasic acid, 417). Dihydrogeodoxin dissolves readily in sodium hydrogen carbonate solution. The solution in ethanol gives a purple colour with ferric chloride.

[7] Hassall, *Org. Reactions*, 1957, **9**, 73.
[8] Eppstein, Meister, Murray, and Peterson, *Vitamins and Hormones*, 1956, **14**, 394.

Dihydrotrimethylgeodoxin, m. p. 121°, was obtained in admixture with another unidentified methylation product, m. p. 151°, when dihydrogeodoxin was treated with dimethyl sulphate and sodium hydroxide solution. The lower-melting compound was found, by mixed m. p. determination and comparison of infrared spectra, to be trimethylerdin hydrate, m. p. 121°, $\lambda_{max.}$ 314 mμ (log ε 3·7).

Reactions of Dihydrogeodoxin.—(a) *With sodium hydroxide.* Dihydrogeodoxin (100 mg.) was refluxed with ethanol (15 c.c.) and N-sodium hydroxide (7·5 c.c.) for 1 hr. Acidification followed by removal of ethanol under reduced pressure afforded a precipitate (100 mg.) which was recrystallised from aqueous ethanol to give pale-yellow prisms, m. p. 232° (decomp.) (Found: C, 47·7; H, 3·0; O, 31·9; Cl, 17·6; OMe, 6·4%; equiv., 208. Calc. for $C_{16}H_{12}O_8Cl_2$: C, 47·6; H, 3·0; O, 31·8; Cl, 17·6; 1OMe, 7·7%; equiv. for dibasic acid, 202). Erdin hydrate prepared from erdin [1d] which had been isolated from the culture fluid of *Aspergillus terreus* Thom (Ac.45) caused no depression of m. p. on admixture with this product. The compounds had identical infrared spectra and gave the same methylation product, m. p. 121°, on treatment with sodium hydroxide solution and dimethyl sulphate.

(b) *With sulphuric acid.* Dihydrogeodoxin (94 mg.) was suspended in 80% sulphuric acid (5 c.c.) and heated slowly to 125°. During 5 min. at this temperature the solution darkened and effervesced. It was cooled rapidly and diluted with water (15 c.c.), yielding a green precipitate (60 mg.) which was purified by washing with sodium hydrogen carbonate solution to remove tar. The major constituent (20 mg.) was obtained from the neutral residue by crystallisation from chloroform–acetone, and from ethanol, as yellow needles, m. p. 317—319° (decomp.) (Found: C, 53·2; H, 3·0; O, 23·7; Cl, 21·2; OMe, 9·8. $C_{15}H_{10}O_5Cl_2$ requires C, 52·8; H, 2·9; O, 23·5; Cl, 20·8, 1OMe, 9·1%). The ultraviolet and infrared spectra are in agreement with the formulation of this compound as 5,7-*dichloro*-2-*hydroxy*-4-*methoxy*-6-*methylxanthone*, $\lambda_{max.}$ 242 (shoulder), 270, 295 (shoulder), 330, 402 mμ (log ε, 3·8, 4·2, 3·3, 3·1, 3·3); the infrared spectrum includes bands at 1640, 1610 (CO), and 1590 cm.$^{-1}$ (aromatic C=C); cf. xanthone,[2] $\lambda_{max.}$ 243, 263, 288, 333 mμ (log ε 4·6, 4·3, 3·7, 3·8) as well as infrared bands at 1660, 1610, and 1585 cm.$^{-1}$ (chloroform solution).

In a second experiment with similar quantities, the green precipitate was dried and sublimed (110°/0·1 mm.) to give a white sublimate (6 mg.), m. p. and mixed m. p. with *methyl* 2,5-*dihydroxy*-3-*methoxybenzoate*, 163—166°. The authentic compound was prepared from the corresponding acid [9] by esterification with dimethyl sulphate and sodium hydrogen carbonate in acetone (Found: C, 54·6; H, 5·1; OMe, 32·3. $C_9H_{10}O_5$ requires C, 54·5; H, 5·1; 2OMe, 31·3%). The products obtained by synthesis and degradation gave identical infrared spectra.

The aqueous solution which remained after removal of the green precipitate was extracted with ether which gave, on evaporation, tarry acid (25 mg.). Sublimation (80°/0·1 mm.) led to the separation of the major constituent, m. p. 170°, which was identified as 2,4-dichloro-orcinol by mixed m. p. determination and comparison of the infrared spectrum with that of authentic material.

DEPARTMENT OF CHEMISTRY, UNIVERSITY COLLEGE OF SWANSEA.
UNIVERSITY COLLEGE OF THE WEST INDIES, JAMAICA. [*Received, March 20th*, 1959.]

[9] Schock and Tabern, *J. Org. Chem.*, 1951, **16**, 1772.