## 940. *The Biosynthesis of Phenols. Part II.* Asterric Acid, a Metabolic Product of* Aspergillus terreus *Thom.*

By R. F. Curtis, C. H. Hassall, D. W. Jones, and T. W. Williams.

Asterric acid, $C_{17}H_{16}O_8$, has been isolated from the culture fluid of *Aspergillus terreus* Thom. Degradation experiments lead to its formulation as (IVa). It has been synthesised from sulochrin by a route that probably simulates that involved in its biosynthesis.

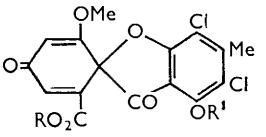
Particular strains of *Aspergillus terreus* Thom. produce the unusual chlorinated metabolites geodin (Ia), erdin [1] (II), and geodoxin [2] (III). During examination of the metabolic products of a new strain of this species it was found that another metabolite which is related to these three compounds could be isolated. It was expected that the elucidation of the structure of this new product, which has been named asterric acid, would contribute to our understanding of the mode of biosynthesis of this group of phenolic compounds.

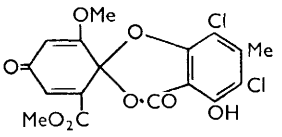
Asterric acid, $C_{17}H_{16}O_8$, behaved on titration as a monocarboxylic, phenolic acid. In accordance with this it yielded diacetyl and di-*O*-methyl derivatives of the acid and corresponding derivatives of the methyl ester. Zeisel estimations indicated two methoxyl groups in asterric acid. One of these was lost on hydrolysis with alkali which gave a dicarboxylic acid. From these facts it was evident that asterric acid contained two free

(I)a: R = Me, R$^1$ = H

(I)b: R = R$^1$ = Me

(II): R = R$^1$ = H




(III)

phenolic hydroxyl, one carboxyl, one methoxycarbonyl, and one methoxyl group. One oxygen atom remained undefined. Since it resisted catalytic hydrogenation at room temperature asterric acid evidently contained no ethylenic linkage. It failed to react with the usual carbonyl reagents. Kuhn–Roth estimation indicated a single *C*-methyl group. This evidence suggested that asterric acid might be a complex diphenyl ether related in structure to geodin and geodoxin. The ultraviolet absorption spectrum was in agreement with this possibility.

Accordingly asterric acid was treated with sulphuric acid under conditions which had proved fruitful in our studies on dihydrogeodoxin.[2] This gave a mixture of products from which, after methylation, trimethylnorgeodin A and B were isolated and identified by comparison with authentic material. The xanthone structures (Va) and (VIa) have been assigned to norgeodin A and norgeodin B respectively,[1] and we have confirmed the structure (Va) for norgeodin A by showing that it can be synthesised by the interaction of 2,3,5-trihydroxybenzoic acid and 3,5-dihydroxytoluene in the presence of zinc chloride and phosphorus oxychloride, a method known to give substitution in the 4-position of the 3,5-dihydroxytoluene nucleus.

These results established the relationship of asterric acid to geodin and, through this, largely defined the orientation of substituent groupings. The fact that the trimethylnorgeodin A and B (Vb, VIb) were formed directly from methyl di-*O*-methylasterrate (IVb) confirmed the presence of a diphenyl ether bridge in asterric acid itself and this was given additional support by the isolation of a further xanthone, presumably (Vc) by sublimation of the dicarboxylic acid (IVe) obtained on alkaline hydrolysis of asterric acid. We were

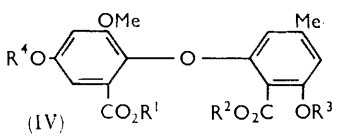
* The paper, *J.*, 1959, 2831, is regarded as Part I.

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

[2] Hassall and McMorris, *J.*, 1959, 2831.

led by this evidence and the knowledge of the structures of geodin and geodoxin to regard (IVa) as likely for asterric acid.

The orientation of substituents in this structure (IVa) is strikingly similar to that in sulochrin (VII), a metabolite of the fungus *Oospora sulphurea-ochracea*.[3] This suggested that sulochrin might be a precursor of asterric acid. It appeared possible that it could be converted *in vivo* by intramolecular free-radical, oxidative coupling into 4-hydroxy-2′-methoxy-6′-methoxycarbonyl-6-methylgris-2′,5′-diene-3,4′-dione (VIII) which would undergo hydrolysis to asterric acid.

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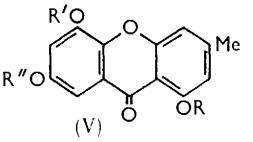


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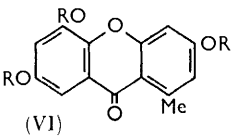


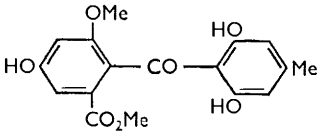

(VI)

(IV) a: $R^1$ = Me; $R^2$ = $R^3$ = $R^4$ = H
(IV) b: $R^1$ = $R^2$ = $R^3$ = $R^4$ = Me
(IV) c: $R^1$ = $R^2$ = H; $R^3$ = $R^4$ = Me
(IV) d: $R^1$ = $R^2$ = Me; $R^3$ = $R^4$ = H
(IV) e: $R^1$ = $R^2$ = $R^3$ = $R^4$ = H

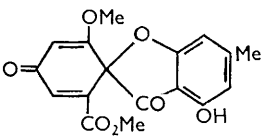
(V) a: R = R′ = R″ = H
(V) b: R = R′ = R″ = Me
(V) c: R = R″ = H; R′ = Me

(VI) a: R = H
(VI) b: R = Me

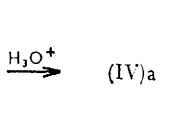
This route has been followed *in vitro*.[4] The first stage utilised oxidation of sulochrin with alkaline potassium ferricyanide, as a result of the observation [5] that geodin methyl ether (Ib) may be prepared in this way from the related 2,4′-dihydroxybenzophenone derivative. The product from sulochrin had properties expected for the spirocoumaran-3-one (VIII). The carbonyl frequencies in the infrared absorption spectrum correspond to those observed in geodin.[1] The reactive 1,4-dienone system accounts for the reaction with



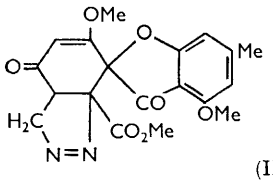

(VII)



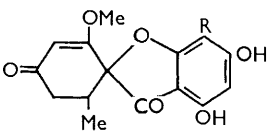

(VIII)




(IV)a




(IX)




(X)

(X) a: R = Cl
(X) b: R = Br
(X) c: R = H

diazomethane to give a pyrazoline which probably has the constitution (IX), similar to that of the corresponding geodin derivative. Acid-catalysed hydrolysis of the spiran ester (VIII) gives a good yield of asterric acid.

This synthesis confirms the structure (IVa) for asterric acid. It also favours the proposal that, in the natural process, the products sulochrin (VII), geodin (Ia), asterric acid (IVa) and geodoxin (III), are produced from one another in turn. It is assumed, in this scheme, that the chlorine atoms in geodin and geodoxin are introduced in secondary reactions. This would be analogous to the case of griseofulvin (Xa) where it has been shown that a bromo- (Xb) and a dechloro-analogue (Xc) are formed when the culture

[3] Nishikawa, *Acta Phytochim., Tokyo*, 1939, **11**, 167.
[4] Curtis, Hassall, and Jones, *Chem. and Ind.*, 1959, 1283.
[5] Scott, *Proc. Chem. Soc.*, 1958, 195.

medium of the fungus *Penicillium griseofulvum* is modified.[6] The conversion of asterric acid into an analogue of geodoxin would involve intramolecular oxidative coupling. Model reactions of this type have been achieved *in vitro*.[7]

## Experimental

M. p.s were determined by means of a Kofler block. Ultraviolet spectra were determined for EtOH solutions in a Beckmann spectrophotometer DU. 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, Swansea) were measured for potassium bromide discs by using a double-beam Grubb–Parsons spectrometer.

*Isolation of Asterric Acid* (IVa).—The strain of *Aspergillus terreus* which was used in these experiments was obtained, by selection, from the mixture of variants produced when *Aspergillus terreus* Thom. (No. 45, L.T.C.C.) was allowed to grow at 30° for long periods on Czapek–Dox agar containing 2% of glucose. Slopes of the selected strain which had been growing for 10 days were shaken with sterilised water, and the spore suspension was then used to inoculate sterile Czapek–Dox medium (Raistrick and Smith modification [8]) (approx. 200 ml.) in flat-sided bottles (approx. 1 l. capacity). These liquid cultures were allowed to grow at 28—30° for 30 days.

The dark brown culture fluid (8 l. batch) was separated from the mycelium. After adjustment to pH 6·6, suspended matter was removed by means of a Sharples supercentrifuge, and the fluid was stirred with activated charcoal (25 g.). After 30 min. the charcoal was removed by filtration and dried. The process was repeated with two further 25 g. portions of charcoal. The combined charcoal fractions were extracted with methanol (4 × 500 ml.). Evaporation gave a light brown powder (1·8 g.).

The filtrate obtained when this powder (15·7 g.) was boiled with ethyl acetate (100 ml.) and filtered was allowed to crystallise in two fractions by adding light petroleum (b. p. 60—80°) in two portions (2 × 50 ml.).

Fraction 1, yellow (1·63 g.; m. p. 188—202°), gave a chloroform-insoluble portion which was recrystallised from ethyl acetate–light petroleum to give (±)-erdin (II), pale yellow needles, m. p. 213° (decomp.), characterised as the (±)-pyrazoline derivative,[1] m. p. 157—158°, and as dihydroerdin,[1] m. p. 256°.

Fraction 2, colourless crystals (9·29 g.; m. p. 196—204°), was recrystallised twice from ethyl acetate–light petroleum to give *asterric acid* (IVa), colourless needles (5·26 g.), m. p. 209—210° (decomp.) [Found: C, 59·1; H, 4·7; *C*-Me, 4·7; OMe, 17·9%; *M* (Rast), 333; equiv., 360. $C_{17}H_{16}O_8$ requires C, 58·6; H, 4·6; 1*C*-Me, 4·3; 2OMe, 17·8%; *M* and equiv. (monobasic acid), 348], $[\alpha]_D$ 0° (*c* 2·74 in acetone), $\lambda_{max.}$ 250, 317 mμ (log ε 4·0; 3·83). The infrared spectrum showed bands at 1686 (methoxycarbonyl) and 1653 cm.$^{-1}$ (*o*-hydroxyphenyl-$CO_2H$).

Asterric acid gave a purple ferric chloride reaction, coupled with diazotised sulphanilic acid, and gave a positive Gibbs reaction.[9] No uptake of hydrogen occurred with palladium–charcoal at room temperature and pressure during 2 hr., and there was no reaction with 2,4-dinitrophenylhydrazine.

With acetic anhydride–pyridine at room temperature in 2 days it gave a *diacetyl derivative* which recrystallised from ethyl acetate–light petroleum (b. p. 60—80°) as colourless prisms, m. p. 147—148° [Found: C, 58·4; H, 4·6; O, 36·8; OMe, 14·3; Ac, 18·9, 19·4%; equiv., 403. $C_{21}H_{20}O_{10}$ requires C, 58·3; H, 4·7; O, 37·0; 2OMe, 14·35; 2Ac, 19·9%; equiv. (for a monobasic acid), 432], $\lambda_{max.}$ 295 mμ (log ε 3·71).

*Methyl Di-O-methylasterrate* (IVb).—Asterric acid (200 mg.) in methanol (50 ml.) with excess of diazomethane in ether gave, after 2 days, *methyl di-O-methylasterrate* (IVb) (224 mg.), m. p. 136—140°. It recrystallised from ethyl acetate–light petroleum (b. p. 60—80°) as short rods, m. p. 148—149° [Found: C, 61·9; H, 5·5; O, 32·8; OMe, 40·4%; *M* (Rast), 378. $C_{20}H_{22}O_8$ requires C, 61·5; H, 5·7; O, 32·8; 5OMe, 39·7%; *M*, 390], $\lambda_{max.}$ 285, 307 mμ (log ε 3·6, 3·65). The infrared spectrum showed no hydroxyl absorption and the compound gave no colour with ferric chloride. With dimethyl sulphate in acetone–sodium hydroxide it gave identical material.

[6] Macmillan, *J.*, 1954, 2585.
[7] Hassall and Lewis, unpublished work.
[8] Raistrick and Smith, *Biochem. J.*, 1936, **30**, 1315.
[9] King, King, and Manning, *J.*, 1957, 563.

The foregoing methyl ester (150 mg.) was boiled with 50% methanolic 2N-sodium hydroxide for 4 hr. Working up gave the corresponding *dicarboxylic acid* (IVc) (108 mg.), prisms [from ethyl acetate–light petroleum (b. p. 60—80°)], m. p. 208—209° [Found: C, 59·2; H, 5·0; O, 35·5; OMe, 27·3%; *M* (Rast), 387; equiv., 185. $C_{18}H_{18}O_8$ requires C, 59·7; H, 5·0; O, 35·3; 3OMe, 25·7%; *M*, 362; equiv. (for a dibasic acid), 181], $\lambda_{max.}$ 297 mμ (log ε 3·68).

*Methyl Asterrate* (IVd).—Asterric acid (100 mg.) was suspended in dry ether (6 ml.) and treated with excess of diazomethane. After the solid had dissolved the mixture was set aside for 10 min. Evaporation gave the *methyl ester* (IVd) (104 mg.), m. p. 176—180°; it recrystallised from ethyl acetate–light petroleum as rods, m. p. 185—186° (Found: C, 59·8; H, 5·1; O, 34·8; OMe, 25·3. $C_{18}H_{18}O_8$ requires C, 59·7; H, 5·0; O, 35·3; 3-OMe, 25·7%), $\lambda_{max.}$ 251, 317 mμ (log ε 4·16, 3·90). The infrared spectrum showed maxima at 3413 (OH), 1698 (aromatic $CO_2Me$), and 1653 cm.$^{-1}$ (*o*-hydroxyphenyl-$CO_2Me$).

*Hydrolysis of Asterric Acid.*—Asterric acid (200 mg.) was heated with 0·5N-sodium hydroxide (10 ml.) for 45 min. Acidification gave the corresponding *dicarboxylic acid* (IVe) (196 mg.), m. p. 209°, that recrystallised from ethyl acetate–light petroleum (b. p. 60—80°) as colourless needles, m. p. 246° [Found: C, 57·8; H, 4·3; O, 37·7; OMe, 9·2%; equiv., 155. $C_{16}H_{14}O_8$ requires C, 57·5; H, 4·2; O, 38·3; 1OMe, 9·2%; equiv. (for a dibasic acid), 167], $\lambda_{max.}$ 310 mμ (log ε 3·98).

Methylation with excess of ethereal diazomethane gave methyl di-*O*-methylasterrate (IVb), m. p. 149—150°, not depressed by authentic material.

*Monomethylnorgeodin A* (Vc).—The foregoing dicarboxylic acid (IVe) (30 mg.) was heated at 230° in a high vacuum. A yellow sublimate (10 mg.) was obtained. Recrystallisation from aqueous ethanol gave *methylnorgeodin A* (6 mg.) as yellow needles, m. p. 300° (decomp.) (Found: C, 65·8; H, 4·5; O, 29·3. $C_{15}H_{12}O_5$ requires C, 66·2; H, 4·4; O, 29·4%), $\lambda_{max.}$ 263, 295, 325, 390 mμ (log ε 4·80, 4·06, 3·84, 3·92). The infrared spectrum showed bands at 1650 (xanthone-carbonyl), 1621 and 1587 cm.$^{-1}$ (aromatic C=C). The compound gave a dark green colour with ethanolic ferric chloride.

*Trimethylnorgeodin A* (Vb) *and B* (VIb).—(a) *From erdin* (II). The crude mixture of norgeodins obtained from erdin and asterric acid could not be purified by fractional crystallisation as described by Calam *et al.*[10] As a result, the compounds were isolated as their methyl derivatives.

Erdin (117 mg.) was heated with hydriodic acid (*d* 1·7; 2 ml.) for 30 min.; the mixture was cooled and diluted with water (1 ml.). The crude precipitate (66 mg.) was treated in acetone with dimethyl sulphate and sodium hydroxide (8%) in the usual way. The pale yellow product was collected, dissolved in benzene (100 ml.), and passed down a column of acid-washed alumina. The first band (visible in ultraviolet light) obtained by elution with benzene (125 ml.) gave trimethylnorgeodin B (52 mg.), m. p. 203—205°, that recrystallised from ethyl acetate–light petroleum (b. p. 60—80°) as colourless needles, m. p. 208—209° (Calam *et al.*[10] record m. p. 198—199°) (Found: C, 68·2; H, 5·5; O, 26·6; OMe, 30·9; *C*-Me, 4·85. Calc. for $C_{17}H_{16}O_5$: C, 68·0; H, 5·4; O, 26·6; 3OMe, 31·0; 1*C*-Me, 5·0%), $\lambda_{max.}$ 257, 284, 307, 365 mμ (log ε 4·5, 4·22, 4·08, 3·7). The infrared spectrum showed bands at 1645 (xanthone-carbonyl), 1610 and 1575 cm.$^{-1}$ (aromatic C=C) with no hydroxyl absorption. There was no colour with ferric chloride.

Further elution (300 ml.) gave trimethylnorgeodin A (17 mg.), m. p. 228°, that recrystallised from ethyl acetate–light petroleum (b. p. 60—80°) as pale yellow rods, m. p. 228—229° (Calam *et al.*[10] record m. p. 216—217°) (Found: C, 67·5; H, 5·4; OMe, 30·3; *C*-Me, 5·1. Calc. for $C_{17}H_{16}O_5$: C, 68·0; H, 5·4; 3OMe, 31·0; 1*C*-Me, 5·0%); $\lambda_{max.}$ 258, 292, 305, 366 mμ (log ε 4·57, 3·79, 3·7, 3·64). The infrared spectrum showed bands at 1646 (xanthone carbonyl), 1609 and 1575 cm.$^{-1}$ (aromatic C=C) with no hydroxyl absorption. There was no colour with ferric chloride.

(b) *From asterric acid* (IVa). Asterric acid (100 mg.), treated with hydriodic acid as for (±)-erdin, gave trimethylnorgeodin B (61 mg.), m. p. 208°, and A (15 mg.), m. p. 228—229°.

(c) *From methyl di-O-methyl asterrate* (IVb). Methyl di-*O*-methylasterrate (100 mg.) with 65% sulphuric acid (3 ml.) at 150° gave a similar yield of trimethylnorgeodin A and B. The samples of trimethylnorgeodin A and B which were prepared by the three procedures were shown to be identical by infrared absorption spectra and mixed m. p. determinations.

[10] Calam, Clutterbuck, Oxford, and Raistrick, *Biochem. J.*, 1947, **41**, 458.

*Synthesis of Trimethylnorgeodin A* (Vb).—2,3,5-Trihydroxybenzoic acid [11] (1·4 g.), orcinol (1·7 g.), freshly fused zinc chloride (5 g.), and phosphorus oxychloride (11 ml.) were heated at 70° for 2 hr. according to the general procedure of Grover, Shah, and Shah.[12] The cooled product was poured into water and extracted with ether (3 × 50 ml.) which was washed with sodium hydrogen carbonate solution, dried, and evaporated to give a yellow oil (1·4 g.). This crystallised from aqueous ethanol to give norgeodin A (47 mg.), pale yellow needles, m. p. 305° (decomp.) (Calam *et al.*[10] give m. p. 305°).

With dimethyl sulphate and alkali in the usual way it gave trimethylnorgeodin A, m. p. 228—229° with no depression on mixed m. p. with the samples already described. Infrared and ultraviolet spectra were identical.

4-*Hydroxy*-2′-*methoxy*-6′-*methoxycarbonyl*-6-*methylgris*-2′,5′-*dien*-3,4′-*dione* (VIII).—4,2′,5′-Trihydroxy-2-methoxy-5-methoxycarbonyl-4′-methylbenzophenone (VII) (sulochrin) (1·0 g.), which had been prepared from the culture fluid of *Oospora sulphurea-ochracea*,[3] was dissolved in de-aerated water (60 ml.) containing sodium carbonate (2·5 g.). The solution was cooled to 0° and potassium ferricyanide (3 g.) in water (80 ml.) was added with vigorous stirring under nitrogen during 30 min. After a further 30 min., the yellow solution was acidified with 4N-hydrochloric acid and shaken with ether. The ether extract was evaporated to give a light, brown solid, which was purified by chromatography in benzene on acid-washed, deactivated alumina. From the benzene eluate (2·5 l.), an amorphous solid (400 mg.) was obtained, having m. p. 173—180°. Repeated recrystallisation from benzene–light petroleum (b. p. 60—80°) gave 4-*hydroxy*-2′-*methoxy*-6′-*methoxycarbonyl*-6-*methylgris*-2′,5′-*dien*-3,4′-*dione* (VIII) as very pale yellow prisms (200 mg.), m. p. 189—191° [Found: C, 61·9; H, 4·2; O, 34·0; OMe, 17·0%; *M* (Rast), 341. $C_{17}H_{14}O_7$ requires C, 61·8; H, 4·3; O, 33·9; 2OMe, 18·8%; *M*, 330], $\lambda_{max.}$ 284 mμ (log ε 4·2). Infrared maxima were at 1739 (C=O in spirocoumaran), 1709 (methoxycarbonyl), 1656 (1,4-dienone), and 1623 cm.$^{-1}$ (enone). The compound gave a green colour with ferric chloride.

(a) *Pyrazoline derivative* (IX). The foregoing dienone (40 mg.) in dioxan (5 ml.) was treated with excess of ethereal diazomethane and kept at 0° for 48 hr. Evaporation and recrystallisation from aqueous methanol gave the *pyrazoline derivative* (IX) as colourless plates (10 mg.), m. p. 235—240° (decomp.) (Found: C, 59·4; H, 4·7; O, 28·5; N, 6·8. $C_{19}H_{18}N_2O_7$ requires C, 59·1; H, 4·7; O, 29·0; N, 7·25%), $\lambda_{max.}$ 275, 330 mμ (log ε 4·10; 3·43).

(b) *Action of sulphuric acid.* The dienone (50 mg.) was dissolved in 65% sulphuric acid (3 ml.) and set aside for 5 min. Dilution with water (7 ml.) gave a solid which crystallised from ethyl acetate–light petroleum (b. p. 60—80°) to give asterric acid (IVa) (33 mg.), m. p. and mixed m. p. 209—210°. The ultraviolet and infrared spectra of the synthetic and the natural asterric acid were identical.

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Department of Chemistry, University College, Swansea. [*Received, May 20th*, 1960.]

[11] Corbett, Hassall, Johnson, and Todd, *J.*, 1950, 1.
[12] Grover, Shah, and Shah, *J.*, 1955, 3982.