Studies in the Biochemistry of Micro-organisms

115. METABOLITES OF *PENICILLIUM FREQUENTANS* WESTLING: ISOLATION OF SULOCHRIN, ASTERRIC ACID, (+)-BISDECHLOROGEODIN AND TWO NEW SUBSTITUTED ANTHRAQUINONES, QUESTIN AND QUESTINOL*

By A. MAHMOODIAN AND C. E. STICKINGS

Department of Biochemistry, London School of Hygiene and Tropical Medicine (University of London), Keppel Street, London, W.C. 1

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The mould *Penicillium frequentans* Westling is, as its name implies, of very common occurrence on all kinds of material. According to Raper & Thom (1949), the Monoverticillate section of the genus *Penicillium* includes a *P. frequentans* series of three species, namely the type species, *P. spinulosum* Thom and a less common species, *P. purpurrescens* (Sopp) n.comb.

Wehmer's classical studies of citric acid production by fungi led him to give the generic name *Citromyces* to the moulds which produced this compound. However, these were later grouped in the genus *Penicillium*, mainly in the *P. frequentans* series (Raper & Thom, 1949). Hetherington & Raistrick (1931) isolated a yellow crystalline substance from several strains of this series, and named it citromycetin. The structure (I) was finally determined by Cavill, Robertson & Whalley (1950).

(I) Citromycetin

Curtis, Hemming & Smith (1951) isolated an antifungal metabolic product, frequentin, from a strain of *P. frequentans* which was also found to produce palitantin, already known as a metabolite of *P. palitans* Westling (Birkinshaw & Raistrick, 1936). The molecular formula and configuration of palitantin (II) were determined by Bowden, Lythgoe & Marsden (1959). Frequentin has recently been shown to be a dehydropalitantin in which the $-CH_2\cdot OH$ group is replaced by $-CHO$ (Grove & Tidd, 1963).

* Part 114: Birkinshaw, Luckner, Mohammed, Mothes & Stickings (1963).

(II) Palitantin

Some strains of *P. spinulosum* were shown by Birkinshaw & Raistrick (1931) to yield a deep-purple pigment which they named spinulosin. It is a substituted benzoquinone (III).

(III) Spinulosin

A morphologically typical strain of *P. frequentans* was isolated a few years ago in this Department, and it was noted that colourless crystalline material could be extracted by ether from the culture filtrate, after growth on Czapek-Dox medium. It was later found that much larger amounts of material were extractable from the mycelium, and a complex mixture of products was obtained. We have not yet separated all the components of the mixture as shown by paper chromatography, but the present paper describes five distinct metabolites that have been isolated, characterized and identified. None of them has previously been reported from *P. frequentans*, although two have been isolated from other moulds, and a third is a dextro-rotatory substance previously isolated from another fungus in its laevorotatory form. The remaining two compounds are new.

A preliminary account of this work has already appeared (Stickings & Mahmoodian, 1962).

The mould grows rapidly on Czapek-Dox medium, giving a complete felt in 3 or 4 days at 24°, and begins to spore immediately. The glucose concentration is decreased to 0.8% in 21 days.

Mycelium from cultures aged 3–5 weeks was dried in a vacuum oven at 45–50°, and extracted first with light petroleum, to remove fats, and then with ether in a percolator. The ether extract deposited a yellow-brown solid (S) which was filtered, and more crops were obtained on further extraction. Evaporation of the ether liquors left a dark gummy semi-solid residue (R).

SULOCHRIN

The solid S was fractionated by extraction with hot chloroform. The chloroform-insoluble residue was crystallized from methanol and then from ethyl acetate, giving pale-yellow crystals, m.p. 266–267°, which gave a green colour with ethanolic ferric chloride. Microanalysis gave the empirical formula $C_{17}H_{16}O_7$, containing 2 methoxyl groups; methylation yielded a dimethyl derivative $C_{19}H_{20}O_7$, m.p. 158–159°, containing four methoxyl groups,

and acetylation gave a triacetate $C_{23}H_{22}O_{10}$, m.p. 164–165°. These properties correspond very closely to those of sulochrin (IV) a metabolite of *Oospora sulphurea-ochracea* van Beyma, isolated by Nishikawa (1936, 1937, 1939), and comparison of the two substances by mixed melting point and infra-red-absorption spectra have shown their identity.

The chloroform-soluble fraction was also purified by crystallization from methanol and sublimation, and yielded a colourless compound, $C_{18}H_{18}O_8$, m.p. 191–193°, containing three methoxyl groups. We identified this as another metabolite of *O. sulphurea-ochracea*, isolated by Nishikawa (1936) and named trimethylosoic acid I (Nishikawa, 1942), and recently identified as the methyl ester of asteric acid (V) (Curtis, Hassall, Natori & Nishikawa, 1961; Natori & Nishikawa, 1962a); asteric acid (VI) is a metabolite of *Aspergillus terreus* Thom (Curtis, Hassall, Jones & Williams, 1960). However, paper chromatography of mycelial extracts of *P. frequentans*, carried out at a later stage of our investigations, revealed no trace of methyl asterate. We conclude that it arose as an artifact due to the use of methanol during purification, since the metabolite bisdechlorogeodin, described below, readily yields methyl asterate in the presence of methanol.

BISDECHLOROGEODIN

When the crude yellow-brown solid S was boiled with benzene, and the benzene evaporated to a small volume, orange-yellow crystals were formed, which on repeated crystallization from ethyl acetate yielded pale-yellow prisms of somewhat indefinite m.p. between 135° and 150°. Analysis of this substance led to the formula $C_{17}H_{16}O_8$, but on careful drying 1 molecule of water was lost, and the anhydrous $C_{17}H_{14}O_7$, m.p. 170–173°, was formed, containing two methoxyl groups. The chemical properties of this compound were identical with those of a third metabolite of *O. sulphurea-ochracea*, called by Nishikawa 'Compound E', and now identified as bisdechlorogeodin, or 4-hydroxy-2'-methoxy-6'-methoxycarbonyl-6-methylgris-2',5'-diene-3,4'-dione (VII) (Natori & Nishikawa, 1962*a*). Thus our compound is readily converted into methyl asterrate on boiling with methanol, and into asteric acid by dissolving in sulphuric acid and pouring into water, or by standing for some hours in sodium carbonate solution. Its ultraviolet-absorption spectrum was also in agreement with this structure. However, the *P. frequentans* metabolite is dextrorotatory, $[\alpha]_D +188^\circ$ in chloroform and $+186^\circ$ in ethanol, whereas the rotation recorded for the *O. sulphurea-ochracea* product was $[\alpha]_D -66^\circ$. We have been in communication with Dr Natori, who has kindly carried out a comparison of infrared-absorption spectra of the two samples, and there is no doubt of their identity other than in optical activity. In view of our results, Dr Natori re-examined the remaining small sample of 'Compound E', m.p. 140–144°. After drying at 90–100°, it had m.p. 143–165°, $[\alpha]_D -103 \pm 5^\circ$ (c 0.1 in ethanol); a mixture with our sample melted at 153–182°, that is, higher than either component (S. Natori, personal communication; see also Natori & Nishikawa, 1962*b*). Synthetic ($\pm$)-bisdechlorogeodin has been prepared by Curtis *et al.* (1960), and melts at 189–191°. It seems probable that the samples of 'Compound E' used for determination of rotation were not the pure (–)-isomer;

they were derived from samples several years old, and may have partly racemized. Unfortunately Dr Natori has so far been unable to obtain more 'Compound E' from available cultures of *O. sulphurea-ochracea*. However, it seems certain that the two species produce bisdechlorogeodin of opposite sign.

Acetylation of (+)-bisdechlorogeodin yielded a monoacetate. A monomethyl ether was obtained by using the methyl iodide-potassium carbonate-acetone method; with diazomethane a pyrazoline derivative was formed. These products are expected by analogy with geodin (Raistrick & Smith, 1936; Barton & Scott, 1958). In both series the acetate and methyl ether are dextrorotatory, whereas the pyrazoline derivative is laevorotatory.

QUESTIN

Paper chromatography of the crude solid S with butanol-borate buffer, pH 9.2, revealed two pink spots. Separation of the two compounds responsible was achieved by adsorption on a magnesium oxide column, followed by countercurrent distribution between ethyl acetate and a sodium carbonate-bicarbonate buffer. In this way two coloured bands could be clearly separated, each giving a red colour in the buffer layer: a fast-moving band A and a slower-moving band B. These fractions were also isolated from the gummy residue R.

From band A, a pure compound was obtained, which separated in two forms from ethyl acetate: either bright-yellow or orange-brown needles, both melting at 301–303°. We have called this substance *questin*. Analysis indicated an empirical formula $C_{16}H_{12}O_5$ with 1 methoxyl group. It was soluble in aqueous sodium carbonate or hydroxide to give a deep-red colour, and in conc. sulphuric acid gave a red colour similar to that given by emodin, $C_{15}H_{10}O_5$ (VIII; $R^1 = R^2 = R^3 = H$). On acetylation it gave a diacetate, m.p. 160–162°, and, when methylated by the dimethyl sulphate-potassium carbonate-acetone method, a dimethyl derivative, m.p. 230–232°, which was shown to be identical

(VIII)

$R^1 = R^2 = R^3 = H$: Emodin
 $R^3 = Me, R^1 = R^2 = H$: Physcion,
                                   parietin
 $R^2 = Me, R^1 = R^3 = H$: Questin

(IX)

$R^1 = R^2 = R^3 = R^4 = H$: Citreorosein, ω -hydroxyemodin
 $R^4 = Me, R^1 = R^2 = R^3 = H$: Teloschistin, fallacinal
 $R^2 = Me, R^1 = R^3 = R^4 = H$: Roseopurpurin, carviolin
 $R^3 = Me, R^1 = R^2 = R^4 = H$: Questinol

with emodin trimethyl ether (VIII; $R^1 = R^2 = R^3 = \text{Me}$). It was therefore clear that questin is a monomethyl ether of emodin, and this was confirmed by demethylation of questin in conc. sulphuric acid, which yielded emodin itself.

By reaction with diazomethane a monomethyl questin was obtained, m.p. 208–210°. This was oxidized by hot alkaline permanganate to give a 25% yield of 3,5-dimethoxyphthalic acid, which was converted into the anhydride for further identification.

The monomethylquestin is therefore emodin 5,7-dimethyl ether (VIII; $R^1 = \text{H}, R^2 = R^3 = \text{Me}$), and questin itself is either the 5- or the 7-methyl ether. The latter compound is physcion (parietin), m.p. 203–207°, long known as a fungal and lichen product (Raistrick, Robinson & Todd, 1937). Apart from the large difference in melting point, other properties distinguish the two substances. Thus questin dissolves readily in aqueous sodium carbonate whereas physcion does not, and the infrared-absorption spectra are different.

Questin is therefore emodin 5-methyl ether, or 4,7-dihydroxy-5-methoxy-2-methylanthraquinone (VIII; $R^1 = R^3 = \text{H}, R^2 = \text{Me}$).

QUESTINOL

From band B, a pure substance was separated as orange needles from methanol, m.p. 280–282°. Analysis gave the empirical formula $\text{C}_{16}\text{H}_{12}\text{O}_6$, with 1 methoxyl group, and colour reactions with alkali and conc. sulphuric acid were very similar to those of questin. Acetylation yielded a triacetate, m.p. 186–188°, and methylation by dimethyl sulphate in aqueous sodium hydroxide yielded a trimethyl derivative, m.p. 188–190°, which was shown to be identical with ω -hydroxyemodin tetramethyl ether (IX; $R^1 = R^2 = R^3 = R^4 = \text{Me}$). The metabolite is therefore a monomethyl ether of ω -hydroxyemodin, and we have called it *questinol*.

The position of the methoxyl group was settled in a manner analogous to that used for questin. A monomethylquestinol yielded 3,5-dimethoxyphthalic acid on oxidation, thus proving that questinol was either the 5- or the 7-methyl ether. The latter compound has been obtained from lichens, as teloschistin from *Teloschistes flavicans* (Seshadri & Subramanian, 1949) and as fallacinol from *Xanthoria fallax* (Asano & Fuziwara, 1936; Murakami, 1956). There has been some dispute over the melting point of this compound: Neelakantan & Seshadri (1954) give m.p. 245–247°, and Murakami (1956) gives m.p. 236–237°. The compound was also prepared by methylation of ω -hydroxyemodin with methyl iodide and sodium methoxide in methanol (Anslow, Breen & Raistrick, 1940): the authors record m.p. 229–231°, and the

substance was later identified with teloschistin (Neelakantan, Rangaswami, Seshadri & Subramanian, 1951) and fallacinol (Murakami, 1956). All these melting points are well below that of questinol (280–282°), and the non-identity was confirmed by differences in the infrared-absorption spectrum between questinol and a sample prepared by Anslow *et al.* (1940) and by the solubility of questinol in aqueous sodium carbonate.

Questinol is therefore ω -hydroxyquestin, or ω -hydroxyemodin 5-methyl ether (4,7-dihydroxy-2-hydroxymethyl-5-methoxyanthraquinone) (IX; $R^1 = R^2 = R^4 = \text{H}, R^3 = \text{Me}$).

ASTERRIC ACID

From the metabolism solution, by concentration and acidification, a crude yellow precipitate was obtained. Part of this was soluble in chloroform, and this fraction on crystallization from ethyl acetate yielded a colourless substance, m.p. 213–215°, which was identified as asterric acid (VI).

DISCUSSION

The anthraquinones. Emodin, known for years as a plant product, has been obtained on only a few occasions from fungi. Kögl & Postowsky (1925) isolated it from the sporophores of the basidiomycete *Dermocybe (Cortinarius) sanguinea* Wulf. It was found also in *Polystictus versicolor* (Shimano, Taki & Gota, 1953), and Agosti, Birkinshaw & Chaplen (1962) have obtained it from laboratory cultures of an artificial mutant of the mould *Cladosporium fulvum*.

Substituted anthraquinones derived from emodin have, however, been known as fungal metabolites for a long time [see (VIII) and (IX)]. Thus physcion, emodin 7-methyl ether (VIII; $R^1 = R^2 = \text{H}, R^3 = \text{Me}$), was obtained from *A. glaucus* by Raistrick *et al.* (1937), and subsequently from other moulds; it was known earlier as a lichen and plant product. Emodin 4-methyl ether (VIII; $R^2 = R^3 = \text{H}, R^1 = \text{Me}$) has not been described. ω -Hydroxyemodin (IX; $R^1 = R^2 = R^3 = R^4 = \text{H}$) was isolated by Anslow *et al.* (1940) from *P. cyclopium*, and from *P. citreo-roseum* Dierckx by Posternak & Jacob (1940), who named it citreorosein. Its 7-methyl ether, teloschistin or fallacinol, has already been mentioned as a lichen product, but has not so far been found in fungi. ω -Hydroxyemodin 4-methyl ether (IX; $R^1 = R^3 = R^4 = \text{H}, R^2 = \text{Me}$) was isolated independently in two different Laboratories: as roseopurpurin from *P. roseo-purpureum* Dierckx (Posternak, 1940) and as carviolin from *P. carmino-violaceum* Dierckx (Hind, 1940*a, b*); the two species are now regarded as identical (Raper & Thom, 1949).

The two anthraquinones, questin and questinol, isolated from *P. frequentans* therefore represent two further additions to this series of substituted emodins.

Emodin is a good example of a structure derivable by self-condensation of a 'polyketomethylene' precursor as envisaged by Birch's acetate hypothesis (see Scheme 1), the only modifications required being oxidation of the resulting anthranol and decarboxylation. Tracer studies have shown that emodin obtained chemically from the dianthraquinone skyrin, a metabolite of *P. islandicum*, is in fact derived from acetate in a manner consistent with this pathway (Gatenbeck, 1958). Recent work suggests that, as with the fatty acids, the polyketomethylenes arise from one terminal molecule of acetyl-coenzyme A condensed with a number of molecules of malonyl-coenzyme A. The modified dianthraquinone, rugulosin, has been shown to incorporate malonate and acetate in agreement with such a route (Shibata & Ikekawa, 1962). No evidence exists as to the chemical nature of any intermediates between acetate (or malonate) and the anthraquinones.

Biosynthetic relationships of the products isolated from Penicillium frequentans. The isolation by Curtis *et al.* (1960) of asteric acid (VI) from cultures of *A. terreus*, an organism known to produce geodin (X), led them to propose that asteric acid was synthesized in Nature from sulochrin (V) via bisdechlorogeodin (VII). Though laboratory syntheses confirmed that this route was chemically possible, there has been no conclusive evidence of its occurrence *in vivo*. The isolation of all three compounds, together with related products, from *O. sulphurea-ochracea* by Natori & Nishikawa

(1962a) (see above) adds support to the biogenetic theory, and is further confirmed by the production of the same group of metabolites by a strain of *P. frequentans*, indicating a similar biosynthetic pathway in three distinct genera of lower fungi.

(X) Geodin

Sulochrin (V) has the acetate pattern of oxygen substitution, but no experimental evidence has been published on its biosynthesis. Aghoramurthy & Seshadri (1954) supposed that sulochrin was produced by a combination of 3,5-dihydroxyphthalic acid (or a methylated derivative) with orcinol. Thomas (1961) suggested branched-chain precursors for a large number of acetate-pattern products, including sulochrin. On the other hand, Gatenbeck (1960) pointed out that sulochrin could well arise by oxidative fission of emodin (Scheme 2, R = H), and quoted in support other cases in which there was experimental evidence of such oxidative breakdown of anthraquinones (see also Money, 1963).

The isolation of questin together with sulochrin from the same culture favours the last pathway, particularly as the methoxyl group is in the right position; the route would thus follow Scheme 2 (R = Me).

Scheme 1. Biosynthesis of emodin.

Scheme 2. Suggested biosynthesis of sulochrin (Gatenbeck, 1960).

EXPERIMENTAL

All melting points are corrected for the emergent stem. Methoxyl determinations are by one of us (A.M.), and all other microanalyses by Dr A. Schoeller, Germany. Ultra-violet-absorption spectra were determined with a Hilger Uvispek instrument, and infrared-absorption spectra on a Perkin-Elmer Infracord instrument. Infrared-absorption maxima are quoted for the ranges 3500–3000 and 1800–1500 cm^{-1} . Bands are strong unless designated as (w) weak or (m) moderate.

History of culture

The culture used was isolated in this Department by Mr G. Agosti in 1956 from a soil sample from Madras, India, and was given the catalogue no. GA. 816. It was identified as a typical *P. frequentans* Westling by Mr G. Smith of this Department. A subculture is maintained by the Commonwealth Mycological Institute, Kew, Surrey (CMI no. 96659).

Cultural conditions

Heavily sporing cultures were produced after a few days growth on carrot-agar slopes. Spore suspensions were made by shaking with sterile water, and inoculated into sterile Czapek-Dox medium of the following composition: glucose, 50.0 g.; NaNO_3 , 2.0 g.; KH_2PO_4 , 1.0 g.; KCl, 0.5 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g.; distilled water, 1 l. The medium was distributed in 350 ml. portions in 1 l. conical flasks plugged with cotton wool.

Batches of 50 flasks were incubated at 24° in the dark. The cultures grew rapidly, the glucose concentration being decreased to about 2% after 2 weeks and to 0.2% after 3½–4 weeks. The upper surface of the mycelium was green after a few days; the lower surface was pale brown, darkening later, with orange-coloured nodules appearing after 2 weeks. The culture fluid turned yellow, and then brown after 3 weeks.

Paper chromatography

Mycelial and culture-filtrate extracts were analysed with various solvent systems (see Table 1). In butanol saturated with aq. 2N-ammonia, two pink spots were observed, R_F 0.48 (questin) and 0.23 (questinol). On drying these faded to yellow, but could be made permanently pink by spraying with 2N- Na_2CO_3 . Similar spots were obtained with butanol saturated with borate buffer, pH 9.2.

Sulochrin and methyl asterrate have high R_F values in these two solvent systems, and can be detected by a diazotized sulphanilic acid–2N- Na_2CO_3 spray, which gives yellow–brown spots. These compounds can be separated by chromatography with toluene, or benzene–2N-acetic acid, in which sulochrin has R_F 0 and methyl asterrate has R_F 0.8–0.9. Asterric acid has R_F 0.3 in butanol–ammonia or butanol–borate buffer, pH 9.2. Bisdechlorogedin streaks badly in the butanol systems, but has R_F 0.8–0.95 in the toluene and CHCl_3 systems (except CHCl_3 – Na_2CO_3). Use of the CHCl_3 – Na_2CO_3 system clearly demonstrated the absence of methyl asterrate from mycelial extracts that had not been treated with methanol.

Extraction

Batches of 50 flasks were grown for 3–5 weeks and harvested in the usual way by separating the culture filtrate from the mycelium.

The culture filtrate from one batch grown for 27 days was adjusted to pH 7, concentrated to 1 l. in a climbing film evaporator and then acidified to pH 2. The yellow precipitate (5.3 g.) was removed and the solution was extracted with ethyl acetate (6 × 1 l.). The extracts were combined and evaporated, leaving an orange–brown gum (6 g.).

The mycelium was extracted by two methods:

Method A. The mycelium was dried in a vacuum oven at 40–50° (wt. 150 g.) and ground to a coarse powder. It was then extracted in a percolator, first with light petroleum (b.p. 40–60°) for 12 hr., then for several days with ether. The petroleum extracts contained lipid material

Table 1. *Paper-chromatographic data for metabolites of Penicillium frequentans*

Equal volumes of immiscible solvent pairs were shaken to equilibrium, and the upper layer was used for development. Where the aqueous member contained buffer, the papers were dipped in this solution and dried before use. A, Ascending; D, descending. A range of values indicates streaking.

Solvent system	$10^2 R_F$					
	Questin	Questinol	(+)-Bisdechlorogedin	Asterric acid	Methyl asterrate	Sulochrin
Butan-1-ol–2N-ammonia (D)	48	23	0–50	29	81	69
Butan-1-ol–0.1 M-borate (D)	56	27	5–53	32	90	86
Chloroform–2N-ammonia (D)	3	0	86	0	95	0 (0–75)
Chloroform–0.1 M-borate (A)	0 (25–75)	0	80	0	95	0 (0–75)
Chloroform–2N- Na_2CO_3	0	0	0–50	0	95	0–50
Benzene–2N-acetic acid (D)	50	0	—	67	90	0
Toluene (A)	0 (0–30)	0	88	0 (0–40)	85	0
Toluene–acetic acid–water (5:5:1, by vol.) (D)	51	6	78	62	80	0 (0–30)
Detection						
Daylight	Yellow	Yellow	Colourless	Colourless	Colourless	Pale yellow
Ultraviolet light	Orange	Orange	Colourless	Blue	Pale blue	Colourless
Sodium carbonate	Pink	Pink	Yellow	Colourless	Colourless	Yellow
Diazotized sulphanilic acid–aq. Na_2CO_3	Pink	Pink	Yellow	Brown	Brown	Yellow

which was not further investigated. During ether extraction, large amounts of solid were deposited and the flask was changed at intervals, the solid being filtered, and the combined ether solutions were evaporated to give more solid, and finally a dark gummy residue. In this way 15–20 g. of yellow-brown solid was obtained per batch.

Method B. The wet mycelium was pressed dry, then macerated at high speed with acetone for 2 min. The mass was filtered and washed with acetone, and the mycelium re-extracted by maceration for a further 2 min. with fresh acetone. The acetone was evaporated under reduced pressure and the residue dried thoroughly on a water pump. It was then triturated with cold light petroleum (b.p. 40–60°), filtered and washed with light petroleum. The yield was about 50 g. per batch, of which 40 g. was insoluble in petroleum. The extracted mycelium, after air-drying, weighed 110 g. This method is more rapid and efficient, but the lido is dark brown in colour and clearly contains other impurities.

Separation and identification of sulochrin, and also of methyl asterrate, an artifact

Solid obtained by method A was extracted with boiling CHCl_3 and filtered. About a quarter of the material did not dissolve, and melted at 235–250°. It was recrystallized several times from ethyl acetate or methanol to give yellow crystals, m.p. 266–267°. For analysis it was sublimed *in vacuo* at 215–220° (Found: C, 61.2; H, 4.9; OMe, 19.0. Calc. for $\text{C}_{17}\text{H}_{18}\text{O}_7$: C, 61.3; H, 4.7; 2 OMe, 18.7%). The compound gives a green colour with ethanolic FeCl_3 . The melting point was not depressed on admixture with an authentic sample of sulochrin from *O. sulphurea-ochracea* (kindly supplied by Dr S. Natori, Tokyo). The infrared-absorption spectra of the two samples in Nujol and the ultraviolet-absorption spectra in ethanol were identical. Nishikawa (1939) records m.p. 262°.

The following derivatives were prepared: Dimethylsulochrin (by the dimethyl sulphate- K_2CO_3 -acetone method), m.p. 158–159° (literature: 158°) (Found: C, 62.85; H, 5.6; OMe, 35.6, 35.9. Calc. for $\text{C}_{19}\text{H}_{20}\text{O}_7$: C, 63.3; H, 5.6; 4 OMe, 34.4%). Triacetylsulochrin (by the acetic anhydride-pyridine method), m.p. 164–165° (literature: 164°) (Found: C, 60.5; H, 4.9; OMe, 28.2, 28.8. Calc. for $\text{C}_{23}\text{H}_{22}\text{O}_{10}$: C, 60.2; H, 4.8; 2 OMe, 28.2%).

The chloroform solution was evaporated to give yellow crystalline material melting at a much lower temperature, usually from 120° upwards. Repeated crystallization from methanol, and subsequently from ethyl acetate, yielded colourless crystals, m.p. 191–193°, which could be sublimed *in vacuo* at 160–165° (Found: C, 59.5, 59.3; H, 5.0, 4.9; OMe, 25.7, 26.0. Calc. for $\text{C}_{18}\text{H}_{18}\text{O}_8$: C, 59.65; H, 5.0; 3 OMe, 25.7%). The compound gave a purple colour with ethanolic FeCl_3 . The melting point was not depressed on mixing with a sample of trimethylsoic acid I (i.e. methyl asterrate) from *O. sulphurea-ochracea*; Nishikawa (1936) gives m.p. 190°; Curtis *et al.* (1960) record m.p. 185–186°. The infrared-absorption spectra of the two samples in Nujol were identical.

The following derivatives were prepared: Methyl dimethylasterrate, m.p. 149–151° (literature: 149°, 148–149°) (by the dimethyl sulphate- K_2CO_3 -acetone method) (Found: C, 61.6; H, 5.6; OMe, 39.8, 40.6. Calc. for $\text{C}_{20}\text{H}_{22}\text{O}_8$: C, 61.55; H, 5.7; 5 OMe, 39.7%). Methyl diacetylasterate, m.p. 119–120° from methanol (literature: 125–126° from

methanol) (by the acetic anhydride-pyridine method) (Found: C, 59.4, 59.5; H, 4.7, 4.7; OMe, 20.7; Ac, 19.9. Calc. for $\text{C}_{22}\text{H}_{22}\text{O}_{10}$: C, 59.2; H, 4.9; 3 OMe, 20.85; 2 Ac, 19.3%); this compound is dimorphic; a methanolic solution seeded with a sample from *O. sulphurea-ochracea* (see below) and allowed to evaporate to dryness gave a solid with m.p. 127–128°; the infrared-absorption spectra of the two forms in Nujol differed considerably. *Methyl dibenzoyl-asterrate*, m.p. 197–198° (from ethyl acetate) (by the benzoyl chloride-pyridine method) (Found: C, 67.1, 67.1; H, 4.7, 4.6; OMe, 16.3. $\text{C}_{32}\text{H}_{28}\text{O}_{10}$ requires: C, 67.3; H, 4.6; 3 OMe, 16.3%).

Hydrolysis yielded norasterric acid (methylsoic acid), m.p. 244–245° (literature: 239°, 246°) (Found: C, 57.5; H, 4.3; OMe, 9.3. Calc. for $\text{C}_{16}\text{H}_{14}\text{O}_8$: C, 57.5; H, 4.2; 1 OMe, 9.3%).

Hydrolysis of methyl dimethylasterrate yielded dimethyl norasterric acid, m.p. 207–208° (literature: 211°, 208–209°) (Found: C, 60.1; H, 5.1; OMe, 25.9. Calc. for $\text{C}_{18}\text{H}_{18}\text{O}_8$: C, 59.65; H, 5.0; 3 OMe, 25.7%).

The methyl dimethylasterrate, methyl diacetylasterate, and norasterric acid thus obtained were compared with samples of pentamethylsoic acid, diacetyltrimethylsoic acid I and methylsoic acid respectively; these authentic samples derived from *O. sulphurea-ochracea* were kindly provided by Dr Natori. In all three cases identity was established by mixed melting points and infrared-absorption spectra.

Isolation of asterric acid from culture filtrate

The crude yellow precipitate obtained by acidification of the concentrated culture filtrate was extracted with CHCl_3 . On evaporation of the extract, a solid separated; after recrystallization from ethyl acetate, the product was obtained as colourless needles, m.p. 213–215°, not depressed on admixture with a sample of dimethylsoic acid I (i.e. asterric acid) derived from *O. sulphurea-ochracea*, kindly provided by Dr Natori (Found: C, 58.6, 58.3; H, 4.85, 4.7; OMe, 18.5. Calc. for $\text{C}_{17}\text{H}_{18}\text{O}_8$: C, 58.6; H, 4.6; 2 OMe, 17.9%). The infrared-absorption spectra were identical.

The identity was confirmed by methylation, giving methyl asterrate, m.p. 188–189° (Found: C, 59.7; H, 4.9; OMe, 25.9. Calc. for $\text{C}_{18}\text{H}_{18}\text{O}_8$: C, 59.65; H, 5.0; 3 OMe, 25.7%).

Isolation and identification of (+)-bisdechlorogodin

Solid obtained from the mycelium by method A was extracted with boiling benzene (100 vol.) and filtered; about 65% of the solid dissolved. The yellow solution was evaporated to about a quarter of its volume and allowed to cool for a few minutes: some orange solid which separated was then filtered off, and the solution left to crystallize overnight. Heavy yellow prisms separated, which were recrystallized by dissolving in ethyl acetate and allowing to evaporate at room temperature. In this way large well-formed pale-yellow prisms of (+)-bisdechlorogodin [(+)-4-hydroxy-2'-methoxy-6'-methoxycarbonyl-6-methylgrise-2',5'-diene-3,4'-dione] were obtained, which melted slowly between 130° and 150° with effervescence; $[\alpha]_D^{25} + 217 \pm 5^\circ$ (c 0.56 in CHCl_3) (Found: after drying for 4 hr. at 70°: C, 58.8; H, 4.7. $\text{C}_{17}\text{H}_{14}\text{O}_7 \cdot \text{H}_2\text{O}$ requires C, 58.6; H, 4.6%).

The compound decomposed partly on drying at 100° *in vacuo*, but on prolonged drying at 77° *in vacuo* over P_2O_5 (loss of wt., 4.8. $\text{C}_{17}\text{H}_{14}\text{O}_7 \cdot \text{H}_2\text{O}$ requires H_2O , 5.2%) the

m.p. was raised to 165–169°; $[\alpha]_D^{20} + 230 \pm 5^\circ$ (c 0.53 in CHCl_3), $+ 218 \pm 5^\circ$ (c 0.43 in ethanol), $[\alpha]_D^{20} + 188 \pm 5^\circ$ (c 0.55 in CHCl_3), $+ 186 \pm 5^\circ$ (c 0.56 in ethanol); λ_{max} in ethanol, approx. 215 and 285 $\text{m}\mu$ (ϵ approx. 30000 and 19500 respectively); λ_{max} in dioxan, 283 $\text{m}\mu$ (ϵ 22500); ν_{max} in Nujol, 1730 and 1700 (methoxycarbonyl and coumaranone), 1660 (cross-conjugated cyclohexadienone), 1625 and 1605 cm^{-1} (carbohylyl-conjugated $\text{C}=\text{C}$) (Found: C, 61.4; H, 4.5; OMe, 18.9. $\text{C}_{17}\text{H}_{14}\text{O}_7$ requires: C, 61.9; H, 4.3; 2 OMe, 18.8%). Further exhaustive drying at 100° *in vacuo* raised the m.p. to 170–173°, with no change in the specific rotation. The infrared-absorption spectrum was identical, except in minor details, with that of a sample of (–)-bisdechlorogeodin obtained from *O. sulphurea-ochracea* both in Nujol and CHCl_3 . The comparison was kindly carried out by Dr S. Natori; details of the optical activity of (–)-bisdechlorogeodin are discussed above (p. 371).

Pyrazoline derivative. (+)-Bisdechlorogeodin (150 mg.) was suspended in dry ether (10 ml.) and gradually treated with excess of diazomethane in ether (85 ml.). After 10 min. at room temperature, further ethereal diazomethane (15 ml.) was added, and the mixture was kept overnight. Some undissolved solid was removed by filtration, and the ether was evaporated; the residue (183 mg.) was crystallized from ethanol as short colourless needles, m.p. 165–166° (70 mg.). Recrystallization several times from ethanol raised the melting point of the *pyrazoline derivative* to a constant 167–168° with effervescence; $[\alpha]_D^{20} - 162 \pm 3^\circ$, $[\alpha]_D^{20} - 179 \pm 4^\circ$, $[\alpha]_D^{20} - 216 \pm 4^\circ$ (c 1.1 in CHCl_3); ν_{max} in Nujol, 1745, 1725, 1675, 1620 and 1590 cm^{-1} (Found: C, 59.4; H, 4.8; N, 7.5; OMe, 24.0. $\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}_7$ requires C, 59.1; H, 4.7; N, 7.3; 3 OMe, 24.1%).

(+)-Bisdechlorogeodin methyl ether. To (+)-bisdechlorogeodin (100 mg.) and anhydrous K_2CO_3 (0.3 g.) were added dry acetone (14 ml.) and methyl iodide (0.2 ml.). The mixture was heated under reflux for 2 hr., after which the supernatant no longer gave a colour with FeCl_3 . After removal of the K_2CO_3 by filtration, the solvents were evaporated, and the residue was crystallized from ethyl acetate (12 ml.). Recrystallization yielded nearly colourless micro-crystals of (+)-bisdechlorogeodin methyl ether, m.p. 247–248° (70 mg.); $[\alpha]_D^{20} + 189 \pm 4^\circ$, $[\alpha]_D^{20} + 221 \pm 4^\circ$ (c 1.1 in CHCl_3); ν_{max} in Nujol, 1725, 1710, 1660, 1620 and 1590 cm^{-1} (Found: C, 62.8; H, 4.8; OMe, 27.1. $\text{C}_{18}\text{H}_{16}\text{O}_7$ requires C, 62.8; H, 4.7; 3 OMe, 27.0%).

(+)-Bisdechlorogeodin acetate. (+)-Bisdechlorogeodin (150 mg.) was treated with acetic anhydride (15 ml.) and sodium acetate (60 mg.) and kept at 22° for 18 hr. The addition of water gave a solid which was recrystallized from methanol to give (+)-bisdechlorogeodin acetate as cream-coloured square tablets, m.p. 158–160°; $[\alpha]_D^{20} + 224 \pm 5^\circ$ (c 0.53 in CHCl_3); ν_{max} in Nujol, 1780 (phenolic acetate), 1740, 1725, 1650, 1625 and 1600 cm^{-1} (Found: C, 61.3; H, 4.5; OMe, 16.6. $\text{C}_{19}\text{H}_{16}\text{O}_8$ requires: C, 61.3; H, 4.3; 2 OMe, 16.7%).

Methanolysis of (+)-bisdechlorogeodin. (+)-Bisdechlorogeodin (50 mg.) was boiled with methanol (20 ml.) for 5 min., then evaporated to about 5 ml. and left to crystallize. Colourless crystals separated, m.p. 190–192° not depressed on admixture with methyl asterate.

Hydrolysis of (+)-bisdechlorogeodin. (a) (+)-Bisdechlorogeodin (30 mg.) was dissolved in conc. H_2SO_4 (1 ml.) and treated with water (6 ml.). The white precipitate was crystallized from methanol and then ethyl acetate, m.p.

212–214° not depressed on admixture with asteric acid. Infrared-absorption spectra in Nujol were identical.

(b) (+)-Bisdechlorogeodin (20 mg.) was dissolved in $\text{N-Na}_2\text{CO}_3$ (5 ml.) and left overnight at room temperature. The solution was acidified to give white crystals, m.p. 210–212° unchanged on admixture with asteric acid.

Isolation of the anthraquinonoid pigments

Solid extracted from the mycelium was dissolved in methanol and passed through a column of MgO , washing with more methanol. The anthraquinones were absorbed as a pink band, spreading slowly downwards. Elution was continued until most of the yellow material had passed through; the pink band was then extruded, dissolved in 2N-HCl and extracted with ethyl acetate, washing with more 2N-HCl to remove small amounts of MgCl_2 . Alternatively, the mycelial solid was dissolved in 0.1N- Na_2CO_3 and passed through a column of MgO ; washing with Na_2CO_3 soln. did not move the red band, but removed much yellow material. The red band was then extracted as described above.

The extracts were evaporated, then separated on a countercurrent apparatus by using ethyl acetate equilibrated with a buffer consisting of equal volumes of 0.5M- NaHCO_3 and 0.5M- Na_2CO_3 . Pink substances separated in the lower layers of two groups of tubes, one being fast-moving, the other slow-moving. The bands were separately acidified and extracted with ethyl acetate, and the solvent was removed to leave orange solids which were purified by crystallization (the yield was about 0.5 g. of each).

Questin

The material from the faster-moving band yielded *questin* (4,7-dihydroxy-5-methoxy-2-methylanthraquinone), which separated from ethanol, methanol, ethyl acetate or acetic acid in needles of two distinct types: (a) yellow and (b) orange. Both forms melted at 301–303°; λ_{max} in ethanol, 224, 248, 285 and 425 $\text{m}\mu$ (ϵ 39000, 13200, 23400 and 9300 respectively); λ_{max} in ethanol-0.1N-NaOH (1:1 v/v), 236, 250 (shoulder), 308 and 503 $\text{m}\mu$ (ϵ 24000, 16100, 20800 and 8500 respectively); ν_{max} of orange form in Nujol, 3250 (phenolic OH), 1670 and 1650 (w, unbonded CO), 1630 (bonded CO), 1580 cm^{-1} ; ν_{max} of yellow form in Nujol, 3250, 1670 (m), 1630 and 1580 cm^{-1} (Found: C, 67.7, 67.4; H, 4.1, 4.4; OMe, 10.9; C-Me, 6.5; acetyl, 0. $\text{C}_{18}\text{H}_{12}\text{O}_6$ requires C, 67.6; H, 4.25; 1 OMe, 10.9; 1 C-Me, 5.3%. Good analysis for methoxyl groups could be obtained only after crystallization from acetic acid). The compound dissolved in aq. Na_2CO_3 or NaOH to give a deep red colour, but was insoluble in aq. NaHCO_3 . In conc. H_2SO_4 a red colour was observed, indistinguishable from that given by emodin.

Monomethylquestin. Questin (60 mg.) was dissolved in ethanol (20 ml.) and treated with excess of diazomethane and left at 0–4° overnight. Evaporation and crystallization twice from ethyl acetate gave 4-hydroxy-5,7-dimethoxy-2-methylanthraquinone as orange rods (43 mg.), m.p. 208–210° unchanged on further crystallization; ν_{max} in Nujol, 1660 (m) (unbonded CO), 1625 (bonded CO) 1590 and 1550 cm^{-1} (m) (Found: C, 68.45, 68.2; H, 5.0, 4.9; OMe, 20.9. $\text{C}_{17}\text{H}_{14}\text{O}_6$ requires C, 68.45; H, 4.7; 2 OMe, 20.8%). The derivative is insoluble in aq. Na_2CO_3 , but dissolves in 2N-NaOH on warming to give a red solution.

Dimethylquestin. Questin (100 mg.), dimethyl sulphate (1.5 ml.), anhydrous K_2CO_3 (2 g.) and acetone (30 ml.) were heated under reflux for 20 hr. Water (30 ml.) was added, and the yellow precipitate was collected and crystallized from ethanol, m.p. 230–232° unchanged by admixture with a sample of emodin trimethyl ether (4,5,7-trimethoxy-2-methylanthraquinone) (Found: C, 69.2; H, 5.1; OMe, 30.2, 29.85. Calc. for $C_{18}H_{16}O_5$: C, 69.2; H, 5.2; 3 OMe, 29.8%). The infrared-absorption spectra were identical throughout the range 4000–650 cm^{-1} in Nujol: ν_{max} , 1660 (unbonded CO), 1600 and 1560 cm^{-1} .

Diacetylquestin. Questin (100 mg.) was mixed with acetic anhydride (5 ml.), and 2 drops of conc. H_2SO_4 were added. The mixture was heated on a boiling-water bath for 5 min., cooled and treated with crushed ice. The precipitate (m.p. 157–160°) was recrystallized from ethyl acetate and then acetic acid, m.p. 160–162°. 4,7-Diacetoxy-5-methoxy-2-methylanthraquinone was also prepared by the acetic anhydride-pyridine method. λ_{max} in ethanol, 219, 258 and 378 $m\mu$; ν_{max} of crystals from ethyl acetate in Nujol, 1765 (phenolic ester), 1660 (unbonded CO) and 1600 cm^{-1} ; ν_{max} of crystals from acetic acid in Nujol, 1765, 1675, 1655, 1605 and 1595 cm^{-1} (Found: C, 65.4, 65.6; H, 4.3, 4.5; OMe, 8.5. $C_{20}H_{16}O_7$ requires: C, 65.2; H, 4.4; 1 OMe, 8.4%). The derivative is insoluble in cold NaOH.

Demethylation. Questin (50 mg.) was mixed with conc. H_2SO_4 (5 ml.) and heated in an oil bath at 150° for 30 min. The solution was cooled and poured on crushed ice. Crystallization from chloroform and then toluene gave yellow-brown needles (36 mg.), m.p. 260–262° unchanged by admixture with a sample of emodin (Found, on a sample sublimed in high vacuum: C, 66.3; H, 4.0. Calc. for $C_{15}H_{10}O_5$: C, 66.7; H, 3.7%). The infrared-absorption spectra in Nujol were identical except for minor details: ν_{max} , 3350 (unbonded OH) 1660 (w) (unbonded CO), 1625 (bonded CO), 1590, 1570 and 1560 cm^{-1} .

Oxidation of monomethylquestin. After preliminary attempts to oxidize the monomethyl derivative (50 mg.) with $KMnO_4$ in acetone, and then with $KMnO_4$ in neutral aqueous solution, had been only partially successful, the reaction solution was made strongly alkaline and heated on the steam bath with excess of $KMnO_4$ for 1 hr. The mixture was cooled, treated with $NaHSO_3$, then HCl until a clear colourless solution was obtained. It was acidified to pH 1–2 and extracted with ether (3 $\times$ 1 vol.).

Removal of the solvent left a mainly crystalline residue, which on recrystallization from water, with a little charcoal, gave nearly colourless plates (10.5 mg.; 25% yield), which gave an infrared-absorption spectrum in Nujol identical with that of 3,5-dimethoxyphthalic acid over the range 4000–650 cm^{-1} . Since the melting point of this compound is not diagnostic (see Oxford & Raistrick, 1933) it was converted into the anhydride by heating at 190° for 5 min., then sublimed at 100° *in vacuo*. The pale-yellow sublimate had m.p. 151–152° unchanged by admixture with an authentic sample of 3,5-dimethoxyphthalic anhydride; the melt reset on cooling and remelted at the same temperature. The infrared-absorption spectra in Nujol were identical, but there was insufficient material for analysis (but see below).

Questinol

The slower-moving band (from the countercurrent distribution) yielded, after several crystallizations, pure

questinol (4,7-dihydroxy-2-hydroxymethyl-5-methoxyanthraquinone) which separated from ethanol, methanol or acetic acid as orange needles, m.p. 280–282°; λ_{max} in ethanol, 224, 247, 286 and 432 $m\mu$ (ϵ 37000, 14300, 21900 and 9000 respectively); λ_{max} in ethanol-0.1N-NaOH (1:1, v/v), 234, 251 (shoulder), 306 and 503 $m\mu$ (ϵ 28000, 17900, 25300 and 8600 respectively); ν_{max} in Nujol, 3500 (m, alcoholic OH), 3150 (m, phenolic OH), 1665 (m, unbonded CO), 1625 (bonded CO), 1605 (m) and 1560 cm^{-1} (Found, on sample crystallized from acetic acid and dried for 10 hr. at 100° *in vacuo*: C, 63.6; H, 4.2; OMe, 10.4; C-Me, 0. $C_{18}H_{12}O_6$ requires: C, 64.0; H, 4.0; 1 OMe, 10.3%. Poor analyses were obtained on samples crystallized from methanol, even after drying at 100°). The colour reactions were indistinguishable from those given by questin.

Monomethylquestinol. ω -Hydroxyemodin 5-methyl ether (50 mg.) was dissolved in methanol (20 ml.), then treated with excess of diazomethane and left overnight at 0–5°. After evaporation, the residue was crystallized successively from methanol, ethyl acetate and ethanol, m.p. 238–240° (35 mg.). For analysis, 4-hydroxy-2-hydroxymethyl-5,7-dimethoxyanthraquinone was crystallized twice more from acetic acid with no change in melting point (Found: C, 64.6; H, 4.6; OMe, 19.7. $C_{17}H_{14}O_6$ requires: C, 64.9; H, 4.5; 2 OMe, 19.75%). The compound is insoluble in aq. Na_2CO_3 , but dissolves in warm aq. NaOH to give a red colour.

Trimethylquestinol. Questinol (57 mg.) was dissolved as far as possible in acetone (5 ml.) and treated with dimethyl sulphate (2.5 ml.). Then 2N-NaOH was added in 1 ml. portions with shaking until the red colour disappeared even in the presence of excess of alkali (20 ml. required). During the addition of the last 5 ml., the yellow product separated. It was filtered off, washed with water, dried and crystallized three times from ethanol, from which trimethylquestinol (4,5,7-trimethoxy-2-methoxymethylanthraquinone) separated in yellow rods, m.p. 188–190° not depressed on admixture with an authentic sample of carviolin trimethyl ether (Hind, 1940a) (Found: C, 66.6; H, 5.5; OMe, 36.3. Calc. for $C_{19}H_{18}O_6$: C, 66.7; H, 5.3; 4 OMe, 36.2%). The infrared-absorption spectra in Nujol were identical: ν_{max} , 1660 (unbonded CO), 1590 and 1560 cm^{-1} (m).

Triacetylquestinol. Questinol (100 mg.) was dissolved in acetic anhydride (1 ml.) and treated with conc. H_2SO_4 (1 drop), then heated for 5 min. on a steam bath. The mixture was cooled and crushed ice added, and the yellow precipitate crystallized several times from ethyl acetate. Triacetylquestinol (4,7-diacetoxy-2-acetoxymethyl-5-methoxyanthraquinone) separated in yellow plates, m.p. 186–188°; λ_{max} in ethanol, 221, 257 and 382 $m\mu$; ν_{max} in Nujol, 1765 (phenolic acetate), 1735 (alcoholic acetate), 1675, 1660 (unbonded CO), 1610, 1595 and 1575 cm^{-1} (Found: C, 61.9; H, 4.25; OMe, 7.3. $C_{22}H_{18}O_9$ requires: C, 61.9; H, 4.25; 1 OMe, 7.3%).

Oxidation of monomethylquestinol. Monomethylquestinol (110 mg.) was dissolved in 0.1N-NaOH (80 ml.), heated on a steam bath. Powdered $KMnO_4$ was added in small portions with shaking and continued heating, until the purple colour persisted for 0.5 hr. After cooling, solid $NaHSO_3$ and 2N-HCl were added until the precipitate was cleared. The solution was yellow, and so it was reoxidized in the same way, giving this time a pale-yellow solution. The acid solution was extracted with ether (3 $\times$ 100 ml.). Evaporation of the ether left a semi-solid residue (50 mg.) which was crystallized from water, with a little charcoal.

Colourless prisms (30 mg.; 35% yield) separated, m.p. indefinite from 160°, but remelting after cooling at 146–147°. The infrared-absorption spectrum in Nujol was identical with that of 3,5-dimethoxyphthalic acid.

The acid was converted into the anhydride as described above. The sublimate was recrystallized from benzene-light petroleum, then resublimed, m.p. 148–149° not depressed on admixture with an authentic sample of 3,5-dimethoxyphthalic anhydride (Found: C, 58.1; H, 4.1; OMe, 29.7. Calc. for $C_{10}H_8O_5$: C, 57.7; H, 3.9; 2 OMe, 29.8%).

SUMMARY

1. A typical strain of *Penicillium frequentans*, grown on Czapek–Dox medium, produces a mixture of metabolites, of which five have been identified.

2. Two of these products are sulochrin and asteric acid, known as metabolites of other fungi.

3. A third component was identified as (+)-bisdechlorogedodin [(+)-4-hydroxy-2'-methoxy-6'-methoxycarbonyl-6-methylgrisi-2',5'-diene-3,4'-dione]. The (–)-isomer was previously isolated, with sulochrin and asteric acid, from *Oospora sulphurea-ochracea*.

4. The remaining two products are two new substituted anthraquinones, which are named questin and questinol.

5. Questin, $C_{16}H_{12}O_8$, m.p. 301–303°, containing one methoxyl group, was shown to be a monomethyl derivative of emodin. Further evidence, including the oxidation of monomethylquestin to 3,5-dimethoxyphthalic acid, proved that questin is emodin 5-methyl ether, or 4,7-dihydroxy-5-methoxy-2-methylanthraquinone.

6. Questinol, $C_{16}H_{12}O_8$, m.p. 280–282°, was similarly shown to be ω -hydroxyquestin, or ω -hydroxyemodin 5-methyl ether (4,7-dihydroxy-2-hydroxymethyl-5-methoxyanthraquinone).

7. The relationship of questin and questinol to other fungal anthraquinones, and the biosynthetic relationships of the five products isolated from *P. frequentans*, are discussed.

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