869. *The Biosynthesis of Phenols. Part IX.*[1] *Asperugin, a Metabolic Product of* Aspergillus rugulosus

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Asperugin, $C_{24}H_{32}O_5$, has been isolated from the culture fluid of a mutant strain of *Aspergillus rugulosus* carrying the genetic marker "fluffy." Degradation experiments lead to the structure (X).

STUDIES utilising the organism *Aspergillus terreus* have established that stages in the pathway of biosynthesis of "non-essential," complex, phenolic compounds may be defined through the investigation of mutants.[2] However, attempts in this connection to prepare a chromosome linkage map incorporating phenol-biosynthesis characters of *A. terreus* were discouraged by difficulties in obtaining recombinants.[3] As a result, *Aspergillus rugulosus* was selected for such an investigation. Our preliminary studies established that this organism produced novel, complex phenols. Moreover, it was closely related to *Aspergillus nidulans*, which has been the subject of detailed study by geneticists.[4]

The investigation of linkage using *Aspergillus rugulosus* involved conventional genetic markers such as morphological features, amino-acid and vitamin requirements, as well as phenol biosynthesis characters. At an early stage it was observed that there was a relationship between the formation of fluffy, ærial mycelium and the biosynthesis of the phenolic compound to which we have assigned the name asperugin. All strains bearing this character "fluffy" produced asperugin, whereas all strains with the wild-type allele produced instead a phenolic compound with very different properties. It was established that the character "fluffy" and the biosynthesis were under genetic control, in the strict sense. This demonstrated a novel relationship between a phenolic metabolite and the morphology of a micro-organism.[5] In this connection we have undertaken an investigation of the molecular structure of asperugin and related metabolites produced by different mutant strains of *A. rugulosus.*

Asperugin was isolated from the mycelium of *Aspergillus rugulosus* grown on a modified Barnes's medium.[5] The crude preparation obtained by extraction of macerated mycelium with ether was purified by countercurrent distribution, followed by chromatography using silica gel. Asperugin, $C_{24}H_{32}O_5$, is an optically inactive, pale yellow, viscous oil with phenolic properties. Kuhn–Roth and Zeisel determinations indicated that there were three *C*-methyl and one methoxyl group; the formation of a disemicarbazone and a monocyano-mono-oxime ($C_{24}H_{32}N_2O_4$) established the presence of two reactive carbonyl groups. Spectroscopic evidence suggested that asperugin was a relatively highly-substituted, monobenzenoid phenol. The ultraviolet absorption spectrum in neutral solvent and the characteristic bathochromic shift of the R band in alkali supported this interpretation. The n.m.r. spectrum was particularly informative. It was possible to make tentative assignments for the 32 protons in the molecule (Table 1). The singlet at τ −2·40 was attributed to one phenolic hydroxyl; this proton, alone, exchanged readily with deuterium in the presence of deuterium oxide. The low value of τ in this case evidently arose from intramolecular hydrogen bonding with one of two aldehyde groups associated with singlets at τ −0·04, −0·75. The presence of a band at 1635 cm.$^{-1}$ in the infrared spectrum also suggests a strongly hydrogen-bonded hydroxyl–formyl system as in 3,6-dihydroxy-4,5-dimethylphthalaldehyde.[6] There are good analogies for the assignments τ 5·98, 2·95 to methoxy and aromatic protons. The remaining peaks in the n.m.r. spectrum

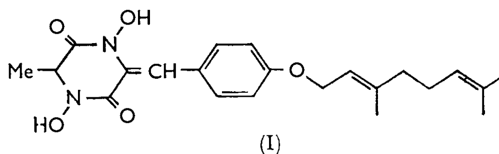
[1] Part VIII, R. F. Curtis, P. C. Harries, and C. H. Hassall, *J.*, 1964, 5382.
[2] R. F. Curtis, P. C. Harries, C. H. Hassall, and J. D. Levi, *Biochem. J.*, 1964, **90**, 43.
[3] J. D. Levi, unpublished results.
[4] G. Pontecorvo, J. A. Roper, L. M. Hemmons, K. D. Macdonald, and A. W. J. Bufton, *Adv. Genet*, 1953, **5**, 141.
[5] C. H. Hassall and K. Lawrence, *J. Gen. Microbiol.*, 1964, **35**, 483.
[6] D. W. Cameron, P. M. Scott, and Lord Todd, *J.*, 1964, 42.

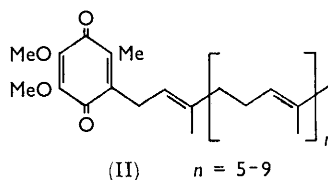
were remarkably similar to those due to simple isoprenoid side-chains in related compounds. Moreover, the occurrence of a doublet at τ 5·35 (J=7 c./sec.) suggested that such a chain was linked through oxygen, as in mycelianamide (I; τ, 5·4; J=7 c./sec.),[7] rather than through carbon, as in ubiquinone (II; τ, 6·82; J=7 c./sec.).[8] This made it possible, tentatively, to assign the partial structure (III) to asperugin.

TABLE 1

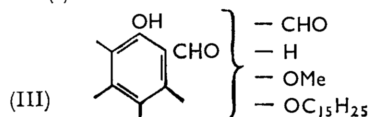
N.m.r. characteristics of asperugin

Chemical shift (τ)	Peaks	Nos. of protons	Assignment
−2·40	Singlet	1	Chelated Ar–OH [9]
−0·75	Singlet	1	Ar–CHO
−0·04	Singlet	1	Ar–CHO
2·95	Singlet	1	Ar–H
4·80	Broad multiplet	3	HC=
5·35	Doublet (J., 7 c./sec.)	2	Ar–O–CH_2–CH=
5·98	Singlet	3	Ar–O–CH_3
7·97	Broad multiplet	8	=C–CH_2–CH_2–C=
8·34	Singlet	12	–C(=)–CH_3
8·41	Singlet		


(I)

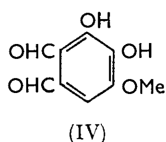


(II) n = 5–9

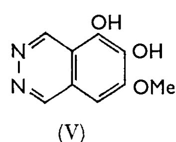


(III)

Asperugin decomposed readily in acid media; this was interpreted as the result of allylic character in the oxygen-linked C_{15} fragment. It was confirmed by cleavage involving both Birch reduction and the use of acid-washed silica gel. Analytical and spectroscopic evidence indicated that the crystalline, phenolic fragment, $C_9H_8O_5$, obtained in the latter case, had one methoxyl, two formyl and two phenolic hydroxyl groups. It reacted with hydrazine to give a crystalline phthalazine derivative and formed, through catalytic hydrogenation, a compound, $C_9H_{12}O_4$, with the n.m.r. spectrum of a substituted 1,3-dihydro-isobenzofuran; evidently the two formyl groups were adjacent. Oxidation of the 1,3-dihydro-isobenzofuran derivative with active lead dioxide followed by condens-



(IV)



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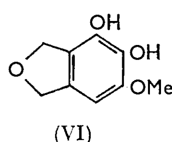

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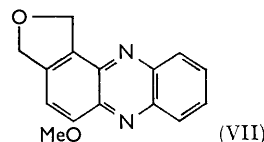
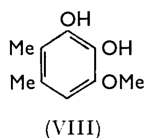
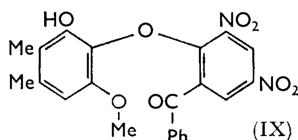


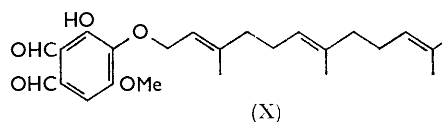
(VII)



(VIII)



(IX)



(X)

ation with *o*-phenylenediamine led to formation of a crystalline phenazine derivative, $C_{15}H_{12}N_2O_2$, and to the conclusion that the two phenolic hydroxyl groups in the fragment

[7] R. B. Bates, J. H. Schauble, and M. Soucek, *Tetrahedron Letters*, 1963, **25**, 1683.

[8] D. E. Wolf, C. H. Hoffman, N. R. Trenner, B. H. Arison, C. H. Shunk, B. O. Linn, J. F. McPherson, and K. Folkers, *J. Amer. Chem. Soc.*, 1958, **80**, 4752.

[9] R. W. Hay and P. P. Williams, *J.*, 1964, 2270.

$C_9H_8O_5$ were adjacent. The structure of this degradation product, the phthalazine derivative, the substituted 1,3-dihydroisobenzofuran and the derived phenazine have been defined as (IV), (V), (VI), and (VII) through the synthesis of (VIII), the Clemmensen reduction product of the fragment $C_9H_8O_5$ (IV) and synthesis of the resulting product (VIII). It was established that the latter was a pyrogallol derivative by the observation that the product of demethylation differed from synthetic 3,4,6-trihydroxy-1,2-dimethylbenzene. The preparation of 3,4-dihydroxy-5-methoxy-1,2-dimethylbenzene utilised a modification of the method employed by Loudon and Summers [10] for the synthesis of 4,5-dimethylcatechol. 4-Hydroxy-5-methoxy-1,2-dimethylbenzene was condensed with 2-chloro-3,5-dinitrobenzophenone to give 2-(4″,5″-dimethyl-2″-methoxyphenoxy)-3,5-dinitrobenzophenone. This was oxidised with hydrogen peroxide to 2-(4″,5″-dimethyl-6″-hydroxy-2″-methoxyphenoxy)-3,5-dinitrobenzophenone (IX) which was converted into the compound (VIII) by the action of phenylhydrazine. This synthetic compound was identical with the product of Clemmensen reduction.

The identification of the oxygen-linked $C_{15}H_{24}$ fragment in asperugin was achieved by hydrogenolysis with sodium and methanol in liquid ammonia. The acid-catalysed cleavage of the molecule gave a complex mixture of products, presumably following a similar course to mycelianamide,[11] but the hydrogenolysis led to the formation of an unsaturated hydrocarbon in excellent yield. Catalytic hydrogenation of this hydrocarbon (3 moles) gave farnesane which was shown to be identical with an authentic sample by comparison of mass spectra, physical constants, and behaviour during vapour phase chromatography. The stereochemistry of the hydrogenolysis product and the $C_{15}H_{25}$ sidechain in asperugin were defined by n.m.r. spectroscopy. Bates and his co-workers [12] have demonstrated significant differences in the methyl absorption bands of the system $A{\cdot}CH_2{\cdot}CMe{:}CH{\cdot}CH_2B$. The differences have been related to the geometrical isomerism of acyclic isoprenoids. In particular, the n.m.r. spectra of the four stereoisomers of farnesol have been differentiated.[13] The *trans-trans* isomer is distinguished unambiguously from the others by proton resonance absorption at τ 8·34, 8·41 (2 : 2). The n.m.r. spectrum of asperugin has similar peaks with equivalent integrals (Table 1.) This, taken with the previous evidence, establishes the structure of asperugin as 4-(*trans-trans*-farnesyloxy)-3-hydroxy-5-methoxyphthalaldehyde (X).

The pyrogallol nucleus is not common in mould metabolites. *Paecilomyces victoriae* produces 4,5-dihydroxy-3-methoxyphthalic acid as co-metabolite of the acetate–malonate derived ustic acid.[14] Gallic acid is synthesised by *Phycomyces blakesleeanus* through the shikimic acid route.[15] Fuscin, dihydrofuscin,[16] fomecin A and B,[17] and flavipin [18] have related patterns of oxygen substitution but there is no evidence bearing on their mode of biosynthesis.

Experimental

Melting points were determined on a Kofler hot-stage apparatus. Ultraviolet spectra were measured in ethanol on a Unicam S.P. 800 spectrophotometer. N.m.r. spectra were determined on a Perkin-Elmer 40 Mc./sec. instrument using tetramethylsilane as internal standard. Infrared spectra were determined for potassium bromide discs, unless otherwise stated, on Perkin-Elmer model 137 or 237 spectrophotometers. Mass spectra were measured using an Associated Electrical Industries model MS 9 spectrometer (we are grateful to Professor G. W.

[10] J. D. Loudon and L. A. Summers, *J.*, 1954, 1134.
[11] C. Gallina, A. Romeo, G. Tarzia, and V. Torto-Rella, *Gazzetta*, 1965, to be published.
[12] R. B. Bates and D. M. Gale, *J. Amer. Chem. Soc.*, 1960, **82**, 5749; R. B. Bates, R. H. Carnigham, R. O. Rakutis, and J. H. Schauble, *Chem. and Ind.*, 1962, 1020.
[13] R. B. Bates, D. M. Gale, and B. J. Gruner, *J. Org. Chem.*, 1963, **28**, 1086.
[14] V. C. Vora, *J. Sci. Ind. Res., India*, 1954, **13**, *B*, 842.
[15] E. Haslam, R. D. Haworth, and P. F. Knowles, *J.*, 1961, 1854.
[16] D. H. R. Barton and J. B. Hendrickson, *J.*, 1956, 1028.
[17] T. C. McMorris and M. Anchel, *Canad. J. Chem.*, 1964, **42**, 1595.
[18] H. Raistrick and P. Rudman, *Biochem. J.*, 1956, **63**, 395.

Kenner and Dr. D. F. Shaw of the University of Liverpool for these determinations). Gas-chromatograms were obtained on a Perkin-Elmer Fractometer model No. 116E using thermistor detectors and 2 m. columns. R_F values refer to thin-layer chromatograms on Kieselgel G (Merck) using either system *A*, benzene–dioxan–acetic acid (90 : 25 : 4 v/v) [19] or system *B*, benzene–methanol–acetic acid (10 : 2 : 1 v/v).[3] The chromoplates were sprayed with diazotised *o*-dianisidine solution followed by ammonia, unless otherwise stated. Light petroleum had b. p. 60—80°.

Extraction and Purification of Asperugin.—A mutant ($w_1pro_1f_1$) [5] of *Aspergillus rugulosus*, derived from I.M.I. strain No. 84338, was grown from a spore suspension for 21 days at 25° on a low-nitrogen medium [5] (200 ml.) using stationary culture in flat-sided bottles (*ca.* 1 l. capacity). The mycelium was separated from the culture fluid using a wire mesh strainer and macerated in a blender for 1 min. The macerate was extracted with ether (250 ml./5 bottles). The ether extract from a batch of 300 bottles was concentrated to 500 ml., keeping the temperature below 40°. Insoluble material was filtered off; the residual solvent was removed under reduced pressure to give a dark brown viscous residue (37·3 g.). This mixture was distributed equally in each of the first ten tubes of a 200 tube automatic countercurrent distribution instrument (H.O. Post Scientific Instrument Co.) and subjected to 495 transfers, with recycling, using 10 ml. phases of the mixture light petroleum–methanol–water (8 : 10 : 1 v/v). Tubes 190—228 contained impure asperugin (5·1 g., R_F 0·65, black spot, system *B*) which was purified by chromatography on silica gel (200 g., 200—300 mesh, L. Light and Co. Ltd.) using gradient elution. Elution with light petroleum–ether (20 : 1 v/v) gave *asperugin* (3·86 g.) in the middle fractions from the column. A portion (650 mg.) was chromatographed under the same conditions to give analytically pure asperugin (600 mg.) as a viscous pale yellow oil, $n_D^{25} = 1·5582$, $[\alpha]_D^{25}$ 0·00° (Found: C, 71·9; H, 8·2. $C_{24}H_{32}O_5$ requires C, 72·0; H, 8·1%), $\nu_{max.}$ (film) 3350, 2960, 2850, 1685, 1635, 1627, 1580, 1507, 1499, 1459, 1420, 1378, 1344, 1300, 1255, 1184, 1140, 1065, 1020, 948, 900, 831, 713 cm.$^{-1}$, $\lambda_{max.}$ (ethanol) 209, 254, 315 mμ (log ε 4·43, 4·21, 3·84), $\lambda_{max.}$ (ethanolic sodium hydroxide) 215, 246, 280, 327, 415 mμ (log ε 4·65, 4·47, 4·15, 4·01, 3·97).

Asperugin was insoluble in water but dissolved readily in most organic solvents. It reacted with ammonium hydroxide to produce a black colour and it was decomposed by treatment with acid.

The ether extract of the mycelium, from which asperugin was obtained, often deposited an insoluble crystalline solid (*ca.* 500 mg./300 bottles) on standing. Recrystallisation from acetone and sublimation (180°/0·5 mm.) gave pale yellow needles which were identified as sterigmatocystin,[20] m. p. and mixed m. p. with authentic material 254—256° (decomp.), $[\alpha]_D^{26}$ − 396° (*c* 0·70 in chloroform) [Found: C, 66·5; H, 3·9; O, 29·7; *O*-Me 9·7%; *M*(Rast), 320. Calc. for $C_{18}H_{12}O_6$: C, 66·7; H, 3·7; O, 29·6; 1(*O*-Me), 9·7%; *M*, 324]. This compound had n.m.r., infrared and ultraviolet spectra that were identical with those of an authentic sample of sterigmatocystin which was kindly supplied by Dr. J. C. Roberts.

Asperugin (158 mg.) formed a *disemicarbazone* (50 mg.) under standard conditions. Recrystallisation from ethanol gave colourless microcrystals, m. p. 177—178° [Found: C, 60·5; H, 7·4; N, 16·1; *O*-Me 6·0; *C*-Me, 7·1%; *M*(Rast), 494. $C_{26}H_{38}N_6O_5$ requires C, 60·7; H, 7·4; N, 16·3; 1(*O*-Me), 5·8; 3(*C*-Me), 8·8%; *M*, 515.] The infrared spectrum contained peaks at 3400 (OH), 3240 (NH), and 1680 (C=N) cm.$^{-1}$.

A *monocyano-mono-oxime derivative* was formed when asperugin (120 mg.), in ethanol (3 ml.), was refluxed for 80 min. with a solution of hydroxylamine hydrochloride (167 mg.) and sodium acetate (159 mg.) in water (2 ml.). The solid that separated after concentration of the reaction mixture was recrystallised twice from aqueous ethanol to give the derivative as colourless needles (20 mg.), m. p. 139—140° [Found: C, 69·7; H, 8·0; N, 6·8; O, 15·7; *O*-Me, 7·6. $C_{24}H_{32}N_2O_4$ requires C, 69·9; H, 7·8; N, 6·8; O, 15·5; 1(*O*-Me), 7·5%]. The infrared spectrum included peaks at 3500 (OH), 3300 (NH), 2240 (C≡N), and 1680 (C=N) cm.$^{-1}$; γ (pyridine) 4·7 (3 protons, multiplet C=C*H*–CH_2–), 5·3 (2 protons, doublet, J = 7 c./sec., Ar–O–CH_2–CH=), 6·20 (3 protons singlet, $ArOCH_3$), 7·93 (8 protons, multiplet, =C–CH_2–CH_2–CH=), and 8·3 [12 protons, broad singlet, =C(Me)–].

Acid-catalysed Cleavage of Asperugin.—Silica gel (40 g., 200—300 mesh, L. Light and Co. Ltd.) after treatment with 5% aqueous acetic acid for 30 min. was washed with water (200 ml.)

[19] G. Pastuska, *Z. analyt. Chem.*, 1961, **179**, 355.

[20] J. E. Davies, D. Kirkaldy, and J. C. Roberts, *J.*, 1960, 2169; E. Bullock, J. C. Roberts, and J. G. Underwood, *J.*, 1962, 4179.

followed by methanol (300 ml.) and dried at 120° under reduced pressure. Asperugin (550 mg.) in light petroleum (20 ml.) was applied to a column of the acid-treated silica gel (40 g.) and subjected to gradient elution. The first few fractions, eluted with light petroleum–benzene were found to contain a mixture of cleavage products from the side-chain as an almost colourless oil (279 mg.). The infrared spectrum showed major peaks at 2950, 1460, 1370, and 883 cm.$^{-1}$ only. Gas–liquid chromatography of the product (10 μl.) on a silicone oil D.C. 200 column at 200°, using a flow rate of 150 ml. of hydrogen per min., indicated that the mixture contained five main components with retention times of 5·7, 6·9, 7·7, 10·8, and 17·0 min. respectively. This mixture was not investigated further.

Elution of the silica-gel column with benzene–ethyl acetate (4 : 1) yielded a yellow solid (191 mg.) which was sublimed at 100°/0·006 mm. to give the *phenolic product of acid-catalysed cleavage of asperugin* as fine yellow needles, m. p. 154—155° [Found: C, 55·4; H, 4·1; *O*-Me, 15·4%; *M* (isothermal distillation), 189. $C_9H_8O_5$ requires C, 55·1; H, 4·1; 1(*O*-Me), 15·8%; *M*, 196], $\nu_{max.}$ 3350 (OH), 1690 (aromatic CHO), and 1635 (hydrogen bonded CHO) cm.$^{-1}$; $\lambda_{max.}$ 219, 264, 333 mμ (log ε 3·95, 4·41, 3·87); τ (deuterochloroform) −2·62 (1 proton, singlet, chelated OH) −0·91 (1 proton, singlet, ArCHO), 0·08 (1 proton singlet, ArCHO), 2·71 (1 proton, singlet, ArOH), 2·91 (1 proton, singlet ArH), and 5·92 (3 proton, singlet, $ArOCH_3$).

The *disemicarbazone* was obtained as pale yellow rods, m. p. 165—166° (Found: N, 27·3. $C_{11}H_{14}N_6O_5$ requires N, 27·1%).

The phthalazine derivative was prepared by heating a mixture of the phenol (52 mg.), water (2 ml.), and hydrazine (0·0125 ml.) for 15 min. at 100°. The yellow crystalline solid which separated on standing at 0° for several days was recrystallised twice from methanol to yield the *phthalazine derivative* (V) as yellow needles, m. p. 228—230° (Found: C, 56·4; H, 4·4. $C_9H_8N_2O_3$ requires C, 56·3; H, 4·2%), $\lambda_{max.}$ 220, 256, 385 mμ (log ε 4·16, 4·58, 3·09).

Reduction of the Phenolic Cleavage Product, $C_9H_8O_5$.—(a) *Catalytic hydrogenation.* A solution of the phenol $C_9H_8O_5$ (400 mg.) in ethyl acetate (100 ml.) was treated with hydrogen at atmospheric temperature and pressure, using 10% palladium–carbon as catalyst (390 mg.). The colourless product (390 mg.) was purified by recrystallisation (ethyl acetate–light petroleum) and sublimation (100°/0·006 mm.) to give the *isobenzofuran* derivative (VI, 220 mg.), colourless needles, m. p. 216—218° (sealed tube), R_F 0·37 (system *A*) (Found: C, 59·2; H, 5·8. $C_9H_{10}O_4$ requires C, 59·3; H, 5·5%). The infrared spectrum showed no carbonyl absorption; τ (pyridine) 4·55 (2 protons, singlet, Ar–CH_2–O), 4·80 (2 protons, singlet, Ar–CH_2–O), and 6·20 (3 protons, singlet, $ArOCH_3$).

A mixture of the hydrogenation product (250 mg.), benzene (150 ml.), and lead dioxide (5 g.) were shaken for 12 min. After the removal of lead dioxide, the red solution that remained was treated for 1 hr. at 25° with a mixture of acetic acid (2 ml.) and *o*-phenylenediamine (150 mg.) in benzene (5 ml.). The solid (54 mg.) obtained from the solution in benzene which had been washed with 5% aqueous hydroxide was purified by sublimation (150°/0·006 mm.), followed by crystallisation from acetone, to give the *phenazine derivative* (VII) as yellow rhombs, m. p. 218—220° (Found: C, 71·3; H, 5·1; N, 11·1. $C_{15}H_{12}N_2O_2$ requires C, 71·4; H, 4·8; N, 11·1%); $\lambda_{max.}$ 237, 273, 370 mμ (log ε 3·41, 3·79, 3·15).

(b) *Clemmensen reduction.* A solution of the phenol, $C_9H_8O_5$ (452 mg.) in ethanol (25 ml.) was added gradually during 10 min. to a boiling suspension of zinc amalgam (from 10 g. zinc) in 22% hydrochloric acid (30 ml.); the mixture was refluxed for 65 min. The crude product obtained through extraction with ether was purified by fractional sublimation (90°/0·007 mm.) which gave as the major product (40 mg.) 3,4-*dihydroxy*-5-*methoxy*-1,2-*dimethylbenzene* (VIII), a colourless microcrystalline solid, m. p. 134—136°, R_F 0·59 (system *A*) (Found: C, 64·0; H, 7·1. $C_9H_{12}O_3$ requires C, 64·3; H, 7·2%). The infrared spectrum showed no carbonyl absorption, $\lambda_{max.}$ 213, 270 mμ (log ε 4·18, 2·86), τ (pyridine) 6·18 (3 protons, singlet, $ArOCH_3$), 7·81 (3 protons, singlet, Ar–CH_3) and 7·88 (3 protons, singlet, $ArCH_3$). The identity of this compound was established by comparison with synthetic material (*vide infra*).

Demethylation of the Clemmensen reduction product was achieved in the usual manner, using 46% hydrobromic acid. The crude product was purified by sublimation (110°/0·007 mm.) to give colourless needles, m. p. 151—152°, R_F 0·38 (system *A*) (Found: C, 62·5; H, 6·6. $C_8H_{10}O_3$ requires C, 62·3; H, 6·5%); $\lambda_{max.}$ 265 mμ (log ε 3·07), τ (pyridine) 7·61 (3 protons, singlet, $ArCH_3$) and 7·80 (3 protons, singlet, $ArCH_3$). Mixed m. p. determination and comparison of n.m.r. infrared and ultraviolet spectra established that this compound was identical with 3,4,5-trihydroxy-1,2-dimethylbenzene, for which Loudon and Summers [10] record m. p.

148°, but it differed from 3,4,6-trihydroxy-1,2-dimethylbenzene, m. p. 146—147°, the synthesis of which is described below.

3,4,6-*Trihydroxy*-1,2-*dimethylbenzene*.—2,3-Dimethyl-*p*-benzoquinone [21] (4 g.) was added in small portions to a stirred solution of conc. sulphuric acid (0·25 ml.) in acetic anhydride (9 ml.), keeping the temperature below 50°. The crude product, obtained by pouring the reaction mixture on ice (50 g.), neutralising with solid sodium hydrogen carbonate, and then extracting into ether, remained after removal of the solvent as a red-brown gum (4·5 g.). It was purified by crystallisation (light petroleum) and sublimation (85°/0·005 mm.) to give 3,4,6-*triacetoxy*-1,2-*dimethylbenzene* as colourless microcrystals, m. p. 89—90° (Found: C, 60·2; H, 5·7. $C_{14}H_{16}O_6$ requires C, 60·0; H, 5·8%).

A solution of the foregoing product (1·01 g.), in anhydrous methanol (10 ml.), was freed from dissolved oxygen by passing a stream of nitrogen for 10 min.; this was mixed with an oxygen-free solution of sodium (0·5 g.) in anhydrous methanol (25 ml.) and refluxed for 70 min. in an atmosphere of nitrogen. The red solution was diluted with water (20 ml.), and sodium dithionite (1 g.) was added to discharge the colour. The product (750 mg.) was obtained by removal of methanol, acidification, and ether extraction. It was purified by recrystallisation (benzene) and sublimation (110°/0·005 mm.) to give 3,4,6-*trihydroxy*-1,2-*dimethylbenzene* as fine needles, m. p. 146—147°, R_F 0·62 (system *A*) (Found: C, 62·4; H, 6·3. $C_8H_{10}O_3$ requires C, 62·3; H, 6·5%), $\lambda_{max.}$ 207, 273 mμ (log ε 4·03, 3·99), τ (pyridine) 7·48 (3 proton, singlet, $ArCH_3$); 7·56 (3 protons, singlet, $ArCH_3$).

2-(4″,5″-*Dimethyl*-2″-*methoxyphenoxy*)-3,5-*dinitrobenzophenone*.—4-Hydroxy-5-methoxy-1,2-dimethylbenzene (55 mg.) [22] and 2-chloro-3,5-dinitrobenzophenone [23] (1·0 g.) in dry pyridine (16 ml.) were allowed to stand for 15 hr. at room temperature. The solid that separated when the mixture was poured into 4N-hydrochloric acid crystallised from benzene and from hexane to give 2-(4″,5″-*dimethyl*-2″-*methoxyphenoxy*)-3,5-*dinitrobenzophenone* as yellow needles, m. p. 141—142° [Found: C, 62·4; H, 4·6; N, 6·4; *O*-Me, 18·0. $C_{22}H_{18}N_2O_7$ requires C, 62·6; H, 4·3; N, 6·6; 1(*O*-Me), 18·5%], τ (deuterochloroform) 1·35 (2 protons, doublet, Ar–H ring with NO_2 groups), 2·40 (5 protons, multiplet, C_6H_5), 3·57 (1 proton, singlet, Ar–H), 3·65 (1 proton, singlet, ArH), 6·50 (3 protons, singlet, $ArOCH_3$), 7·95 (3 protons, singlet, $ArCH_3$) and 8·08 (3 protons, singlet, Ar–CH_3).

3,4-*Dihydroxy*-5-*methoxy*-1,2-*dimethylbenzene*.—2-(4″,5″-Dimethyl-2″-methoxyphenoxy)-3,5-dinitrobenzophenone (500 mg.) was dissolved in a mixture of conc. sulphuric acid (1·25 ml.) and acetic acid (20 ml.), and treated with a solution of 30% hydrogen peroxide in acetic acid (1 : 2 v/v, 0·5 ml.). After 15 min. the mixture was poured on crushed ice (500 g.). The light yellow solid (412 mg.) that precipitated was collected. As the infrared spectrum of this compound showed a peak at 3490 $cm.^{-1}$ (OH) it was not characterised further.

A solution of this light yellow, hydroxy-compound (400 mg.) in benzene (40 ml.) was treated with phenylhydrazine (0·8 ml.) at room temperature, for 15 hr. The resultant solution was extracted with 2N-sodium hydroxide (2 × 50 ml.). The aqueous layer was acidified immediately with 4N-hydrochloric acid (100 ml.) and extracted with ether (2 × 250 ml.). The product obtained from the ether extract was sublimed (90°/0·007 mm.) and crystallised from benzene to furnish 3,4-*dihydroxy*-5-*methoxy*-1,2-*dimethylbenzene* (52 mg.) as colourless needles, m. p. (and mixed m. p. with the product of Clemmensen reduction of the phenol, $C_9H_8O_5$, derived from asperugin), 134—136° [Found: C, 64·3; H, 7·3; *O*-Me, 8·0. $C_9H_{12}O_3$ requires C, 64·3; H, 7·2; 1(*O*-Me), 8·2%]. A comparison of the ultraviolet, infrared, and n.m.r. spectra established that this compound was identical with the product of Clemmensen reduction of the phenol, $C_9H_8O_5$, derived from asperugin.

Cleavage of Asperugin with Sodium and Methanol in Liquid Ammonia.—Asperugin (1·18 g.) in methanol (30 ml.) was added to liquid ammonia (250 ml.) with the formation of a black solution. Sodium (10 g.) was added in small pieces during 1 hr. After the mixture had been stirred for a further 4 hr., the excess of sodium was destroyed by the addition of ammonium chloride (*ca.* 5 g.) followed by water (100 ml.). The oily product, which was obtained by extraction with ether in the usual way (491 mg.), was dissolved in chloroform (100 ml.) and treated with hydrogen at atmospheric temperature and pressure in the presence of Adams catalyst (64 mg.). When the hydrogen uptake (3·0 mol.) had ceased, the product was obtained as a colourless liquid

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(480 mg.) by removal of catalyst and solvent. Fractional distillation using micro-apparatus [24] indicated that only one major component was present. This fraction (260 mg.) had b. p. 124—126°/14 mm., n_D^{25} 1·4303 [Found: C, 85·1; H, 14·9%; *M* (mass spectrometry), 212. $C_{15}H_{32}$ requires C, 84·8; H, 15·2%; *M*, 212], $\nu_{max.}$ (thin film) 2950, 2920, 2865, 1465, and 1375 cm.$^{-1}$, τ (carbon tetrachloride) 8·80 (17 protons, broad singlet, $-CH_2-$ and $-CH-$), and 9·16 (15 protons, broad multiplet, $-C-CH_3$). Gas–liquid chromatography on Perkin-Elmer column "C" (silicone oil DC. 200) at 212° using nitrogen as a carrier gas at a flow rate of 80 ml./min. showed only one sharp peak, retention time 10·0 min. The major peaks in the mass spectrum are described in Table 2.

TABLE 2

m/e	212	197	183	169	155	141	127	113
Relative ion abundance % of base peak	3·4	2·1	6·1	1·6	2·6	2·5	15·8	13·0
m/e	99	85	71	57	43	29	18	
Relative ion abundance % of base peak	8·0	33·5	84·0	100	51	14·8	15·2	

The reduction product was identified as 2,6,10-trimethyldodecane (farnesane) by direct comparison with authentic material prepared from commercial farnesol (Fluka A.G.) by reduction using sodium and methanol in liquid ammonia, followed by catalytic hydrogenation, as in the case of the degradation product. The authentic 2,6,10-trimethyldodecane, b. p. 124—126°/14 mm. n_D^{25} 1·4303 [Found: C, 85·1; H, 15·2%; *M* (mass spectrometry), 212. Calc. for $C_{15}H_{32}$: C, 84·8; H, 15·2%; *M*, 212] had infrared n.m.r., and mass spectra and v.p.c. retention time identical with those of the product derived from asperugin.

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