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Studies in the Biochemistry of Micro-Organisms

94. THE COLOURING MATTERS OF SPECIES IN THE *ASPERGILLUS NIDULANS* GROUP PART I. ASPERTHECIN, A CRYSTALLINE COLOURING MATTER OF *ASPERGILLUS QUADRILINEATUS* THOM & RAPER

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One of the outstanding characteristics of the *Aspergillus nidulans* group of species of moulds is that they usually produce ascospores contained in dark-coloured perithecia. The ascospore members of the group consist of six recognized species or varieties (Thom & Raper, 1945) in cultures of which the colony reverse, the perithecial walls and the ascospores are usually all reddish purple to dark purple-red in colour. The ascospores are characterized by equatorial bands which are generally two in

number, but number four in one member of the group, *A. quadrilineatus* Thom & Raper—hence its specific epithet. We have investigated the colouring matters present in two highly pigmented strains of this species which were isolated in this Department from different samples of Australian soil, and have isolated from each of them in pure crystalline form a hitherto undescribed anthraquinone colouring matter for which we propose the name asperthecin. However, since the crude colouring matters isolated

from the two strains, S.M. 200 and S.M. 297, showed considerable chemical differences they will be described separately.

A. quadrilineatus strain S.M. 200 was cultivated on Raulin-Thom solution. Microscopic examination of these mature cultures, mounted in dilute aqueous sodium bicarbonate solution, showed that the dark purple-red colour of the perithecia and ascospores diffused and gave a purple-blue solution. No such effect was observed in cultures mounted in water. Further, when portions of mature mycelium, pressed as dry as possible on filter paper, were immersed in cold concentrated sulphuric acid a very intense purple colour was immediately produced.

The isolation, in a pure crystalline form, of the colouring matter, asperthecin, responsible for these reactions, was found to be unusually difficult and proved to be impossible by solvent extraction of the vacuum-dried mycelium. It was finally accomplished by disintegrating the freshly harvested and washed mycelium in aqueous *N* hydrochloric acid in a high-speed macerator. The resulting suspension was filtered, washed with water and drained thoroughly on the pump. The brown friable mass, while still moist, was extracted with large volumes of cold absolute ethanol. The orange-brown extract was evaporated to low bulk *in vacuo* and gave crude asperthecin in orange-brown needles. The average yield obtained corresponded to about 15 g./100 flasks containing a total of 35 l. of Raulin-Thom solution and is calculated to be, on the average, about 2.5 % of the dry weight of the mycelium.

Purification of the crude crystalline asperthecin by direct crystallization proved to be unsatisfactory. It was, however, readily purified via the crystalline hexaacetate, which was then hydrolysed to the parent colouring matter by boiling in methanol containing a little sulphuric acid. The recovered asperthecin then crystallized readily either from methanol as chestnut-brown needles or from dioxan as bright red needles containing solvent of crystallization.

Asperthecin, $C_{15}H_{10}O_8$, darkens but does not melt when heated to 370°. It contains no methoxyl group, or methyl group attached to carbon. It is almost insoluble in water and is not readily soluble in any organic solvent except pyridine. Its reddish orange solutions in organic solvents, including glacial acetic acid, do not fluoresce in daylight nor does its stable, intensely purple solution in cold concentrated sulphuric acid. It gives a stable purple ferric colour in ethanolic solution. It is readily soluble in dilute sodium bicarbonate, sodium carbonate and sodium hydroxide, giving purple-red to purple solutions which are increasingly unstable in air with rise in pH, so that the initially purple solution in aqueous sodium hydroxide fades

in a few seconds to orange and becomes colourless overnight.

The following crystalline functional derivatives of asperthecin were prepared and characterized. (a) Hexaacetylasperthecin, $C_{15}H_4O_2(O.CO.CH_3)_6$, by heating with acetic anhydride and a little concentrated sulphuric acid; pale yellow needles, m.p. 228°, optically inactive and insoluble in aqueous sodium carbonate. (b) Pentamethylasperthecin, $C_{15}H_8O_3(OCH_3)_5$, by prolonged methylation with dimethyl sulphate and anhydrous potassium carbonate in acetone; orange plates, m.p. 134–135°, which are insoluble in alkalis, even aqueous sodium hydroxide. (c) Dimethylasperthecin, $C_{15}H_8O_6(OCH_3)_2$, by brief methylation with diazomethane in acetone-ether; bright red blades, m.p. 236–237°, which are readily soluble in aqueous sodium carbonate. (d) Dimethylasperthecin tetraacetate, $C_{15}H_4O_2.(OCH_3)_2.(O.CO.CH_3)_4$, by acetylation of (c) with acetic anhydride and concentrated sulphuric acid; yellow rods, m.p. 229–230°.

Consideration of the properties of asperthecin already described shows that the pigment displays the character of a polyhydroxyquinone. Further, since it has also been shown that, on distillation with electrolytic zinc dust, asperthecin gives 2-methylanthracene, it follows that it must be a hexahydroxyanthraquinone since, as reported above, it gives, on acetylation, a hexaacetyl derivative, $C_{15}H_4O_2(O.CO.CH_3)_6$. Hence the functions of all the eight oxygen atoms are accounted for. So far as we are aware no natural polyhydroxyanthraquinone of biological origin, other than asperthecin, contains six hydroxyl groups.

The following evidence indicates that the side chain present in asperthecin which gives rise to a methyl group on zinc dust distillation is a methylol (CH_2OH) group. (i) Asperthecin does not contain a carbon-methyl ($C-CH_3$) grouping as was shown by a Kuhn-Roth estimation. (ii) Asperthecin does not contain a carboxyl ($COOH$) group, since its hexaacetate is not soluble in aqueous sodium carbonate. (iii) Asperthecin only gives a penta- and not a hexa-methyl ether on prolonged methylation with dimethyl sulphate and anhydrous potassium carbonate in acetone, and this pentamethyl ether is not soluble in aqueous sodium hydroxide.

The partial structure of asperthecin may therefore be represented as structure (I), and it now remains to determine the orientation of the five nuclear hydroxyl groups. The first evidence on this point is provided by the finding that when asperthecin is heated with concentrated hydriodic acid and red phosphorus in glacial acetic acid solution, a reduction product (almost certainly emodin anthranol) is formed which gives emodin (4:5:7-trihydroxy-2-methylanthraquinone, II) on oxidation with chromic acid in acetic acid solution.

Hence, assuming that no migration of hydroxyl groups occurs during the reduction, the partial structure of asperthecin may be expanded to (III).

The evidence available at present for the orientation of the two remaining hydroxyl groups is as follows. In spite of repeated attempts, carried out under a variety of experimental conditions, we have completely failed to detect any fluorescence in daylight of any solutions of asperthecin, including its reddish orange solution in glacial acetic acid and its intense purple solution in cold concentrated sulphuric acid. Raistrick, Robinson & Todd (1934, p. 567) compared the colours and fluorescences of solutions in glacial acetic acid of eight synthetic and seven natural hydroxyanthraquinones and concluded that fluorescence is characteristic of quinizarin (1:4-dihydroxyanthraquinone) derivatives. In the intervening years a number of other polyhydroxyanthraquinones of established structure have been examined and have confirmed the validity of this generalization. The only way in which two nuclear hydroxyl groups can be introduced into structure (III) which will avoid formulating asperthecin as a derivative of quinizarin is structure (IV). Hence, unless asperthecin is an exception to the above generalization, the two remaining hydroxyl groups must be in positions 3 and 6. This conclusion is supported by the fact that, although solutions of asperthecin did show a weak fluorescence when viewed in ultraviolet light, this fluorescence was similar in colour to, but slightly less intense than, that given by emodin (II) in the same solvents.

On this evidence asperthecin is 3:4:5:6:7-pentahydroxy-2-hydroxymethylanthraquinone (structure IV) and as will be shown later this structure illustrates certain properties of asperthecin which are unique among polyhydroxyanthraquinones of mycological origin and rare among those from any natural source.

Structure (IV), however, does not clearly illustrate the ultraviolet and infrared absorption spectra of asperthecin. Birkinshaw (see Appendix to this paper) has determined the ultraviolet spectra of a

number of natural and synthetic polyhydroxyanthraquinones of established structure and has shown that, of nine polyhydroxyanthraquinones examined, the ultraviolet spectrum of catenarin, 1:4:5:7-tetrahydroxy-2-methylantraquinone (Anslow & Raistrick, 1940, 1941) is most closely related to that of asperthecin. Since the ultraviolet spectrum of the unknown ω -hydroxycatenarin (V) would be expected to be practically the same as that of catenarin in the $\lambda 200$ – 600 m μ . region, as is known to be the case with emodin and ω -hydroxy-emodin, we may deduce from this evidence that asperthecin is a hydroxy derivative of ω -hydroxycatenarin of which three are possible, i.e. (a) 3-hydroxy-, (b) 6-hydroxy-, and (c) 8-hydroxy- ω -hydroxycatenarin.

Of the three possibilities (c), the 8-hydroxy compound, is the least probable structure for asperthecin since it contains only one β -hydroxyl group and hence, unlike asperthecin, would probably be insoluble in aqueous sodium bicarbonate. Further, (c) would be a simple derivative of the known 8-hydroxycatenarin, 1:4:5:7:8-pentahydroxy-2-methylantraquinone, which dissolves in concentrated sulphuric acid to a pure blue solution with a red fluorescence (Raistrick *et al.* 1934, p. 570), whereas asperthecin gives a purple non-fluorescent solution in the same reagent. It is also improbable that asperthecin is the 3-hydroxy derivative (a), since the closely related 1:3:5:7-tetrahydroxy-2:6-dimethylantraquinone is, unlike asperthecin, insoluble in aqueous sodium bicarbonate (Kusaka, 1935, quoted by Asahina & Shibata, 1954, p. 156). The 6-hydroxy derivative (b) (structure VI) therefore seems to be the most probable of the three possible hydroxy derivatives, (a), (b) and (c), of ω -hydroxycatenarin. On this

evidence asperthecin is 1:4:5:6:7-pentahydroxy-2-hydroxymethylanthraquinone.

Briggs, Nicholls & Paterson (1952) found that in the ultraviolet absorption spectra of hydroxy-anthraquinones the major absorption band due to the carbonyl group of the quinonoid nucleus is greatly affected by the number of free, i.e. unsubstituted, α -hydroxyl groups, the presence of β -hydroxyl groups having little effect. Thus, in hydroxyanthraquinones containing no free α -hydroxyl groups this band occurs at 356–362.5 $m\mu$, with one free α -hydroxyl group at 407.5–413.5 $m\mu$, and with two such groups at 427.5–432.5 $m\mu$. These figures are broadly confirmed by Birkinshaw (see Appendix to this paper) and amplified by the observation that in three anthraquinones, each having three free α -hydroxyl groups, the above-mentioned bands give place to new bands in the 490 and 510 $m\mu$ regions, i.e. islandicin (1:4:5-trihydroxy-2-methylanthraquinone) at 492 and 513 $m\mu$; helminthosporin (4:5:8-trihydroxy-2-methylanthraquinone) at 480, 490 and 510 $m\mu$; and catenarin (1:4:5:7-tetrahydroxy-2-methylanthraquinone) at 488.5 and 508 $m\mu$. It is therefore perhaps significant that asperthecin has bands at 484 and 510 $m\mu$ and that structure (VI) has three free α -hydroxyl groups while structure (IV) has only two. On this evidence structure (VI) is to be preferred to structure (IV). On the other hand, since any anthraquinone having three free α -hydroxyl groups must of necessity be a derivative of quinizarin (1:4-dihydroxyanthraquinone) it is curious that while islandicin, helminthosporin and catenarin all give markedly fluorescent solutions in glacial acetic acid, asperthecin shows no such fluorescence.

The infrared spectrum of solid asperthecin has also been examined. Flett (1948) examined two mono-, five di-, one tri-, and one tetra-hydroxy-anthraquinone in the solid state and concluded that a band in the region of 1670–1680 cm^{-1} in the infrared absorption spectrum is characteristic of a carbonyl group which is not chelated with an α -hydroxyl group, while a band in the 1630–1640 cm^{-1} region indicates a carbonyl group chelated with an α -hydroxyl group. The infrared spectrum of solid asperthecin has a broad absorption band in the double-bond stretching region with a maximum at 1606 cm^{-1} and a shoulder at about 1575 cm^{-1} . No band was, however, observed near 1670 cm^{-1} . Hence, if one accepts Flett's correlation rule it follows that both the C=O groups in asperthecin must be chelated with α -hydroxyl groups, so that structure (IV) for asperthecin, which contains only one chelated carbonyl group, would be untenable. However, workers at the Butterwick Research Laboratories of Imperial Chemical Industries Ltd. (L. A. Duncanson & J. F. Grove, private com-

munication) have examined 19 polyhydroxy-anthraquinones and derivatives in the solid state and have shown that the correlation rules worked out by Flett on simple compounds cannot always be applied to polyhydroxyanthraquinones. Thus, although alizarin (1:2-dihydroxyanthraquinone) shows a strong absorption band at 1664 cm^{-1} , anthragallol (1:2:3-trihydroxyanthraquinone) shows only a weak shoulder at about 1660 cm^{-1} . Hence, since structure (IV) for asperthecin contains the 1:2:3-trihydroxyanthraquinone structure, it appears certain that structure (IV) cannot be eliminated on the infrared evidence alone.

However, analysis of the Butterwick Laboratory infrared spectra seems to indicate a correlation between the frequency of the strongest absorption band in the 6 μ . (1670 cm^{-1}) region and the number and position of free α -hydroxyl groups in polyhydroxyanthraquinones produced by moulds and containing two or more α -hydroxyl groups. Thus, in cynodontin with four α -hydroxyl groups (1:4:5:8-tetrahydroxy-2-methylanthraquinone) the band is at 1580 cm^{-1} ; in four substances with three α -hydroxyl groups the band is close to 1600 cm^{-1} , i.e. helminthosporin (4:5:8-trihydroxy-2-methyl-) at 1602, islandicin (1:4:5-trihydroxy-2-methyl-) at 1598, catenarin (1:4:5:7-tetrahydroxy-2-methyl-) at 1600, and erythroglaucon (1:4:5-trihydroxy-7-methoxy-2-methyl-) at 1600; and in four substances with two hydroxyl groups in the α -position to the same carbonyl group the band is close to 1625 cm^{-1} , i.e. emodin (4:5:7-trihydroxy-2-methyl-) at 1623, ω -hydroxyemodin (4:5:7-trihydroxy-2-hydroxymethyl-) at 1625, physcion (4:5-dihydroxy-7-methoxy-2-methyl-) at 1627, and chrysophanol (4:5-dihydroxy-2-methyl-) at 1627. Since the corresponding band in asperthecin is at 1606, it seems logical to conclude that asperthecin contains, in addition to other substituents, the 1:4:5-trihydroxy grouping, a conclusion which is in agreement with the ultraviolet absorption spectra findings and with structure (VI) for asperthecin.

It is thus clear that, while both structures (IV) and (VI) illustrate many of the properties and reactions of asperthecin, neither structure explains all of them. It now remains to examine which of these structures best illustrates the other unusual properties of asperthecin previously referred to, which are:

(a) Asperthecin has the unusual property that it is readily soluble in aqueous sodium bicarbonate although it does not contain a $-\text{COOH}$ group, as is indicated by its empirical formula and by the fact that its hexaacetate is not soluble in sodium carbonate. It is a generally accepted rule that polyhydroxyanthraquinones having only α -hydroxyl groups, i.e. in positions 1, 4, 5 or 8, dissolve in aqueous sodium hydroxide but do not dissolve in

aqueous sodium carbonate or in sodium bicarbonate, while those having β -hydroxyl groups, i.e. in positions 2, 3, 6 or 7, whether with or without α -hydroxyl groups, dissolve in aqueous sodium hydroxide or carbonate but rarely in sodium bicarbonate. It appears probable that this generalization must be modified in the case of those polyhydroxyanthraquinones which have two or more β -hydroxyl groups. Thus, while anthragallol (1:2:3-trihydroxyanthraquinone) with two β -hydroxyl groups is insoluble in sodium bicarbonate, the following are all soluble in this reagent: rufigallic acid (VII), synthesized by Robiquet (1836) from gallic acid, which has four β -hydroxyl groups; asperthecin (if IV) which has three β -hydroxyl groups; asperthecin (if VI) and 1:3:6:8-tetrahydroxyanthraquinone (Shibata, 1941) and rhodocladonic acid (VIII), from a number of lichen species in the genus *Cladonia* (Zopf, 1907; Koller & Hamburg, 1936; Shibata, 1941), which have two β -hydroxyl groups. Rufigallic acid and asperthecin decompose very rapidly in strongly alkaline solution. This remarkable instability towards alkalis is generally believed to be due to the presence of three vicinal hydroxyl groups, a belief which is illustrated in both structures (IV) and (VI) for asperthecin.

(VII)
Rufigallic acid

(VIII)
Rhodocladonic acid

(b) Asperthecin is a powerful mordant dyestuff and dyes white wool, mordanted with chromium, aluminium or tin, an intense indigo blue, deep violet or dull red colour respectively. This property which, among polyhydroxyanthraquinones, is characteristic of derivatives of alizarin (1:2-dihydroxyanthraquinone), is in conformity with structure (IV) for asperthecin which contains two 1:2-dihydroxy groupings and is therefore doubly an alizarin derivative, and also with structure (VI).

Chemical work on the constitution of asperthecin is proceeding. In the meantime we believe that asperthecin is either 3:4:5:6:7- or 1:4:5:6:7-penta-hydroxy-2-hydroxymethylanthraquinone of structures (IV) or (VI) respectively. We have at present

no evidence to decide conclusively between these structures.

The second strain of *A. quadrilineatus*, strain S.M. 297, was cultivated on Czapek-Dox or Raulin-Thom solutions and gave mycelia from each culture medium which, when freshly harvested, gave an intense prussian blue colour in cold concentrated sulphuric acid which was quite different from the deep purple colour given by mycelia of strain S.M. 200 in the same reagent. Isolation of the colouring matters of strain S.M. 297 by extraction of the pressed, disintegrated mycelium with ethanol, exactly as was done with strain S.M. 200, resulted in a light brown, mainly crystalline solid in a yield of about 8 g./100 flasks which gave the typical intense prussian blue sulphuric acid colour observed with the intact mycelium. However, all attempts to purify this crude material and to isolate from it the pure colouring matter giving the blue sulphuric acid colour have so far failed and have led instead to the isolation of asperthecin in all cases. The methods used for purification included direct crystallization from dioxan; acetylation, which furnished an acetate having the same melting point and mixed melting point as hexaacetylasperthecin and giving pure asperthecin on hydrolysis; chromatography on calcium carbonate, which did indeed give two different coloured bands, which were separated mechanically, but yielded asperthecin in each case. Further, when the fresh ethanolic extract of the mycelium of strain S.M. 297 was diluted with ether and kept in the laboratory for a week, the initially reddish solution had changed colour to orange and the sulphuric acid colour of the colouring matter contained in it had changed from deep blue to purple.

It therefore seems very probable that the colouring matter giving the blue sulphuric acid colour is very unstable and is readily converted into asperthecin, possibly by tautomeric change to which both structures (IV) and (VI) would appear to lend themselves readily.

A preliminary survey has been carried out on a number of authentic strains of *Aspergillus nidulans* (Eidam) Wint. and other species in the *A. nidulans* group, many of which have long been in laboratory culture and were not highly pigmented. None of them proved to be nearly as rich in asperthecin as *A. quadrilineatus* strain S.M. 200. However, small amounts of crystalline material having the properties of asperthecin (colour; crystal form; solubility in sodium bicarbonate to a purple-red solution; deep purple solution in cold concentrated sulphuric acid) were obtained from cultures on Czapek-Dox solution of one strain each of *A. nidulans*, *A. nidulans* mut. *alba* Yuill and strain N.R.R.L. 521 of *A. quadrilineatus* from Dr K. B. Raper. Small amounts of amorphous material giving the colour

reactions of asperthecin were isolated from two other strains of *A. nidulans* and one strain of *A. rugulosus* Thom & Raper. There is thus good presumptive evidence for the belief that asperthecin will prove to be of frequent occurrence in species in the *A. nidulans* group, particularly in cultures recently isolated from natural sources.

EXPERIMENTAL

Cultures

Two strains of *Aspergillus quadrilineatus* Thom & Raper, both isolated here by Mrs S. Marcus, were used in this work. They were (a) Strain L.S.H.T.M. catalogue no. S.M. 200. Isolated in October 1950 from Australian soil sample 257-1. This light clay Stony Desert surface soil (0-1 in.), pH 6-8, was collected about 15 miles west of Tibooburra, New South Wales. This strain, which was identified by Mr G. Smith to whom we are indebted for the following notes, is predominantly perithecial on all culture media. Cultures show little green colour, being mostly thick grey layers of hülle cells in which the purple perithecia are embedded. The ascospores are quite typical, the four equatorial bands (the origin of the specific epithet) being quite normal as evident as in the type strain N.R.R.L. 201 received from Dr Kenneth B. Raper. (b) Strain L.S.H.T.M. catalogue no. S.M. 297. Isolated in February 1951 from Australian soil sample 221-1. This very loose red sandy surface soil (0-½ in.), pH 7-4, was collected from the sand ridge country on the New South Wales-South Australian border. The culture was identified by Dr K. B. Raper as a somewhat abnormal strain of *A. quadrilineatus*. It is quite normal in colony appearance, but is somewhat abnormal in the unusual indistinctness of the two outer of the four ridges on the ascospores, demonstration of which with certainty demands the limit of resolution of the microscope.

It is of interest to note that both soils were from the most arid situations of the very low rainfall area of New South Wales.

We wish to thank Professor E. G. Hallsworth, now Head of the Department of Agricultural Sciences of the University of Nottingham, for the gift of a number of Australian soil samples including the two samples referred to above.

THE COLOURING MATTER OF

A. QUADRILINEATUS STRAIN S.M. 200

We have found, by microscopic examination of cultures of this strain of various ages and mounted in a variety of media, that the characteristic dark red or purple colour of the perithecial walls and ascospores is due to the presence, probably in the form of a salt, of a hitherto undescribed colouring matter for which we propose the name *asperthecin*. Thus, immature cultures, containing only hyphae and hülle cells, showed no colour when mounted in any medium. Mature perithecia however appeared dark blue to black in aqueous suspension and orange in lacto-phenol. In dilute aqueous NaHCO_3 the colour diffused rapidly from the perithecia and more slowly from the ascospores, giving a purple-blue solution.

Cultural conditions and characteristics

Raulin-Thom solution (glucose, 75.0 g.; tartaric acid, 4.0 g.; ammonium tartrate, 4.0 g.; $(\text{NH}_4)_2\text{HPO}_4$, 0.6 g.; $(\text{NH}_4)_2\text{SO}_4$, 0.25 g.; K_2CO_3 , 0.6 g.; MgCO_3 , 0.4 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.07 g.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.07 g.; distilled water, 1500 ml.) was distributed in 350 ml. amounts in 1 l. conical flasks. The flasks were plugged with cotton wool, sterilized and inoculated with a suspension in water prepared from mature cultures on slopes of Czapek-Dox or beer wort agar of *A. quadrilineatus* strain S.M. 200. The inoculated flasks were incubated for varying periods at 24° in the dark. During growth the surface of the mycelium became deeply folded, granular in texture and a dark buff colour, with a dark brown reverse. The culture fluid became a dull yellow colour. A number of cultural details including yields of asperthecin are collected in Table 1, in which, for strain S.M. 200, each batch contained 25 flasks. The weight of mycelium if dried is estimated to be about 150 g./25 flasks.

At intervals during the incubation period portions of the mycelium were pressed between filter papers and immersed in cold conc. H_2SO_4 or aqueous $\text{N-Na}_2\text{CO}_3$ or aqueous N-NaOH . In H_2SO_4 the mycelium gave after 7 days a moderate purple, after 14 days a strong violet and after 21 days a very dark purple colour. In Na_2CO_3 and NaOH the mycelium gave little or no colour after 7 or 14 days, but after 21 days gave a moderate purple in both reagents. Single flasks, examined after 21 days' growth, yielded approximately 6 g. of washed and dried mycelium.

Table 1. *Cultural details and yields of asperthecin or crude colouring matter from Aspergillus quadrilineatus strains*

Batch no.	Incubation period (days)	Residual glucose by polarimeter (%)	pH	Wt. of crude crystalline asperthecin (g.)
Strain S.M. 200				
1	21	0.75	5.4	3.49
2	26	0.4	6.0	3.49
3	24	0.9	5.9	3.14
4	28	0.7	6.1	4.17
5	32	0.6	6.3	3.63
6	36	0.4	6.2	4.92
Strain S.M. 297				
1	28	0.7	8.0	1.44*
2	38	0.5	8.1	1.97*

* Crude colouring matter.

Isolation of asperthecin

Considerable difficulty was experienced in attempting to isolate the colouring matter from the mycelium. Yields satisfactory in quantity and quality could be obtained only by careful attention to the following points. (i) Drying of the mycelium, even *in vacuo* and at temperatures below 45°, led to poor yields of impure material on subsequent solvent extraction. (ii) The use of hot solvents yielded material similar to that obtained in (i). (iii) The pigment could not be recovered satisfactorily from CaCO_3 chromatograms, being accompanied by a dark tarry impurity or decomposition product. (iv) Asperthecin is present in the mycelium, at least in part, as a salt which is insoluble in water and in organic solvents. Hence the mycelium must be treated with acid before the bulk of the colouring matter can be removed. (v) Asperthecin is contained very largely or perhaps wholly in the perithecia and ascospores. Hence mechanical disintegration of the perithecia before solvent extraction considerably facilitates removal of the colouring matter.

The best method of isolation of asperthecin was therefore found to be the following. At the end of the incubation period (for batch 6, 36 days at 24°) the culture fluid was separated from the mycelium by decanting through muslin and the mycelium was washed once with water. The mycelial mats were then suspended in aqueous N-HCl and disintegrated, five at a time, in a Top Drive Macerator (Townson and Mercer, Croydon). After 10 min. treatment, microscopic examination of the brown suspension showed the absence of intact cells apart from a few hülle cells and ascospores. The suspension was filtered through a hardened paper, washed with water until free from acid and drained thoroughly on the pump. The mycelium at this stage was a brown friable mass containing about 50% solids. It was roughly crumbled and vigorously stirred for 4 hr. with ethanol (2 l.). After standing overnight, the dark orange-brown ethanolic extract was separated, the solid re-stirred during 1 working day with ethanol (2.5 l.) and kept overnight. A total of six such extracts was made, the last being of a very pale brown colour. Each extract was evaporated *in vacuo*, the first to about 100 ml., the last to about 20 ml. In each case the solid which separated consisted mainly of orange-brown needles, accompanied in the first extract by a few colourless needles and rods and in the succeeding extracts by large quantities of 'fat'. The concentrated extracts were filtered separately, and the solids were washed first with a little ethanol and then copiously with light petroleum (b.p. 40–60°) until free from 'fat'. Yields of brown crystalline solid from batch 6 (1) 2.49 g.; (2) 1.08 g.; (3) 0.39 g.; (4) 0.34 g.; (5+6) 0.62 g.; total 4.92 g. from 25 flasks, corresponding to approximately 3.3% of the estimated wt. of dry mycelium. Only small amounts of very impure material could be obtained from the dark brown ethanol mother liquors, and 'fat' (21 g.) was left on evaporation of the light petroleum washings.

Purification and properties of asperthecin

Purification by direct crystallization of the crude crystalline product obtained above could not be effected with certainty and it was more convenient to convert it into the acetate, which was purified by crystallization and hydrolysed (see p. 482). The product of the hydrolysis of the pure acetate was a brownish red crystalline solid which could be crystallized from methanol or dioxan. Asperthecin forms chestnut-

brown needles from methanol which darken but do not melt when heated to 370°. (Found, on material dried in high vacuum at 110° without loss in wt.: N, S, halogens, OCH_3 , nil; C, 56.7; H, 3.2. $\text{C}_{15}\text{H}_{10}\text{O}_8$ requires C, 56.6; H, 3.2%.) Asperthecin separates from dioxan as bright red needles containing solvent of crystallization. (Found: loss on drying in high vacuum at 130°, 21.3%. $\text{C}_{15}\text{H}_{10}\text{O}_8 \cdot \text{C}_4\text{H}_8\text{O}_2$ requires 21.7%. Found on dried material: OCH_3 , C-CH_3 , nil; C, 56.6; 56.6, H, 3.2, 3.2. $\text{C}_{15}\text{H}_{10}\text{O}_8$ requires C, 56.6; H, 3.2%.)

Asperthecin is almost insoluble in water, light petroleum, CHCl_3 or benzene; slightly soluble in methanol or ethanol, and moderately soluble in acetone, glacial acetic acid and dioxan. Its solutions in organic solvents are reddish orange in colour and do not fluoresce in daylight. It dissolves very readily in pyridine, forming a pyridine salt which separates as purple needles on standing. Asperthecin dissolves in cold conc. H_2SO_4 to a stable purple non-fluorescent solution, which appears redder by transmitted light and bluer in thin layers, and in cold conc. HNO_3 to a blue solution which becomes purple, and then fades, on standing. It gives a stable purple ferric colour in ethanolic solution. It dissolves in boiling aqueous aluminium sulphate giving, after filtration, a clear purple, non-fluorescent solution which is almost indistinguishable in colour from an aqueous solution of potassium permanganate.

It is noteworthy that asperthecin is soluble in 0.5N NaHCO_3 to a stable purple-red solution. It dissolves in $\text{N-Na}_2\text{CO}_3$ to a purple-red solution, which is bluer than that in NaHCO_3 and fades overnight on standing in air with the formation of a blue precipitate; in aqueous N-NaOH it gives a purple solution which fades within a few seconds on exposure to air to a dull pale orange and becomes colourless overnight. Portions of a dilute ethereal solution of asperthecin were shaken with equal volumes of 0.2M phosphate or borate buffers. At pH 4.0 and below the aqueous layer was colourless; at pH 5.0 it was very pale purple and at pH 6.0 a pale purple. At pH 7.2 the ether layer was pale orange and the aqueous layer a strong purple-red; with increasing pH the ether layer became still paler orange in colour until at pH 8.0 and above the ether layer was quite colourless and the aqueous layer a strong purple-red.

Addition of ethanolic potassium acetate (5%, w/v) to a hot ethanolic solution of asperthecin caused the immediate precipitation of the potassium salt as an amorphous, deep violet solid, insoluble in ethanol and organic solvents but readily soluble in water.

Asperthecin is chromatographically homogeneous, giving a uniform purple-pink band on CaCO_3 columns and on MgCO_3 a bluer more intensely coloured band, when chromatographed in ethanolic solution. It is reduced by zinc dust in glacial acetic acid solution, the colour changing quickly from orange to purple and then to pale yellow. $\text{Na}_2\text{S}_2\text{O}_4$ is without effect when added to a NaHCO_3 solution, but an excess of the reagent added to a solution in $\text{N-Na}_2\text{CO}_3$ changes the colour from purple-red to orange.

Derivatives of asperthecin

Hexaacetylasperthecin. Crude crystalline asperthecin (2.0 g.) was mixed with acetic anhydride (16 ml.) containing conc. H_2SO_4 (0.3 ml.). The mixture became warm, and yellow needles separated from the clear solution. After 10 min. the mixture was heated at 100° for 5 min. to complete the reaction. The brown solution was kept at 0° and the crystals were collected on a sintered glass filter, washed with

glacial acetic acid then with much water and dried. The slightly orange-yellow product (2.4 g.), m.p. 212–215°, was crystallized twice from glacial acetic acid (25 ml.) and a portion (0.10 g.) of the resulting material was purified for analysis by crystallizing several times from ethanol (100 ml.) giving *hexaacetylasperthecin* as pale yellow needles, m.p. 228°. (Found, on material dried in high vacuum at 110°: OCH₃, nil; C, 56.9, 56.85; H, 4.0, 3.8; CH₃CO, 45.2; mol.wt. (cryoscopic in camphor) 531, 529. C₂₇H₂₂O₁₄ requires C, 56.8; H, 3.9; 6CH₃CO, 45.3%; mol.wt. 570.) Hexaacetylasperthecin shows no optical activity in CHCl₃ solution (c, 3.90), is insoluble in 0.5N-NaHCO₃ and N-Na₂CO₃ and gives no ferric colour in ethanol.

The original acetic anhydride filtrate and acetic acid washings were treated with ice water in the usual way to yield crude hexaacetylasperthecin as an orange amorphous solid (0.7 g.) which melted indefinitely from 185° upwards and was most conveniently purified by crystallizing from a small volume of the acetic anhydride-conc. H₂SO₄ mixture.

Hydrolysis of pure hexaacetylasperthecin A solution of pure hexaacetylasperthecin (0.78 g.) in methanol (70 ml.) containing 3% (v/v) of conc. H₂SO₄ was boiled under reflux for 1.5 hr. The solid dissolved to an orange solution during 5 min. and asperthecin began to separate as reddish needles after 30 min. The crystals were collected after storing overnight at 0° giving purified asperthecin as a brownish red solid (0.40 g.) which was crystallized from methanol or dioxan (see p. 481). The orange-red methanol filtrate was poured into hot water (350 ml.) from which the rest of the colouring matter (0.04 g.) separated on cooling.

Pentamethylasperthecin. A mixture of asperthecin (0.10 g.), dry acetone (80 ml.), dimethyl sulphate (1.0 ml.) and freshly ignited K₂CO₃ (2.0 g.) was boiled under reflux. The mixture soon went purple and then slowly became brown. After 14 hr. boiling, the blue solid was removed by filtration and the solvent was evaporated from the filtrate giving an oily orange residue which was shaken with aqueous 0.2N-NaOH to remove dimethyl sulphate. The residue was sublimed in high vacuum at 180°, giving an oily orange sublimate which was crystallized twice from ethanol-light petroleum (b.p. 60–80°). *Pentamethylasperthecin* was obtained as large flat orange plates, m.p. 134–135°. (Found, on material dried in high vacuum at room temp.: C, 61.4, 61.7; H, 5.4, 5.2; OCH₃, 39.6, 39.5. C₃₀H₃₀O₈ requires C, 61.85; H, 5.2; 5 OCH₃, 40.0; C₂₁H₂₂O₈ requires C, 62.7; H, 5.5; 6 OCH₃, 46.3%.) Pentamethylasperthecin is insoluble in alkalis, gives a stable purple-blue solution in cold conc. H₂SO₄ and a red solution in cold conc. HNO₃.

Dimethylasperthecin. Asperthecin (1.2 g.) was ground under acetone (30 ml.) to a fine suspension. An ethereal solution (400 ml.) of diazomethane (from 3 g. of nitroso-methylurea) was quickly added. A brick-red precipitate of needles separated, and after standing for 2 hr. at room temp. the mixture was concentrated to 50 ml. and kept overnight at 0°. The crystals were collected as a bright brick-red solid (1.07 g.) which was purified by crystallization from dioxan and then ethanol giving *dimethylasperthecin* as clusters of thin blades, bright red in bulk, m.p. 236–237°. (Found, on material dried in high vacuum at 120°: C, 58.8, 58.9; H, 4.2, 4.2; OCH₃, 18.0, 18.2. C₁₇H₁₄O₈ requires C, 58.9; H, 4.1; 2 OCH₃, 17.9%.) Dimethylasperthecin gives generally similar colour reactions to asperthecin itself, except that it is not soluble in 0.5N-NaHCO₃. In N-Na₂CO₃ it gives a red-violet solution, and in aqueous N-NaOH a violet solution,

both solutions being slightly bluer and more stable than the corresponding asperthecin solutions; cold conc. H₂SO₄, stable purple-blue solution; cold conc. HNO₃, blue solution; ethanolic FeCl₃, stable purple solution, slightly browner than that given by asperthecin. Unlike asperthecin it is readily reduced by Na₂S₂O₄. The reduction product gives an orange-yellow solution in ether with a green fluorescence, and a yellow solution in N-Na₂CO₃.

Dimethylasperthecin tetraacetate. Dimethylasperthecin (0.9 g.) was heated for 15 min. at 100° with acetic anhydride (3 ml.) containing 2% (v/v) conc. H₂SO₄. Yellow crystals soon began to separate, and, after chilling, the semi-solid mass was transferred to a sintered glass filter and washed first with glacial acetic acid and then with much water. The dried product (1.17 g.) was crystallized from acetic anhydride (7 ml.) yielding *dimethylasperthecin tetraacetate* as yellow pointed rods, m.p. 229–230°. (Found, on material dried in high vacuum at 120°: C, 58.3; H, 4.5; OCH₃, 12.3; CH₃CO, 32.2. C₂₈H₂₂O₁₂ requires C, 58.35; H, 4.3; 2 OCH₃, 12.1; 4 CH₃CO, 33.5%.)

Hydrolysis of dimethylasperthecin tetraacetate. Dimethylasperthecin tetraacetate (0.20 g.) was boiled under reflux with methanol (15 ml.) containing 3% (v/v) conc. H₂SO₄. The hydrolysis proceeded in a similar manner to that of hexaacetylasperthecin (see this page); after 1.5 hr. the mixture was cooled and the precipitate of bright red needles was collected (0.12 g.), washed with methanol and then water. When crystallized from dioxan this product formed bright red thin blades, identical in all respects with dimethylasperthecin; m.p. and mixed m.p. 236–237°.

Degradation of asperthecin

By zinc dust distillation. Isolation of 2-methylanthracene. An intimate mixture of asperthecin (0.20 g.) and electrolytic Zn dust (15 g.) was heated in portions of approx. 1 g. in special glass tubes as described by Kögl & Postowsky (1924). The pale yellow crystalline distillates were dissolved in ether, dried over KOH and the solution was evaporated. The residue (10 mg.) was sublimed *in vacuo* at 110° and crystallized twice from ethanol to yield almost colourless leaflets, m.p. 195–197°. A mixture with authentic 2-methylanthracene, m.p. 203–204°, melted at 201–202°. The ultraviolet absorption spectrum of the product was compared with that of authentic 2-methylanthracene, with the following results. Zn dust product: light absorption max., 253.5, 310, 326.5, 342, 359 and 379 mμ. (log ε 5.18, 3.30, 3.53, 3.75, 3.89 and 3.83 respectively). Found on authentic 2-methylanthracene: light absorption max., 254, —, 326.5, 342, 359.5 and 379 mμ. (log ε 5.20, —, 3.42, 3.69, 3.81 and 3.76 respectively). There was no maximum at 310 mμ.

With HI and red phosphorus. Isolation of Frangula emodin. A mixture of asperthecin (0.50 g.), red P (0.50 g.), glacial acetic acid (15 ml.) and HI (3 ml., sp.gr., 1.7) was boiled under reflux for 5 hr. The brown mixture was cooled, poured into water (500 ml.) and decolorized with a little NaHSO₃. The resulting purple-brown precipitate was collected, washed with water, dried and extracted with boiling glacial acetic acid (50, 20, 20 ml.). Concentration of the combined brown extracts to 15 ml. yielded a light brown micro-crystalline product (0.28 g.) which gave the colour reactions of an anthranol.

The crude anthranol (0.26 g.) was dissolved in glacial acetic acid (20 ml.) by warming, and to the cooled solution was added a solution of CrO₃ (0.3 g.) in glacial acetic acid

(15 ml.). After standing for 30 min. at room temp. the brown mixture was diluted with much water and extracted 3 times with ether. The orange ethereal extracts were freed from acetic acid and a trace of coloured material by shaking repeatedly with aqueous NaHCO_3 , and were then washed with water and evaporated to dryness. The brown-orange residue (0.10 g.) was sublimed in high vacuum at 180° and the orange sublimate was crystallized from toluene (25 ml.) giving orange needles, m.p. 256° alone or mixed with authentic *Frangula* emodin, m.p. 256° . (Found, on sample sublimed at 180° in high vacuum: C, 66.8, 66.9; H, 3.8, 3.7. Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_5$: C, 66.7; H, 3.7%.) The product and authentic emodin gave indistinguishable solutions in $\text{N-Na}_2\text{CO}_3$ (slightly bluish red) and cold conc. H_2SO_4 (red). For confirmation, a portion of the product was converted into the acetate, which crystallized from ethanol as yellow needles, m.p. $196\text{--}197^\circ$, not depressed on admixture with authentic triacetylemodin, m.p. $197\text{--}198^\circ$.

Tests for fluorescence in asperthecin solutions

Raistrick *et al.* (1934, p. 567) showed that acetic acid solutions of polyhydroxyanthraquinones which are derivatives of quinizarin (1:4-dihydroxyanthraquinone) are strongly fluorescent in daylight, whereas those which do not contain the 1:4-dihydroxy grouping are not fluorescent. Hence, fluorescence was regarded as being characteristic of quinizarin derivatives. Because of the importance of this test in deducing the structure of asperthecin, solutions of asperthecin in glacial acetic acid and in cold conc. H_2SO_4 were examined for fluorescence on a number of occasions. No fluorescence in daylight was ever observed, although a number of other mould colouring matters, known to be derivatives of quinizarin, all showed strong fluorescence when compared side by side with asperthecin. Moreover, when examined in ultraviolet light, these solutions fluoresced brilliantly, whereas asperthecin showed only a pale dull red fluorescence in H_2SO_4 and a pale cream fluorescence in glacial acetic acid. Both these solutions were similar in colour to, but slightly less intense than, those shown by emodin (4:5:7-trihydroxy-2-methylanthraquinone, II) when examined under the same conditions. Further, when a little solid magnesium acetate was added to an ethanolic solution of asperthecin a strong purple colour was formed but without fluorescence (Shibata, 1941, p. 105). In the same test a number of quinizarin derivatives gave magenta coloured solutions which had a red fluorescence.

Dyeing tests with asperthecin and dimethylasperthecin

White wool was mordanted with chromium, tin or aluminium, using respectively $\text{K}_2\text{Cr}_2\text{O}_7$, SnCl_4 or aluminium acetate, as described by Cain & Thorpe (1913). A solution of the substance under test was made in dioxan (10 mg./ml.). This solution (0.3 ml.) was added to water (5 ml.) with shaking, and the mordanted wool (0.1 g. approx.) was immersed in it immediately. The tube was placed in a bath of cold water which was gradually heated to boiling and kept there for 1 hr. The dyed wool was then washed successively in running water, hot soap solution and finally in running water. The following results were obtained. Asperthecin, intense coloration of the fibre with all three mordants; Cr, indigo blue; Al, deep violet; Sn, dull red. Dimethylasperthecin, feeble dyeing of the fibre with all three mordants; Cr, pale blue; Al, pale violet; Sn, flesh pink.

When compared with alizarin, asperthecin gave colours on wool of equal intensity and brilliance, but for each mordant the colour was bluer than that of alizarin. On the other hand, the colours given by dimethylasperthecin were equal in intensity only to those given by a hydroxyanthraquinone which is not a mordant dyestuff, e.g. in this case, islandicin.

THE COLOURING MATTER OF

A. QUADRILINEATUS STRAIN S.M. 297

Preliminary experiments, for which we are indebted to Mrs S. Marcus, indicated that freshly harvested mycelia of this strain gave colour reactions, particularly with cold conc. H_2SO_4 , which were significantly different from those given by strain S.M. 200 (*q.v.*). Thus, pressed portions of the mycelium of strain S.M. 297 grown for 21 days on either Czapek-Dox or Raulin-Thom solutions, gave an immediate, intense prussian blue colour when immersed in cold conc. H_2SO_4 (strain S.M. 200 gives a deep purple colour) and intense purple-violet colours in $\text{N-Na}_2\text{CO}_3$ and aqueous N-NaOH which, particularly with the latter reagent, faded quickly. Extraction of the mycelium with cold organic solvents also gave solutions which were much redder and less orange than corresponding extracts of strain S.M. 200. Finally, the culture filtrate of strain S.M. 297, grown for 33 days on Raulin-Thom solution, quickly gave a heavy dark orange precipitate (0.35 g./flask) with Brady's reagent (0.32% 2:4-dinitrophenylhydrazine in aqueous 2N-HCl). The precipitate, which is being investigated in this laboratory, is insoluble in NaHCO_3 , gives a purple-red Neuberg reaction and crystallizes from acetone as orange-red needles, m.p. 235° (decomp.). Only a small precipitate is slowly given by strain S.M. 200 under comparable conditions.

All attempts to isolate, in a pure crystalline condition, the colouring matter giving the prussian blue H_2SO_4 colour have so far failed, owing, we believe, to the ease with which it is converted into asperthecin. In attempting to isolate and purify it, however, batches of 1 l. conical flasks, each containing 350 ml. of Czapek-Dox solution (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, 1000 ml.), were sterilized, inoculated with *A. quadrilineatus* strain S.M. 297 and incubated at 24° . At the end of the incubation period the mycelium was folded, pale brownish grey on the surface with a dark brownish purple reverse. The pigments present in the harvested mould were extracted with ethanol from the acidified and disintegrated mycelium exactly as is described on p. 481 for the isolation of asperthecin. The results obtained in two typical batches are summarized in the lower part of Table 1. Since the average weight of dry mycelium was approximately 6.5 g./flask, the yields of colouring matter obtained correspond to 1.0% of the dry weight of the mycelium for batch 1 (22 flasks) and 1.4% for batch 2 (21 flasks). The crude colouring matter obtained was a light brown, mainly crystalline solid and all batches of it showed the intense blue colour with cold conc. H_2SO_4 which is characteristic of the colour given by the whole mycelium. In other respects, however, the fractions behaved in the same way as similar fractions isolated from *A. quadrilineatus* strain S.M. 200.

The following attempts have been made to purify the crude crystalline pigment (Table 1, column 5) and to isolate from it the colouring matter giving the prussian blue H_2SO_4 colour.

By direct crystallization. A portion was crystallized from dioxan. As purification proceeded, the H_2SO_4 reaction of the crystals obtained became more and more purple. No material giving a blue H_2SO_4 colour could be obtained from the mother liquors.

By acetylation. A portion (0.30 g.) was acetylated with acetic anhydride and conc. H_2SO_4 as described for the crude pigment from *A. quadrilineatus* strain S.M. 200 (see p. 481). Yellow crystals separated from the reaction mixture and were collected (0.21 g.). A further quantity of amorphous yellow acetate (0.23 g.) was obtained by shaking the filtrate with water. Neither fraction gave the pure blue H_2SO_4 colour given by the starting material; the crystalline fraction gave a blue-purple and the amorphous fraction a purple-blue.

Purification of the crystalline fraction by crystallization first from glacial acetic acid and then from ethanol yielded pale yellow needles, m.p. 228° , alone or mixed with authentic hexaacetylasperthecin, m.p. 228° , prepared from *A. quadrilineatus* strain S.M. 200. (Found, on material dried in high vacuum at 90° : C, 56.6; H, 4.0. Calc. for $\text{C}_{27}\text{H}_{22}\text{O}_{14}$: C, 56.8; H, 3.9%.) The product gave a purple colour in cold conc. H_2SO_4 which was indistinguishable from that given by hexaacetylasperthecin in the same reagent. The purified acetate, m.p. 228° , was hydrolysed by boiling in methanol- H_2SO_4 (see p. 482), and the resulting crystalline product was recrystallized from dioxan to yield asperthecin as clusters of bright red needles. (Found, on material dried to constant weight at 130° : C, 56.5; H, 3.4. Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_8$: C, 56.6; H, 3.2%.) The product and authentic asperthecin from *A. quadrilineatus* strain S.M. 200 were indistinguishable in their reactions towards the following reagents: cold conc. H_2SO_4 , purple; cold conc. HNO_3 , blue; 0.5N- NaHCO_3 , stable, purple-red coloured solution; 0.5N- Na_2CO_3 , purple-red solution, fading overnight; 0.5N- NaOH , purple solution, fading in a few seconds; ethanolic FeCl_3 , purple.

Crystallization of the amorphous acetate failed to yield any product giving a blue H_2SO_4 colour, but yielded only impure specimens of hexaacetylasperthecin.

By chromatography. A solution of the crude pigment in acetone was passed through a column of CaCO_3 , which was then well washed with the same solvent. Two main bands—an upper and a lower—were formed and were separated mechanically, dissolved in dilute HCl and ice, and extracted with ether. The upper band, violet in colour, gave a reddish orange ether extract, which on evaporation gave an amorphous dirty brown solid (H_2SO_4 blue) from which, on acetylation, impure hexaacetylasperthecin (H_2SO_4 purple) was obtained. The lower band, violet-red in colour, gave an orange ether extract from which, on concentration, needles of asperthecin (H_2SO_4 purple) separated.

The instability of the pigment giving the blue H_2SO_4 colour was further demonstrated as follows. A little of the ethanolic extract of the mycelium of *A. quadrilineatus* strain S.M. 297 (H_2SO_4 blue) was diluted with ether, and the reddish solution was kept on the bench for 1 week. At the end of this time, the colour of the solution had changed to orange, and a portion of it, when dried and dissolved in cold conc. H_2SO_4 , gave a purple solution. The conclusion therefore seems inevitable that the colouring matter of *A.*

quadrilineatus strain S.M. 297, which gives the prussian blue H_2SO_4 colour, is very unstable and is readily converted, possibly by tautomeric change, into asperthecin.

SUMMARY

1. The purple-red colouring matter of the perithecia and ascospores of two different strains of *Aspergillus quadrilineatus* Thom & Raper has been isolated in pure crystalline form and has been named asperthecin.

2. Evidence is presented that asperthecin is the colouring matter which is characteristic of the purple-red perithecia and ascospores of a number of other species in the *Aspergillus nidulans* group.

3. Asperthecin, $\text{C}_{15}\text{H}_{10}\text{O}_8$, forms chestnut-brown needles, m.p. above 370° .

4. A number of functional derivatives and degradation products of asperthecin are described.

5. Asperthecin is believed to be either 3:4:5:6:7- or 1:4:5:6:7-pentahydroxy-2-hydroxymethylanthraquinone.

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