hydroxide and the oxygen taken up is replaced by electrolytically generated oxygen, which is added in small equal-sized volumes. The frequency of their addition is a measure of the respiration rate.

2. The oxygen is produced by electrolysis of a solution of sodium hydroxide by using a large palladium cathode to absorb the hydrogen.

REFERENCES

- Bennet-Clark, T. A. (1932). *Sci. Proc. R. Dublin Soc.* **20**, 281.
 Fernandes, D. S. (1923). *Rec. Trav. bot. neerl.* **20**, 107.
 Mapson, L. W. & Burton, W. G. (1962). *Biochem. J.* **82**, 19.
 Ulrich, A. (1940). *Plant Physiol.* **15**, 527.
 Wager, H. G. (1957). *New Phytol.* **56**, 230.
 Wetzell, K. (1927). *Planta*, **4**, 476.

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Studies in the Biochemistry of Micro-organisms

109. THE STRUCTURE OF ASPERTHECIN*

BY J. H. BIRKINSHAW AND RACHEL GOURLAY

Department of Biochemistry, London School of Hygiene and Tropical Medicine, University of London

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Asperthecin, $C_{15}H_{10}O_8$, the polyhydroxyanthraquinone derived from *Aspergillus quadrilineatus* Thom & Raper, was first isolated by Howard & Raistrick (1955). They prepared various derivatives and advanced a partial structure based on emodin. As a result of further degradative work by Neelakantan, Pocker & Raistrick (1957), the structure of asperthecin was reduced to two possibilities, (I) and (II), but the evidence was insufficient to determine which was correct.

Further work on asperthecin, here presented, has afforded proof that the structure of asperthecin is represented by (II).

The evidence previously available, supporting structure (II), was mainly based on the visible absorption spectrum. The location of the main absorption band in this region at $\lambda_{484m\mu}$ (Birkinshaw, 1955) is strong evidence for the presence of three α -hydroxyl groups (cf. Birkinshaw & Gourlay, 1961). A further indication in favour of structure (II) was the fact that methylation of asperthecin with diazomethane in ether for a limited period afforded a di-*O*-methylasperthecin. Prolonged methylation in presence of methanol pro-

duced tetra- and penta-methylasperthecin, but a trimethylasperthecin was not isolated (Neelakantan *et al.* 1957). This suggests that asperthecin has only two β -hydroxyl groups.

The main evidence against structure (II) and favouring structure (I) was the fact that solutions of asperthecin showed no fluorescence in daylight. Since fluorescence was considered to be diagnostic for quinizarin derivatives the presence of a 1,4 arrangement of hydroxyl groups as in structure (II) was unlikely (Howard & Raistrick, 1955).

Further evidence favouring structure (II) has now been obtained by partial (short-time) acetylation with acetic anhydride, by the method used in investigating dermocybin (Birkinshaw & Gourlay, 1961). After 12 min. in boiling acetic anhydride asperthecin afforded mainly a red triacetate with some orange tetra-acetate. The first stage of acetylation should be confined to the nuclear β -hydroxyl groups and the ω -hydroxyl group. Since a triacetate is first formed, the inference is that asperthecin has only two β -hydroxyl groups and hence three α -hydroxyl groups. In accordance with this, triacetylation of asperthecin produces only a small shift in the main visible absorption maximum from 484 to 478 $m\mu$, the latter value being still

* Part 108: Stickings & Townsend (1961).

(I)

(II)

within the range expected for three free α -hydroxyl groups. The absorption peaks in both the visible and ultraviolet spectra of triacetylasperthecin, which, on the basis of structure (II), should have free hydroxyl groups in the 4,5,8 positions, are very close to those obtained for helminthosporin (4,5,8-trihydroxy-2-methylanthraquinone) and islandicin (1,4,5-trihydroxy-2-methylanthraquinone) (Birkinshaw, 1955).

The production of a tetra-acetate as the next stage in acetylation would then be due to acetylation of an α -hydroxyl group. Since, when three α -hydroxyl groups are present, two must occupy positions *peri* to one and the same carbonyl group, the hydrogen-bonding of each member of this pair is correspondingly weakened, rendering one of these two hydroxyl groups more susceptible to acetylation. In accordance with this the tetra-acetate shows visible absorption at $\lambda_{\max}$ 432 $m\mu$, indicative of two free α -hydroxyl groups [cf. anthrarufin (1,5-dihydroxyanthraquinone), $\lambda_{\max}$ 430 $m\mu$; emodin, $\lambda_{\max}$ 437 $m\mu$]. This behaviour is to be expected from structure (II), whereas structure (I), with three β -hydroxyl groups and one ω -hydroxyl group, should most readily afford a tetra- and then a penta-acetate.

It is not possible to say which of the hydroxyl groups 4 and 5 is acetylated in the tetra-acetate. The fact that this acetate in acetic acid shows daylight fluorescence would suggest that it has free hydroxyl groups in the 5,8 (quinizarin) positions, which would indicate acetylation of the 4-hydroxyl group. A comparison of the visible and ultraviolet-absorption peaks, however, indicates that the tetra-acetate is more closely related to anthrarufin (1,5-dihydroxyanthraquinone) than to quinizarin (see Table 1). This evidence favours

acetylation of the 5-hydroxyl group leaving free the 4- and 8-hydroxyl groups.

The weight of evidence in favour of structure (II) led us to attempt to confirm this structure by synthesis. In order to simplify this approach the hydroxymethyl group was eliminated from asperthecin by oxidation of the group to a carboxyl group with nitrous acid in sulphuric acid by the method of Hill & Richter (1936), to yield asperthecic acid, $C_{15}H_8O_9$, followed by decarboxylation of the asperthecic acid to the pentahydroxyanthraquinone. Synthesis of the latter substance was achieved as follows.

The first stage was the condensation of hemipinic anhydride with *m*-methoxyphenol (present in large excess to act as solvent) in the presence of aluminium chloride to form 2-(2'-hydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid (III). The method of linkage of the two moieties was settled by decarboxylation in quinoline with a copper chromite catalyst, which also caused monodemethylation and ring-closure with the production of a dimethoxyxanthone that must have structure (IV). 3,5-Dimethoxyxanthone (IV) has been synthesized by Noyce & Weldon (1952). The production of this xanthone decides the manner of linkage of the two benzene nuclei in the benzoylbenzoic acid (III), since the new ether linkage must have occurred (with elimination of methanol) between a hydroxyl and a methoxyl group, both *ortho* to the common carbonyl link.

Oxidation with alkaline persulphate (Elbs method) converted (III) into the 2-(2',5'-dihydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid (V), affording the pentamethoxy derivative (VI) on methylation. It was then found that a more direct approach was possible, since by allowing hemipinic

Table 1. *Light-absorption spectra of polyhydroxyanthraquinones in ethanol*

Substituted anthraquinones	$\lambda_{\max}$ ($m\mu$) with log ϵ (in parentheses)						
	233 (4.37)	256 (4.28)	290* (4.00)	—	—	478 (3.93)	545-555* (3.24)
1 Triacetylasperthecin	229 (4.61)	255 (4.38)	280-290* (4.20)	—	432 (4.06)	—	—
2 Tetra-acetylasperthecin	225 (4.64)	253 (4.31)	286 (4.02)	—	430 (4.04)	—	—
3 Anthrarufin (1,5-dihydroxyanthraquinone)	225 (4.34)	249 (4.49)	279 (4.01)	325 (3.39)	450 (3.93)	—	—
4 Quinizarin (1,4-dihydroxyanthraquinone)	221 (4.20)	245 (4.33)	298 (4.09)	388 (3.49)	—	491 (4.18)	515 (4.11)
5 Asperthecic acid	238 (4.44)	259 (4.46)	286.5 (4.14)	311 (4.03)	—	485 (4.25)	512 (4.22)
6 Decarboxyasperthecic acid	238 (4.44)	259 (4.45)	286.5 (4.15)	311 (4.02)	—	485 (4.25)	512 (4.21)
7 3,4,5,7,8-Pentahydroxyanthraquinone							

* Inflection.

anhydride to react with 1,2,4-trimethoxybenzene monodemethylation occurred and a monohydroxy-tetramethoxybenzoylbenzoic acid (VIII) was obtained. A by-product of this reaction, formed in considerable yield, appeared from its analysis to be 3,4-dimethoxy-1,2-di-(2',4',5'-trimethoxybenzoyl)-benzene. The hydroxytetramethoxybenzoylbenzoic acid (VIII) gave on methylation the same pentamethoxybenzoylbenzoic acid (VI) as had been obtained by the method involving the Elbs oxidation. The conversion of the acid (VIII) into a trimethoxyanthrone (VII) by decarboxylation (with elimination of methanol) indicated that demethylation involved in the synthesis of the benzoylbenzoic acid had occurred at one of the groups *ortho* to the carbonyl link. Since the hemipinic anhydride moiety had not been demethylated when condensed with *m*-methoxyphenol, it appears to be stable to the reaction conditions and the demethylation must have occurred on the trimethoxybenzene moiety. Hence the hydroxytetramethoxybenzoylbenzoic acid is formulated as (VIII).

Treatment of (VIII) with a fused mixture of aluminium chloride and sodium chloride afforded the 3,4,5,7,8-pentahydroxyanthraquinone (IX), which was purified through the penta-acetate. On comparison of properties and absorption spectrum of this synthetic product with those of decarboxy-asperthecic acid, the two were found to be identical. The melting points of their penta-acetates agreed and were not depressed on mixing. Asperthecin,

therefore, has the structure 3,4,5,7,8-pentahydroxy-2-hydroxymethylantraquinone (II).

The synthetic 3,4,5,7,8-pentahydroxyanthraquinone and other pentahydroxyanthraquinones synthesized (Birkinshaw & Gourlay, 1961), namely 1,4,5,6,7-pentahydroxy-2-methyl-, 1,4,6,7,8-pentahydroxy-2-methyl-, 4,5,6,7,8-pentahydroxy- and 4,5,6,7,8-pentahydroxy-2-methyl-anthraquinone, all have the quinizarin grouping, yet none of them showed any daylight fluorescence when dissolved in acetic acid, and in this respect behaved like asperthecin and asperthecic acid. The absence of fluorescence, therefore, can no longer be regarded as a reliable indication of the absence of a quinizarin grouping in the polyhydroxyanthraquinone molecule.

EXPERIMENTAL

The C and H determinations were made by Dr A. Schoeller; OMe and acetyl by one of us (R.G.). All melting points are uncorrected. The light-absorption values of the various derivatives and synthetic products, where not given below, are recorded in Table 1.

Derivatives of asperthecin

Triacetylasperthecin. Asperthecin (0.1 g.) was refluxed with acetic anhydride (5 ml.) for 8 min., when solution was effected. After refluxing for a further 4 min. the solution was cooled and a mixture of red and orange crystals (0.11 g.) separated. Recrystallization from acetic anhydride, avoiding excessive heating, afforded *triacetylasperthecin* as bright-red needles (0.08 g.), m.p. 248–250°

(decomp.) (Found: C, 55.3; H, 3.7; acetyl, 29.0. $C_{21}H_{16}O_{11}$, $\frac{1}{2}H_2O$ requires C, 55.6; H, 3.8; 3 acetyl, 28.5%).

Tetra-acetylasperthecin. The mother liquors from the preceding reaction, when poured into water, gave an orange-red precipitate, which was repeatedly crystallized from acetic acid, giving *tetra-acetylasperthecin* as bright-orange needles (0.02 g.), m.p. 251–253° (decomp.). The tetra-acetate shows slight but distinct fluorescence when dissolved in acetic acid. An improved yield (0.07 g.) of the tetra-acetate was obtained by refluxing asperthecin (0.1 g.) with acetic anhydride (5 ml.) for 20 min. (Found: C, 56.5; H, 3.7; acetyl, 35.2. $C_{23}H_{18}O_{12}$ requires C, 56.8; H, 3.7; acetyl, 35.4%).

Oxidation of asperthecin; asperthecin acid penta-acetate. Asperthecin (0.2 g.), H_3BO_3 (1 g.), $NaNO_2$ (0.5 g.) and conc. H_2SO_4 (25 ml.) were heated rapidly to 180° (oil-bath temp.), cooled to 145° and maintained at 145° for 80 min. The cooled solution was poured into water (250 ml.) and heated to boiling to coagulate the product, and the ppt. (0.17 g.) filtered off and dried. The crude product was acetylated by heating with acetic anhydride (5 ml.) and conc. H_2SO_4 (0.1 ml.) at 90° for 5 min. The solution was cooled and poured into water and the brown ppt. (0.19 g.), m.p. 190–193°, was filtered off, dried and recrystallized three times from methanol, giving *asperthecin acid penta-acetate* (0.05 g.), m.p. 218–220° (decomp.) (Found, on sample dried at 100° *in vacuo*: C, 55.0; H, 3.6; acetyl, 40.4. $C_{25}H_{18}O_{14}$ requires C, 55.3; H, 3.3; 5 acetyl, 39.7%).

Asperthecin acid. The penta-acetate (0.06 g.) was hydrolysed by refluxing with methanol (5 ml.) containing conc. H_2SO_4 (0.15 ml.). The acetate dissolved and after heating for 1 hr. red crystals separated. The product was filtered off and washed with methanol giving *asperthecin acid* (30 mg.), m.p. (decomp.) above 350° (Found, on material dried at 100° *in vacuo*: C, 54.5; H, 2.7. $C_{14}H_8O_6$ requires C, 54.2; H, 2.4%).

Decarboxylation of asperthecin acid. Asperthecin acid (0.35 g.), quinoline (15 ml.) and copper chromite (0.2 g.) were heated to 200° in a current of N_2 . The effluent gases were passed through standard aq. $Ba(OH)_2$ solution. The reaction as measured by CO_2 evolved was 65% complete in $7\frac{1}{2}$ hr. and was stopped at this stage. The cooled solution was added to 2N-HCl (250 ml.), and the ppt. filtered off and dried. The product was extracted with methanol (3×150 ml.). The orange methanolic solution was evaporated to 200 ml. and chromatographed on a column of $CaCO_3$. Ethanol was used as the eluent. Two distinct coloured bands were obtained, an orange and a deep pink, shading to violet towards the base. The column was removed and cut into three portions, orange, pink and violet, and each portion dissolved in 5N-HCl. The solutions were heated to boiling and cooled, and the anthraquinone fractions filtered off and dried giving fraction 1, 0.07 g., fraction 2, 0.10 g. and fraction 3, 0.04 g. Purification of fractions 2 and 3 by acetylation gave the acetyl derivatives, m.p. 242–243°, undepressed when mixed with each other or with an authentic sample of 3,4,5,7,8-penta-acetoxyanthraquinone. The acetate (60 mg.) was hydrolysed by refluxing for $1\frac{1}{2}$ hr. with methanol (5 ml.) and conc. H_2SO_4 (0.1 ml.). *Decarboxyasperthecin acid* was precipitated as red needles, m.p. 353–354°. No depression in melting point occurred when this material was mixed with synthetic 3,4,5,7,8-pentahydroxyanthraquinone (Found, on sample dried *in vacuo* at 100°: C, 58.35; H, 2.8. $C_{14}H_8O_6$ requires C, 58.3; H, 2.8%). Compare u.v. spectra in Table 1.

Syntheses

2-(2'-Hydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid (III). Hemipinic anhydride (from hemipinic acid, 5 g.) was dissolved in *m*-methoxyphenol (23 g.). Powdered anhydrous $AlCl_3$ (8 g.) was added in portions and after the initial reaction had subsided the mixture was heated at 70–75° (oil-bath temp.) for 6 hr. After standing overnight, the viscous red mass was treated with ice and 2N-HCl (50 ml.) and the mixture extracted with ether (3×150 ml.). The ether was washed twice with $NaHCO_3$ solution (100 ml., 50 ml. 10%). Acidification of the bicarbonate extract precipitated a resinous material. This material when dissolved in ether (200 ml.) and the volume reduced to 50 ml. gave a pale-yellow crystalline compound (2.2 g.; m.p. 204–207°). One recrystallization from methanol gave needles of 2-(2'-hydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid, m.p. 211–212°, raised by sublimation to 215° (Found, on sublimed sample: C, 61.0; H, 4.8; OMe, 27.8. $C_{17}H_{16}O_7$ requires C, 61.4; H, 4.8; OMe, 28.0%).

2-(2',5'-Dihydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid (V). 2-(2'-Hydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid (III) (2.20 g.) was dissolved in water (20 ml.) containing NaOH (1.33 g.). $K_2S_2O_8$ (1.80 g.) was added in portions to the solution at 35–40° during 2.5 hr. After 4 days at room temp. the deep-red solution was made faintly acid to Congo red with 5N- H_2SO_4 . The unchanged acid (1.35 g.), m.p. 203–206°, was collected and the filtrate was made strongly acid with conc. HCl (10.0 ml.). The crystalline product (0.66 g.), m.p. 225–231°, was collected after 3 days. Recrystallization from methanol-water afforded 2-(2',5'-dihydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid (V) as prisms, m.p. 257–258° (decomp.), raised by sublimation to 261–262° (decomp.) (Found: C, 58.5; H, 4.7; OMe, 26.7. $C_{17}H_{16}O_8$ requires C, 58.6; H, 4.6; OMe, 26.7%).

3,4-Dimethoxy-2-(2',4',5'-trimethoxybenzoyl)benzoic acid (VI). 2-(2',5'-dihydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid (0.15 g.) was dissolved in acetone (20 ml.) and K_2CO_3 (1 g.) and dimethyl sulphate (1.0 ml.) were added. The solution was refluxed for 7 hr., kept overnight and then refluxed for a further 7 hr. after the addition of dimethyl sulphate (1.0 ml.). The cooled solution was filtered and the acetone evaporated to give a pale-yellow oil. The oil was suspended in 2N-NaOH (25 ml.) and aq. 2N- NH_3 (5 ml.) was added. The solution was refluxed for 2 hr. to hydrolyse any ester, cooled, acidified and extracted with ether. A colourless solid (0.074 g.), m.p. 227–229°, was isolated from the ether extract. Two recrystallizations from methanol gave colourless needles (0.032 g.), m.p. 230–231°, of 3,4-dimethoxy-2-(2',4',5'-trimethoxybenzoyl)benzoic acid (Found, on sample dried at 100° *in vacuo*: OMe, 41.6. $C_{19}H_{20}O_8$ requires OMe, 41.2%).

Decarboxylation of 2-(2'-hydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid; 3,5-dimethoxyxanthone (IV). The benzoylbenzoic acid (1.1 g.), quinoline (12 ml.) and copper chromite (0.4 g.) were heated at 190–200° under the same conditions as above. The reaction was 80% complete in 4 hr., when it was stopped. The cooled solution was poured into 2N-HCl (100 ml.) and the mixture extracted with ether (3×100 ml.). The ether solution was reduced in volume and the pale-yellow solid which separated crystallized from ethanol in long colourless needles (0.15 g.), m.p. 170–171°, of 3,5-dimethoxyxanthone. Noyce & Weldon (1952)

report the m.p. of synthetic 3,5-dimethoxyanthrone as 173.5–174.5° (corr.). (Found: C, 70.4; H, 4.7; OMe, 24.4. $C_{15}H_{12}O_4$ requires C, 70.3; H, 4.7; OMe, 24.2%). Light-absorption values in ethanol: $\lambda_{\max}$ 236, 246, 267, 304 and 330 m μ ; $\log \epsilon$ 4.51, 4.52, 4.24, 4.10 and 3.85 respectively.

2-(2'-Hydroxy-4',5'-dimethoxybenzoyl)-3,4-dimethoxybenzoic acid (VIII). Hemipinic anhydride (from hemipinic acid, 2 g.) was dissolved in 1,2,4-trimethoxybenzene (10 ml.) by warming. The solution was cooled rapidly to room temp., powdered anhydrous $AlCl_3$ (4.0 g.) was added and the mixture warmed at 75–80° (oil-bath temp.) for 5½ hr. After 18 hr. at room temp. the viscous green product was warmed with 2N-HCl (50 ml.), cooled and extracted with ether. The ether solution was shaken with $NaHCO_3$ solution (2 x 100 ml., 10%). A crystalline solid separated from the ether during the extraction. This product crystallized from ethanol in colourless needles, m.p. 176° (Found, on sample dried *in vacuo* at 60°: C, 63.8; H, 5.6; OMe, 46.9; mol.wt. 540. $C_{22}H_{20}O_8$ requires C, 63.8; H, 5.7; 8 OMe, 47.1%; mol.wt. 526). This by-product appears to be 3,4-dimethoxy-1,2-di-(2',4',5'-trimethoxybenzoyl)benzene. The resinous product obtained on acidification of the $NaHCO_3$ solution was dissolved in ether. The ether was evaporated to small volume, when a pale-yellow material separated. Crystallization from methanol–water afforded a cream-coloured product (0.75 g.), m.p. 225°, consisting of 2-(2'-hydroxy-4',5'-dimethoxybenzoyl)-3,4-dimethoxybenzoic acid (Found, on sample dried at 100° *in vacuo*: C, 59.6; H, 4.9; OMe, 34.3. $C_{18}H_{16}O_8$ requires C, 59.7; H, 5.0; OMe, 34.3%).

Methylation of the hydroxytetramethoxybenzoylbenzoic acid. The pentamethyl ether (VI) of the hydroxytetramethoxybenzoylbenzoic acid was prepared by the method used for the methylation of 2-(2',5'-dihydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid. The product was isolated and crystallized from methanol, giving colourless needles, m.p. 230–231°, undepressed when mixed with the methylation product of 2-(2',5'-dihydroxy-4'-methoxybenzoyl)-3,4-dimethoxybenzoic acid (Found, on sample dried at 100° *in vacuo*: C, 60.5; H, 5.4; OMe, 41.4. $C_{19}H_{20}O_8$ requires C, 60.6; H, 5.4; OMe, 41.2%).

Decarboxylation of 2-(2'-hydroxy-4',5'-dimethoxybenzoyl)-3,4-dimethoxybenzoic acid; 2,3,5-trimethoxyanthrone (VII). The benzoylbenzoic acid (1.25 g.), quinoline (12.5 ml.) and copper chromite (0.4 g.) were heated at 200° in an atmosphere of N_2 for 4½ hr. The effluent gases were passed through standard aq. $Ba(OH)_2$ solution. The CO_2 produced in this time was 72% of the theoretical amount. The cooled solution was poured into 2N-HCl (250 ml.) and the mixture was filtered. The dried residue was extracted with ether, and the ether extract washed with bicarbonate and evaporated to small volume, when a cream-coloured solid separated. Recrystallization from methanol gave 2,3,5-trimethoxyanthrone as cream-coloured needles, m.p. 232–235°. Sublimation raised the m.p. to 238° (Found, on sublimed sample dried at 100° immediately before analysis: C, 66.6; H, 4.8; OMe, 32.1. $C_{16}H_{14}O_6$ requires C, 67.1; H, 4.9; OMe, 32.5%). Light-absorption values in ethanol: $\lambda_{\max}$ 219, 249, 314 and 330 m μ ; $\log \epsilon$ 4.39, 4.68, 4.00 and 4.05 respectively.

3,4,5,7,8-Penta-acetoxyanthraquinone. $AlCl_3$ (5 g.) and NaCl (1 g.) were fused at 180°, cooled to 120° and 2-(2'-hydroxy-4',5'-dimethoxybenzoyl)-3,4-dimethoxybenzoic acid (0.56 g.) was added with stirring. The flask was transferred to an oil bath at 190° and heating continued for

5 min. The cooled mixture was treated with ice and conc. HCl (20 ml.) and diluted with water (400 ml.). The ppt. coagulated on boiling and was filtered off, washed and dried. The brown product (0.38 g.) was heated for 10 min. at 70–80° with acetic anhydride (5 ml.) containing conc. H_2SO_4 (0.1 ml.). On cooling, pale-yellow needles separated. The mixture was filtered and the product (0.39 g.) recrystallized from methanol, affording lemon-yellow needles (0.32 g.) of 3,4,5,7,8-penta-acetoxyanthraquinone, m.p. 241–242°. A further recrystallization raised the m.p. to 243° (Found, on sample dried to constant wt. *in vacuo* at 100°: C, 57.9; H, 3.9; acetyl, 42.1. $C_{24}H_{18}O_{12}$ requires C, 57.8; H, 3.6; 5 acetyl, 43.3%).

3,4,5,7,8-Pentahydroxyanthraquinone. The penta-acetoxyanthraquinone (0.27 g.) was refluxed for 2 hr. with methanol (25 ml.) containing conc. H_2SO_4 (0.75 ml.). The acetate dissolved and after 30 min. red needles separated. The ppt. was filtered off and washed with methanol, giving 3,4,5,7,8-pentahydroxyanthraquinone as light-red-brown needles (0.15 g.), m.p. 353–355° (Found, on sample dried at 100° *in vacuo*: C, 58.2; H, 2.9. $C_{14}H_8O_7$ requires C, 58.3; H, 2.8%).

SUMMARY

1. Several new derivatives of asperthecin have been prepared. Acetylation of asperthecin with acetic anhydride has given the triacetate, m.p. 248–250° (decomp.), and the tetra-acetate, m.p. 251–253° (decomp.). Asperthecin acid has been obtained by the oxidation (nitrous acid in concentrated sulphuric acid) of the hydroxymethyl group of asperthecin to a carboxyl group. Asperthecin acid has been decarboxylated to a pentahydroxyanthraquinone.

2. The condensation of hemipinic anhydride with 1,2,4-trimethoxybenzene has given 2-(2'-hydroxy-4',5'-dimethoxybenzoyl)-3,4-dimethoxybenzoic acid. Subsequent ring closure and demethylation of the benzoylbenzoic acid has afforded 3,4,5,7,8-pentahydroxyanthraquinone, identical with decarboxyasperthecin acid.

3. Asperthecin is, therefore, represented by the structure 3,4,5,7,8-pentahydroxy-2-hydroxymethylanthraquinone.

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REFERENCES

- Birkinshaw, J. H. (1955). *Biochem. J.* **59**, 485.
- Birkinshaw, J. H. & Gourlay, R. (1961). *Biochem. J.* **80**, 387.
- Hill, R. & Richter, D. (1936). *J. chem. Soc.* p. 1714.
- Howard, B. H. & Raistrick, H. (1955). *Biochem. J.* **59**, 475.
- Neelakantan, S., Pocker, A. & Raistrick, H. (1957). *Biochem. J.* **66**, 234.
- Noyce, D. S. & Weldon, J. W. (1952). *J. Amer. chem. Soc.* **74**, 5144.
- Stickings, C. E. & Townsend, R. J. (1961). *Biochem. J.* **78**, 412.