19. *The Chemistry of* Aspergillus *Colouring Matters. Part I.*

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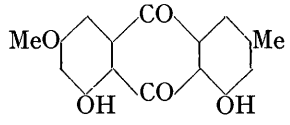
Gould and Raistrick (*Biochem. J.*, 1934, **28**, 1640) have described yellow, orange and red crystalline pigments occurring in the mycelia of various species of *Aspergillus* of the *A. glaucus* series when grown on a modified Czapek–Dox medium with glucose as the source of carbon.

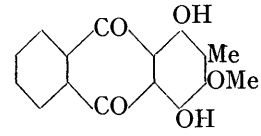
The yellow and orange colouring matters are related, and have been termed flavoglaucin ($C_{19}H_{28}O_3$) and auroglaucin ($C_{19}H_{22}O_3$) respectively; on the other hand rubroglaucin,

$C_{16}H_{12}O_5$, has the character of a polyhydroxyanthraquinone and does not in the least resemble the other pigments.

In working up crude flavoglaucin from *A. glaucus* Link, we have been able to isolate a small quantity of a fourth colouring matter, which proved to be identical with the emodin monomethyl ether isolated from *Ventilago madraspatana* Gärtn. by A. G. Perkin and Hummel (J., 1894, **65**, 943) and later found widely distributed in nature. This substance, often called physcion, was obtained by Jowett and Potter (J., 1903, **83**, 1330) by the methylation of emodin with methyl iodide and methyl-alcoholic sodium methoxide. According to Eder and Hauser (*Helv. Chim. Acta*, 1925, **8**, 140) physcion is 4 : 5-dihydroxy-7-methoxy-2-methylanthraquinone (I) and this suggestion is very probably correct in

(I.)





(II.)

view of the synthesis of *Frangula* emodin itself (Eder and Widmer, *Helv. Chim. Acta*, 1923, **6**, 966; Adams and Jacobson, *J. Amer. Chem. Soc.*, 1924, **46**, 1316) and the methylation mentioned above.

A specimen obtained by Perkin and Hummel had been preserved in the collection of the Department of Colour Chemistry and Dyeing at Leeds University, and we are grateful to Professors A. G. Perkin and F. M. Rowe for the opportunity to make a direct comparison with our material.

Physcion has been known for many years as one of the so-called lichen acids occurring particularly in different species of *Xanthoria* and of *Placodium*. The isolation of physcion from species of *Aspergillus* is the first recorded instance of any mould metabolic product which is identical with one of the lichen acids, although several very closely related substances have been isolated. This observation is of importance, since it indicates that it is the fungal and not the algal half of the lichen symbiont which synthesises the lichen acids.

Rubroglaucin.—The melting point of rubroglaucin is now found to be 180—181° (Gould and Raistrick, *loc. cit.*, gave m. p. 172—173°), but the identity of the substance is not in doubt because most of the material examined was derived from *A. ruber* following the earlier procedure. It has also been isolated in small relative quantity from *A. albidus* and *A. glaucus mut. alba*. This substance, $C_{16}H_{12}O_5$, contains a methoxyl group and has the composition and properties of a dihydroxymethoxymethylanthraquinone. Actually it affords a diacetyl derivative and gives 2-methylanthracene on distillation with zinc dust.

Some further light on its constitution is thrown by the absence of adjective dyeing properties (no vicinal hydroxyls) and by its insolubility in aqueous sodium carbonate (no β-hydroxyl groups). On demethylation, however, a mordant dye is obtained and this is soluble in alkali carbonates. Furthermore, the solutions of rubroglaucin in acetic acid and some other solvents are yellow with a green fluorescence; this indicates the quinizarin group (compare the present authors, *Biochem. J.*, 1934, **28**, 567). It would appear that rubroglaucin should be a methoxy-1 : 4-dihydroxy-β-methylanthraquinone and we thought (II) the most likely of the various isomerides because the solution of rubroglaucin in sulphuric acid is bluer in tone than that of purpurin in this solvent. The inclusion of the methyl group in the hydroxylated ring of purpurin seemed to be the only plausible way of providing an explanation of this contrast, always assuming that demethylated rubroglaucin is a homologue of purpurin.

Accordingly, 1 : 3 : 4-*trihydroxy-2-methylanthraquinone* was synthesised and this substance proved to be very similar to purpurin in its properties and quite different from demethylated rubroglaucin.

Hence it is very probable that demethylated rubroglaucin, despite its mordant dyeing properties, is not a methylpurpurin and, as it must be a quinizarin, it cannot be an alizarin derivative. Its solubility in aqueous alkali carbonates shows that it is not a 1 : 4 : 5-trihydroxyanthraquinone and consequently it must be a 1 : 4 : 6-trihydroxy-β-methyl-

anthraquinone. Rubroglaucin must be a 6-methoxy-1 : 4-dihydroxy-β-methylanthraquinone and the methyl group may be in one of the positions 2, 3, and 7. A decision will be sought by synthesis. Demethylated rubroglaucin could not be freed from a molecule of water, even by sublimation, and the analytical figures tallied with the formula $C_{15}H_{12}O_6$ ($C_{15}H_{10}O_5,H_2O$). A very small amount was available and the triacetate could not be recrystallised to constant melting point; its analysis, however, was satisfactory, agreeing with the formula $C_{21}H_{16}O_8$ or $C_{15}H_7O_2(OAc)_3$. Our synthetic 2-methylpurpurin was also found to retain $1H_2O$ very tenaciously, and there is no reason to doubt the view that demethylated rubroglaucin is a trihydroxymethylanthraquinone.

Flavoglaucin and Auroglaucin.—The compositions attributed to the yellow and the orange pigment of Gould and Raistrick (*loc. cit.*) have been confirmed by fresh analyses and by the preparation of derivatives. Flavoglaucin is $C_{19}H_{28}O_3$ and auroglaucin is $C_{19}H_{22}O_3$. The difference is accounted for by the fact that auroglaucin contains three double bonds more than flavoglaucin. Partial hydrogenation ($3H_2$ to flavoglaucin and $6H_2$ to auroglaucin) gave one and the same colourless solid, m. p. 111°, which was not quite homogeneous but was composed mainly of an unsaturated alcohol, $C_{19}H_{32}O_2$, yielding a characteristic, homogeneous, *phenylurethane*.

On microhydrogenation in presence of platinum, auroglaucin and flavoglaucin took up volumes of hydrogen corresponding with 10 and 7 molecular proportions respectively. On a larger scale flavoglaucin absorbed rather more than $7H_2$ and afforded a colourless liquid giving analytical figures agreeing with $C_{38}H_{76}O$, and probably consisting of a mixture of $C_{19}H_{38}O$ with $C_{19}H_{38}$. The relevant equation is $C_{19}H_{28}O_3 + 7H_2 = C_{19}H_{38}O + 2H_2O$, and reduction to $C_{19}H_{38}$ would account for the hydrogen absorbed in excess of the theoretical volume. Oxidation of flavoglaucin by means of potassium permanganate in pyridine or acetone solution afforded oxalic acid, *n*-octoic acid, probably acetic acid, and unidentified acids giving high-boiling methyl esters.

Attempts to determine the functions of the oxygen atoms showed that the substances are ketonic (dinitrophenylhydrazones) and the Zerewitinoff determinations indicated the presence of two hydroxyl groups in flavoglaucin and auroglaucin. In addition a crystalline *phenylurethane* was obtained from auroglaucin. The third oxygen may be present as a protected hydroxyl group or as a readily ruptured ether linkage. In the latter case the Zerewitinoff results must be interpreted as due to enolisation of the ketone. Auroglaucin condenses with *o*-phenylenediamine with elimination of only $1H_2O$, but the product cannot be diazotised; hence it would appear that auroglaucin may be an αβ-unsaturated ketone.

These data do not allow us to advance a constitutional formula, but it may be pointed out that full hydrogenation provides some evidence of the existence of one carbocyclic structure and it is not excluded that this may be a benzene ring. On the other hand, no degradation product supporting this hypothesis has been isolated.

The formation of *n*-octoic acid by permanganate oxidation of flavoglaucin shows that the group $CH_3{\cdot}[CH_2]_6{\cdot}C{:}$ is contained in the molecule and the full structure might be that of a benzene derivative containing a long aliphatic chain or chains, two hydroxyl groups, a carbonyl group, and a double bond. One of the hydroxyl groups might be replaced by a readily ruptured cyclic ether linkage. On the benzenoid hypothesis the double bond would then need to be saturated. If the colouring matters do not contain a benzene nucleus, the number of possible isomerides is greatly increased, and it is difficult to devise a plausible formula. The investigation is being continued.

The flavoglaucin used in this research was obtained from *A. amstelodami*, *A. glaucus* Link, *A. novus*, *A. mongolicus*, *A. albidus*, *A. glaucus mut. alba* and *A. oriolus*. The auroglaucin was derived from *A. novus*, *A. Scheelei*, *A. Dierckxii*, *A. fumigatoides*, *A. mollis*, *A. profusus*, *A. argillaceus*, *A. disjunctus*, *A. pseudoglaucus*, and *A. oriolus*.

Experimental.

Flavoglaucin.—The crude pigment from the various species is difficult to purify on account of its ready solubility in many solvents and gummy impurities hinder the crystallisations. The following method was adopted in order to obtain pure flavoglaucin. The crude material

was extracted (Soxhlet) with light petroleum (b. p. 40—60°); flavoglaucin separated from the extract on cooling. This recrystallisation by extraction was repeated, and the product further purified by repeated crystallisation from 75% alcohol. It was obtained as pale yellow platelets, m. p. 100°, sufficiently pure for most purposes. Final purification by crystallisation from hexane gave a product, m. p. 103° [Found: C, 74·9, 75·0; H, 9·0, 8·9; *M* (Rast), 299, 306. Calc. for $C_{19}H_{28}O_3$: C, 75·0; H, 9·3%; *M*, 304].

Flavoglaucin contains no methoxyl groups, and estimation of hydroxyl groups (Zerewitinoff) gave OH, 10·9, 11·3% in pyridine solution (Calc. for 2OH, 11·2%). Estimation of side-chain methyl groups (Kuhn and Roth) indicated the presence of one such group (CrO_3 oxidation gave 0·9, 0·9 mol. of acetic acid).

Flavoglaucin is insoluble in water but readily soluble in organic solvents with the exception of light petroleum, in which it is but sparingly soluble in the cold. Its solutions are intensely yellow. The following reactions were noted: (1) In alcoholic solution it gives a dull green colour with ferric chloride which quickly fades and becomes dirty brown. (2) It is not immediately soluble in aqueous sodium hydroxide but dissolves slowly to a solution which, at first orange, fades to yellowish-brown, becomes turbid and froths on shaking. A similar effect is produced after prolonged standing with sodium carbonate solution. (3) The brownish solution in concentrated sulphuric acid darkens rapidly on heating. (4) A fine ruby-red coloration is produced on the addition of a saturated solution of antimony trichloride in chloroform. (5) A solution in glacial acetic acid is very slowly decolourised by hydrogen peroxide. (6) Potassium permanganate is instantly decolourised in acetone solution, as also is bromine in chloroform solution. (7) In alcoholic solution it is readily reduced by zinc dust and a little concentrated hydrochloric acid with formation of a colourless crystalline substance soluble in alcohol or ether to a colourless solution with a violet fluorescence. This product is gradually oxidised in the air (or by means of ferric chloride) to a brownish substance which is not identical with the original material. (8) Micro-zinc-dust-distillation gave traces of a brown oil with a pungent odour which did not crystallise or form a picrate.

Flavoglaucin and o-*Phenylenediamine.*—A solution of flavoglaucin (37 mg.) and *o*-phenylenediamine (16 mg.) in alcohol (5 c.c.) was refluxed for 45 minutes; on dilution with a little water a yellow crystalline solid separated. Recrystallised from alcohol, it formed pale yellow needles, m. p. 161° (Found in material dried in a vacuum over phosphoric oxide: C, 75·8; H, 8·4; N, 7·3. $C_{25}H_{34}O_2N_2$ requires C, 76·1; H, 8·6; N, 7·1%). The *substance*, although appearing to react with nitrous acid in some way, did not undergo diazotisation. An attempt to obtain a crystalline acetyl derivative from it by acetylation afforded a gummy product.

Flavoglaucin 2 : 4-*Dinitrophenylhydrazone.*—Flavoglaucin (50 mg.), dissolved in a little warm methyl alcohol, was added to a solution of 2 : 4-dinitrophenylhydrazine (70 mg.) in methyl alcohol containing a little hydrogen chloride. The solution immediately became orange-red and after heating on the steam-bath for 15 minutes it was cooled. The crystalline product which separated was recrystallised from chloroform–alcohol. It formed small, deep red needles, m. p. 179—181°, which on drying over phosphoric oxide in a vacuum became opaque, possibly by loss of solvent of crystallisation. This *substance* seems to exist in two modifications, as it crystallised initially from the reaction mixture as orange needles, m. p. 186—187° (Found: C, 61·8; H, 6·1; N, 12·2. $C_{25}H_{32}O_6N_4$ requires C, 62·0; H, 6·1; N, 11·6%).

Other Derivatives of Flavoglaucin.—Acetylation with acetic anhydride gave an oil, as also did treatment with phenyl *iso*cyanate. Flavoglaucin forms a colourless or only very faintly coloured oxime, but it is difficult to crystallise. The phenylhydrazone was obtained as a yellowish crystalline solid which was rather unstable and tended to decompose partly during purification.

Flavoglaucin (40 mg.) was heated on the steam-bath during 30 minutes with a solution of excess of phenylhydrazine in 90% acetic acid. On addition of a little water and cooling, the *product* separated as yellowish crystals. It separated from dilute acetic acid as small yellowish prisms, m. p. 137° (Found: N, 7·4. $C_{25}H_{34}O_2N_2$ requires N, 7·1%).

Auroglaucin.—The crude auroglaucin is readily purified by recrystallisation from alcohol, forming fine orange-red needles, m. p. 153° [Found: C, 76·5, 76·7; H, 7·2, 7·3; *M* (Rast), 297, 298. Calc. for $C_{19}H_{22}O_3$: C, 76·5; H, 7·5%; *M*, 298].

Auroglaucin contains no methoxyl groups and estimation of hydroxyl groups (Zerewitinoff) gave a value between one and two hydroxyls (Found: OH, 8·9, 9·5 at 24°; 9·5, 9·8 at 95° in anisole. $C_{19}H_{22}O_3$ requires 1OH, 5·7; 2OH, 11·4). Estimation of side-methyl groups by chromic acid oxidation (Kuhn–Roth) gave 1·3 mols. of acetic acid, corresponding probably to one side-methyl group and some volatile acid from the end of the chain (see p. 82).

Auroglaucin is insoluble in water and rather sparingly soluble in most organic solvents, but it dissolves readily in chloroform. Its solutions are intensely yellow at moderate concentrations—much more intense than corresponding solutions of flavoglaucin. The following properties were noted: (1) With ferric chloride in alcoholic solution it gives a red colour, fading to brown. (2) It is not immediately soluble in aqueous sodium hydroxide but, like flavoglaucin, dissolves slowly. The solution is at first cherry-red, but becomes dirty brown and turbid. (3) With concentrated sulphuric acid a reddish purple coloration is produced; on warming, charring occurs. (4) Antimony trichloride in chloroform gives a brownish-red colour, fading rapidly to greenish-brown and becoming olive-green on standing. (5) Glacial acetic acid solutions are very slowly decolourised by hydrogen peroxide. (6) Auroglaucin in acetone decolourises potassium permanganate instantaneously; bromine is decolourised by auroglaucin in chloroform solution. (7) The behaviour on reduction with zinc and hydrochloric acid and on zinc-dust distillation was the same as that of flavoglaucin.

Auroglaucin and o-Phenylenediamine.—A solution of auroglaucin (100 mg.) and *o*-phenylenediamine (50 mg.) in alcohol (*ca.* 10 c.c.) was refluxed for 45 minutes. On cooling, small yellow crystals separated, which were collected and recrystallised from alcohol. The *product* formed small yellow rods, m. p. 185° (decomp.); on exposure to the air they gradually become brown (Found: C, 74·0; H, 7·1; N, 7·2; loss at 100° in a high vacuum over phosphoric oxide, 4·2. $C_{25}H_{28}O_2N_2,H_2O$ requires C, 73·9; H, 7·4; N, 6·9; H_2O, 4·4%).

A solution of the substance in acetic acid developed a red colour on the addition of concentrated hydrochloric acid; addition of sodium nitrite to this solution caused some change, a brownish substance being formed, but the product did not couple with β-naphthol in alkaline solution.

Auroglaucin 2 : 4-Dinitrophenylhydrazone.—A solution of auroglaucin (40 mg.) in warm methyl alcohol was added to a similar solution of 2 : 4-dinitrophenylhydrazine (56 mg.) in methyl alcohol containing a little hydrogen chloride. An orange-red precipitate was almost immediately formed. The mixture was heated on the steam-bath for 1 hour to ensure complete reaction and some decomposition occurred, the liquid becoming greenish-brown. On cooling, the *product* separated as small brick-red needles, which were recrystallised from chloroform–alcohol; m. p. 223—224° (Found: C, 62·6; H, 5·4; N, 12·0. $C_{25}H_{26}O_6N_4$ requires C, 62·7; H, 5·4; N, 11·8%).

Auroglaucin Phenylurethane.—A solution of auroglaucin (0·1 g.) and phenyl *iso*cyanate (0·5 g.) in dry benzene (*ca.* 8 c.c.) was heated under reflux for 4 hours. After distillation of half the solvent, the solution was cooled; the *phenylurethane* then separated. Recrystallised from alcohol, it formed pale yellow, slender rods, m. p. 161° (Found: C, 74·7; H, 6·4; N, 3·6. $C_{26}H_{27}O_4N$ requires C, 74·8; H, 6·5; N, 3·4%).

Acetylation of Auroglaucin.—When auroglaucin (0·1 g.) was boiled for 5 minutes with acetic anhydride (6 c.c.) and anhydrous potassium acetate (0·1 g.), a pale yellow solution was formed. The crystalline precipitate obtained on pouring into water was collected, washed with a little water, and recrystallised from alcohol as rapidly as possible. The product formed sheaves of yellowish needles, m. p. 108—109°. On keeping in a corked tube the crystals rapidly became opaque and slowly developed an orange colour. Analysis of the compound gave values quite unlike those for any simple acetate of auroglaucin [Found: C, 60·6, 60·5; H, 5·7, 5·5; CH_3·CO (by CrO_3 oxidation), 32·3, 32·3. Allowing for the 1·3 mols. of acetic acid produced from auroglaucin under the same conditions, we get CH_3·CO, 10·2%].

Catalytic Hydrogenation of Flavoglaucin and Auroglaucin.—In order to obtain good results in the hydrogenation of both pigments, it seems necessary to use considerable quantities of catalyst. Using ordinary platinum-black, it is possible to stop the hydrogenation at an intermediate stage as is described below. For complete hydrogenation a platinum–silica catalyst (Membranfilter A.G. No. 17) or highly active platinum-black should be used.

(A) *Partial hydrogenation.* (1) *Flavoglaucin.* A solution of flavoglaucin (0·257 g.) in glacial acetic acid (*ca.* 30 c.c.) was shaken with hydrogen in presence of platinum-black (0·5 g.). Absorption of hydrogen was at first very rapid, the equivalent of 2 mols. being taken up in 10 minutes, the third mol. requiring almost ½ hour more. The hydrogenation was stopped at this stage, the solution being only very faintly coloured (observed absorption, 63 c.c. Calc. for $3H_2$, 60 c.c.). The filtered solution was evaporated in a vacuum, and the pale yellowish residue was recrystallised several times from ether–light petroleum (b. p. 40—60°). The reduction *product* formed colourless needles (0·19 g.), m. p. 111° [Found: C, 76·9, 76·8; H, 10·8, 10·8; *M* (Rast), 260, 269. $C_{19}H_{32}O_2$ requires C, 78·1; H, 11·0%; *M*, 292].

The neutral substance is strongly unsaturated to potassium permanganate and non-ketonic.

It gives no coloration with ferric chloride in alcoholic solution. It yields on acetylation a colourless oil, but gives a crystalline bisphenylurethane with phenyl *iso*cyanate and hence contains two hydroxyl groups.

The substance (70 mg.) was dissolved in dry benzene (5 c.c.) and after addition of phenyl *iso*cyanate (0·3 g.) the solution was refluxed for 4 hours, and half the solvent then removed by distillation. On cooling and keeping overnight, the *phenylurethane* separated as colourless crystals. After recrystallisation from alcohol it formed globular aggregates of colourless needles, m. p. 160—161° after slight sintering at 157° (Found: C, 74·7; H, 7·9; N, 5·6. $C_{33}H_{42}O_4N_2$ requires C, 74·7; H, 7·8; N, 5·3%).

(2) *Auroglaucin.* A suspension of auroglaucin (0·254 g.) in glacial acetic acid (*ca.* 30 c.c.) was shaken with hydrogen in presence of platinum-black (0·5 g.). Hydrogen was at first rapidly absorbed, a volume corresponding to 4 mols. being taken up in some 15 minutes, the suspended material passing meanwhile into solution. During the next hour a further 2 mols. were absorbed; the hydrogenation was then stopped (absorption, 123 c.c. Calc. for $6H_2$, 120 c.c.). On evaporation of the filtered solution in a vacuum a pale yellow crystalline solid was obtained, which after several recrystallisations from ether–light petroleum (b. p. 40—60°) formed colourless needles (0·2 g.), m. p. 111° (Found: C, 76·9, 76·6; H, 11·0, 10·9%). This product was identical in all its properties with the partial reduction product of flavoglaucin already described and a mixture of the two substances showed no depression of melting point. The identity was confirmed by the preparation of the bisphenylurethane in the manner already described. The colourless needles after recrystallisation from alcohol had m. p. 160—161° after slight sintering at 157° and had all the properties of the phenylurethane obtained from the product of partial reduction of flavoglaucin; a mixed melting point with the latter showed no depression (Found: C, 74·4; H, 8·0; N, 6·0%).

From the composition of the bisphenylurethane it appears that the reduction product consists mainly of a substance $C_{19}H_{32}O_2$. The analysis figures quoted for the reduction product are obtainable with all samples irrespective of the mode of purification and it seems probable that the material obtained represents mixed crystals of $C_{19}H_{32}O_2$ with a small amount of another product containing 3 oxygen atoms. The possibility that the substance might contain less than 19 carbon atoms was entertained, but it is discounted by the result of reducing molten flavoglaucin in a stream of hydrogen in presence of platinum-black; the only volatile product which could then be detected was water.

(B) *Complete hydrogenation.* (1) Micro-hydrogenation (Dr. A. Winterstein, Heidelberg), with a platinum–silica catalyst containing 17% of platinum (differential method), gave the following results: Auroglaucin, $C_{19}H_{22}O_3$, absorbed 10 mols. of hydrogen; flavoglaucin, $C_{19}H_{28}O_3$, absorbed 7 mols. of hydrogen.

(2) *Hydrogenation of flavoglaucin.* Flavoglaucin (1 g.), dissolved in glacial acetic acid (60 c.c.), was shaken with hydrogen in presence of highly active platinum-black (2 g.). Absorption was at first rapid, but slowed down to a steady rate after some 3 mols. of hydrogen had been taken up. At this stage the solution became colourless and a quantity of colourless crystalline material separated, which dissolved again as the reduction proceeded. After 6 hours, absorption had practically ceased (observed absorption, 580 c.c. Calc. for $7H_2$, 550 c.c.). The filtered solution was evaporated in a vacuum on the steam-bath and the colourless viscous residue was fractionated in a vacuum; after two distillations it had b. p. *ca.* 173—175°/12 mm. (Found: C, 83·8; H, 13·7%). The analytical values suggested that this liquid was a mixture of an alcohol containing one oxygen atom with a hydrocarbon. Accordingly it was again shaken with hydrogen in acetic acid solution in presence of platinum-black, but very little hydrogen was absorbed; on working up as before and subjecting the product to two fractionations, we obtained a colourless liquid, b. p. *ca.* 173—175°/12 mm. (Found: C, 83·6; H, 13·8%). The product is apparently quite saturated, as it does not decolourise potassium permanganate or bromine water.

Oxidation of Flavoglaucin with Potassium Permanganate.—A preliminary experiment with flavoglaucin (1 g.) in acetone solution showed that oxidation with potassium permanganate produced mainly a mixture of liquid acids, from which an amide, m. p. 102—103°, was ultimately obtained. The oxidation was accordingly carried out with a larger quantity of material.

Flavoglaucin (5 g.) was dissolved in pyridine (200 c.c.) and treated with 4% aqueous potassium permanganate in portions of 46·25 c.c. (equiv. to 1 atom of oxygen) at room temperature, the mixture being shaken after each addition until the colour disappeared. After 12 portions had been added decolourisation of the added permanganate was less rapid and it became exceedingly slow when a total of 15 portions had been added. After keeping over-night, the

solution was decolourised by means of sulphur dioxide, filtered from some crystalline inorganic material, made slightly alkaline with potassium hydroxide, and concentrated to about half its bulk in a vacuum on the steam-bath. After being made strongly acid, the solution was extracted continuously with ether during about 24 hours. The ethereal extract was thrice shaken with small quantities of concentrated potassium hydroxide solution and then with a little water, the aqueous layer being added to the alkali extracts. After drying over anhydrous sodium sulphate, the ether was removed and a trace of a colourless oil, which had a strong odour resembling that of rue, was obtained. It did not possess ketonic properties, however, and did not crystallise. On account of the small amount produced, it was not further investigated.

The alkaline solution was acidified with concentrated hydrochloric acid and extracted seven times with ether and the combined ethereal extracts were dried over anhydrous sodium sulphate. After removal of most of the ether on the steam-bath, the residue, which had a rancid odour, was treated with excess of diazomethane in ethereal solution. The ether was removed from the solution of esters produced, by cautious distillation on the water-bath through a fractionating column. After removal of the ether the residue was distilled first at the ordinary pressure. A trace of ester, possibly methyl acetate, distilled with the last traces of ether at about 55°, but nothing further came over till the bath temperature rose to about 180°; the distillation was then continued under diminished pressure, affording : (1) b. p. 90—100°/14 mm. Colourless liquid with slight fruity odour (0·5 g.) (Found : C, 62·7; H, 9·7%); (2) b. p. 100—108°/14 mm., similar colourless liquid (0·5 g.) (Found : C, 61·4; H, 10·3%); (3) b. p. 150—180°/14 mm. Brownish thick liquid (0·45 g.), whose distillation was accompanied by some decomposition. The residue in the flask charred on further attempted distillation.

From the analytical results it seemed probable that fractions (1) and (2) were fatty ester mixtures of rather similar composition; fraction (3) was deemed too impure to render analysis of any value. The various fractions were converted into amides.

On standing for 4 weeks with concentrated aqueous ammonia (5 c.c., *d* 0·880) in a stoppered flask, the fraction (1) dissolved gradually and the liquid became filled with colourless plates mixed with a white powdery material. The solid material was collected, dried, extracted with a little hot benzene and filtered (the residue—a white powder—did not melt below 320° but underwent gradual sublimation above 300°; it appeared from its properties to be oxamide). To the hot benzene solution an equal volume of light petroleum (b. p. 60—80°) was added, and the solution allowed to cool; colourless platelets then separated. After several such recrystallisations the amide was obtained as glistening rectangular platelets, m. p. 103—104°, identical in properties with the product from fraction (2) (see below), with which a mixed m. p. gave no depression.

The liquid fraction (2) was kept for 4 weeks with concentrated aqueous ammonia (5 c.c., *d* 0·880); it behaved exactly as fraction (1) and was worked up in the same way. A small quantity of oxamide was obtained, less than from (1), but the main product was the amide, m. p. 103—104° (Found : C, 66·7; H, 11·8; N, 9·8. Calc. for $C_8H_{17}ON$: C, 67·1; H, 11·9; N, 9·8%). *n*-Octoamide has m. p. 105° and comparison with a specimen of this substance prepared from synthetic *n*-octoic acid showed the above amide to be identical with it. A mixture of the two substances melted at 104—105°.

Isolation of Emodin Monomethyl Ether (Physcion) from the Metabolic Products of A. glaucus *Link.*—It was observed in working up the crude flavoglaucin (4·5 g.) from *A. glaucus* Link by light petroleum extraction that the extract had a pale reddish colour. On cooling and standing overnight, the flavoglaucin crystallised as usual, but in addition a small quantity of reddish crystalline material was observed to be present. The mixed solids were collected and digested with warm 75% alcohol; the flavoglaucin readily dissolved, leaving the red substance as a practically insoluble residue. This, recrystallised several times from chloroform–alcohol, formed brownish-orange needles (8 mg.), m. p. 203—204° (Found : C, 67·8; H, 4·8; MeO, 11·3. Calc. for $C_{16}H_{12}O_5$: C, 67·6; H, 4·2; 1MeO, 10·9%). The substance dissolved in concentrated sulphuric acid to a reddish-purple solution, was insoluble in cold dilute sodium carbonate solution but soluble in caustic alkalis with a ruby-red colour, and was a very feeble mordant dye. These properties correspond to those of emodin monomethyl ether, which has m. p. 203—204°, and comparison with an authentic specimen of this substance (from *Ventilago madraspatana*) established the identity of the material isolated from *A. glaucus* Link. A mixed melting point of the two samples showed no depression.

Rubroglaucin.—The material used was derived from *A. ruber* (Gould and Raistrick, *loc. cit.*). Purification can be effected by recrystallisation from butyl alcohol (or from alcohol, but the crystals then appear to be solvated), the colouring matter forming small red needles, m. p.

180—181° [Found : C, 67·2; H, 4·3; MeO, 10·7; *M* (Rast), 336. Calc. for $C_{16}H_{12}O_5$: C, 67·6; H, 4·2; MeO, 10·9%; *M*, 284]. The total amount of purified material available was some 300 mg.

Rubroglaucin is practically insoluble in water and is only sparingly soluble in organic solvents apart from chloroform, which dissolves it readily. It has all the properties of a hydroxyanthraquinone derivative. Insoluble in cold aqueous sodium carbonate, it dissolves readily in sodium hydroxide to a red-violet solution. Its solution in concentrated sulphuric acid is violet with a blue tinge, and its solution in acetic acid is yellow with a green fluorescence. Rubroglaucin possesses practically no dyeing properties when applied to mordanted cotton.

Rubroglaucin from A. albidus.—The orange-coloured extract of crude flavoglaucin from *A. albidus* in light petroleum (b. p. 40—60°) showed a green fluorescence. On cooling and keeping, the flavoglaucin which separated was contaminated with a red crystalline substance present in small quantity. When the solid material was digested with warm 80% alcohol, the flavoglaucin dissolved, leaving the red substance. The latter, after several recrystallisations from alcohol, formed small red needles, m. p. 180—181°, identical in all properties with rubroglaucin; a mixed m. p. with the latter showed no depression (Found : C, 66·5, 66·5; H, 4·3, 4·3. Calc. for $C_{16}H_{12}O_5,0{\cdot}5C_2H_5{\cdot}OH$: C, 66·4; H, 4·9%). The quantity obtained in this way was approximately 30 mg.

The crude flavoglaucin from *A. glaucus mut. alba* gave on similar treatment some 6 mg. of rubroglaucin.

Rubroglaucin Acetate.—Rubroglaucin (20 mg.) was heated for a few minutes with acetic anhydride containing a trace of sulphuric acid. The yellow solution was poured into water, and the *acetate* collected and recrystallised twice from dilute acetic acid. The yellow needles, m. p. 226—228°, appeared to contain water of crystallisation (Found : C, 62·6; H, 4·4; MeO, 7·9. $C_{20}H_{16}O_7,H_2O$ requires C, 62·2; H, 4·7; MeO, 8·0%).

2-*Methylanthracene from Rubroglaucin.*—Rubroglaucin (90 mg.) was subjected to zinc dust distillation in six portions of 15 mg. each, and the combined distillates, which took the form of a faintly yellowish, crystalline solid with a greenish fluorescence, were recrystallised several times from alcohol, affording colourless leaflets with a weak greenish fluorescence, m. p. 198°. A mixture of the substance with 2-methylanthracene (m. p. 204°) melted at 202°, but a mixture with anthracene (m. p. 212°) melted at 182—188°. There can be little doubt but that this product is 2-methylanthracene.

Demethylation of Rubroglaucin.—Rubroglaucin (100 mg.) was heated with concentrated sulphuric acid (2 c.c.) at 140—150° during 20 minutes. The solution was cooled, diluted with water, and the mixture boiled to coagulate the brownish precipitate, which was then collected, washed with water, dried, and recrystallised from chloroform–alcohol. Some decomposition had evidently occurred during the heating with sulphuric acid, as a large proportion of the crude product was dark in colour and did not dissolve in chloroform. The recrystallised product formed red needles, m. p. 217—218°, and two further crystallisations, followed by sublimation in a vacuum at 180°, were carried out before analysis. The bright red needles had m. p. 220° (Found : C, 62·7, 62·7; H, 4·2, 4·1. $C_{15}H_{10}O_5,H_2O$ requires C, 62·5; H, 4·2%). The *triacetate* crystallised from acetic acid in yellow needles (Found in material dried at 150° in a high vacuum : C, 63·4; H, 4·1. $C_{21}H_{16}O_8$ requires C, 63·6; H, 4·0%).

The demethylated product, presumably a methyltrihydroxyanthraquinone, retaining $1H_2O$, resembles rubroglaucin in its colour reactions, but, unlike the latter, it is readily soluble in cold aqueous sodium carbonate solution. It is, moreover, a good mordant dye.

Synthesis of 1 : 3 : 4-*Trihydroxy*-2-*methylanthraquinone.*—2-*Hydroxy*-4-*methoxy*-3-*methylacetophenone.* Resacetophenone (20 g., 1 mol.) was dissolved in a solution of potassium hydroxide (25 g., 6 mols.) in methyl alcohol (250 c.c.) contained in a flask fitted with a reflux condenser. Methyl iodide (62·5 g., 6 mols.) was then added in three portions, the flask being immersed in water at about 15°. When all had been added, the mixture was gently refluxed until the alkaline reaction of the liquid disappeared (4—5 hours; a little more methyl iodide may be added towards the end if necessary). After removal of the methyl alcohol, water was added to the residue, and the mixture extracted with ether. The ethereal extract was shaken once with excess of potassium hydroxide solution, dried, and evaporated. The residual reddish oil solidified almost at once, and on recrystallisation from light petroleum (b. p. 80—100°) furnished 2-hydroxy-4-methoxy-3-methylacetophenone as almost colourless platelets, m. p. 83—84° (yield, 5—6 g.); the alkaline extract on acidification and steam-distillation gave paeonol (*ca.* 3 g.).

2 : 3 : 6-*Trimethoxytoluene.* 2-Hydroxy-4-methoxy-3-methylacetophenone (10·8 g.) was

suspended in a mixture of *N*-sodium hydroxide (60 c.c.) and methyl alcohol (30 c.c.), and 3% hydrogen peroxide (51 c.c.) added under coal gas. After a short time the mixture became warm and much of the suspended material passed into solution, the liquid meanwhile acquiring a brownish tint. When the reaction had subsided and the flask cooled to room temperature, water (*ca.* 25 c.c.) was added, and unchanged initial material (3 g.) separated. The filtrate was acidified and extracted several times with ether, the extracts dried, and the ether removed, leaving a dark oily residue. This was dissolved in xylene (100 c.c.) and, after addition of anhydrous potassium carbonate (20 g.) and methyl sulphate (15 g.), the mixture was refluxed for 6 hours and then distilled in steam. The xylene came over first, followed by the desired methyl ether. The xylene layer was separated, dried, and the solvent removed cautiously by distillation through a short column. The residue, a colourless odourless liquid, had b. p. 145—147°/14 mm. (yield, 4 g.) (Found: MeO, 49·8. $C_{10}H_{14}O_3$ requires 3MeO, 51·1%).

1 : 3 : 4-*Trihydroxy-2-methylanthraquinone.* Powdered anhydrous aluminium chloride (2 g.), was added to a mixture of phthalic anhydride (1 g.), 2 : 3 : 6-trimethoxytoluene (2 g.), and carbon disulphide (30 c.c.), and the whole heated under reflux during 24 hours. The carbon disulphide was removed, the residue decomposed with cold dilute hydrochloric acid, and unchanged initial materials removed by distillation in steam. The semi-solid residue was taken up in a large volume of ether, and the solution dried and concentrated; the crude benzoylbenzoic acid separated as yellowish crystals. Recrystallised from ether, it formed colourless prisms, m. p. 205—208° (Found: C, 65·7; H, 5·5. $C_{18}H_{18}O_6$ requires C, 65·5; H, 5·4%). The acid gave no coloration with ferric chloride in alcoholic solution.

The above benzoylbenzoic acid (0·4 g.) was heated with concentrated sulphuric acid (2 c.c.) at 150—160° for 30 minutes. The solution acquired a deep red-violet colour, and on cooling and pouring into water a red-brown precipitate was produced; this was boiled with water for a few minutes to coagulate, collected, washed with hot water, dried, and purified by sublimation in a high vacuum and crystallisation from 96% alcohol. The product (yield, *ca.* 20 mg.) formed red needles, m. p. *ca.* 268—270°, with partial sublimation; it appeared to contain water of crystallisation (Found: C, 62·6; H, 4·4. $C_{15}H_{10}O_5,H_2O$ requires C, 62·5; H, 4·2%).

1 : 3 : 4-*Trihydroxy-2-methylanthraquinone* is readily soluble in aqueous sodium hydroxide or carbonate solution with a bluish-crimson colour. Its solution in concentrated sulphuric acid is bluish-crimson—very reminiscent of purpurin, which it resembles also in the colour of its acetic acid solutions—yellow with but feeble greenish fluorescence. It is, however, a weaker adjective dye than purpurin.

The yield of anthraquinone in this synthesis was poor, since the major part of the crude product was a mixture of colourless prisms and needles. The nature of this acidic substance has not yet been determined.

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