CLVIII. STUDIES IN THE BIOCHEMISTRY
OF MICRO-ORGANISMS
LXII. THE CRYSTALLINE COLOURING MATTERS OF
SPECIES IN THE *ASPERGILLUS GLAUCUS* SERIES.
PART II

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GOULD & RAISTRICK [1934] reported the isolation of three new crystalline colouring matters which they obtained by extraction with light petroleum of the dried mycelium of 25 species in the *Aspergillus glaucus* series. The colouring matters and the empirical formulae provisionally assigned to them were: (a) flavoglaucin, $C_{19}H_{28}O_3$, lemon-yellow needles; (b) auroglaucin, $C_{19}H_{22}O_3$, orange-red needles; (c) rubroglaucin, $C_{16}H_{12}O_5$, ruby-red needles. Further investigation of these colouring matters has confirmed the views expressed by Gould & Raistrick in the cases of flavoglaucin and auroglaucin and provisional structural formulae have now been assigned to them [Raistrick *et al.* 1937; Cruickshank *et al.* 1938].

The case is different with rubroglaucin. In the earlier work only very small amounts of crude rubroglaucin were available for purification and chemical examination, but investigation of this material by Raistrick *et al.* [1937] appeared to confirm the results of Gould & Raistrick [1934] and to indicate that rubroglaucin is a dihydroxymonomethoxymethylanthraquinone. In order to settle this point larger quantities of rubroglaucin have now been isolated from more recently prepared mycelium of the same strain of *Aspergillus ruber* (Spieckermann & Bremer) Thom & Church as was used by Gould & Raistrick three years previously for the preparation of this colouring matter. Exhaustive fractionation of the crude red pigment obtained resulted in the isolation of two hydroxyanthraquinones, i.e. physcion, 4:5-dihydroxy-7-methoxy-2-methylanthraquinone, and a hitherto undescribed trihydroxymonomethoxymethylanthraquinone, of at present undetermined molecular constitution, for which the name *erythroglaucin* is proposed. The question then immediately arose as to whether this finding was to be explained (a) by a change in the metabolic processes of this strain of *A. ruber* after 3-4 years' cultivation on laboratory media, or (b) by the fact that the substance previously described as rubroglaucin is in fact not an entity but a mixture of physcion and erythroglaucin which is unusually difficult to separate into its components. Possibility (a) seems to be ruled out by the facts that both physcion and erythroglaucin have now been isolated from the mycelium of the Gould & Raistrick strain of *A. ruber* when this was grown on two different media and after incubation periods of 28, 41 and 100 days, and also from a number of other different strains of *A. ruber* obtained from widely different sources. No *pure* substance having the properties

recorded for rubroglaucin was encountered though mixtures of physcion and erythroglaucin having these properties were observed. Possibility (b) was shown to be correct by the actual isolation of physcion and erythroglaucin from a very small sample of rubroglaucin originally prepared by Dr B. S. Gould in 1934. Hence rubroglaucin must now be regarded as a mixture of physcion and erythroglaucin and the word rubroglaucin becomes a *nomen nudum*.

Gould & Raistrick [1934] suggested that the 25 species of moulds in the *A. glaucus* series which they examined might be conveniently divided into three groups on the basis of the pigments, soluble in light petroleum (B.P. 40–50°), which they contain. Group I, containing 13 species, consisted of those species giving flavoglaucin and auroglaucin only; group II contained 2 species which give flavoglaucin, auroglaucin and an unidentified red pigment; group III contained 10 species which give flavoglaucin and at least one red pigment which in four cases was believed to be rubroglaucin. No auroglaucin was isolated from members of group III. It was also reported that, in addition to the highly coloured pigments flavoglaucin, auroglaucin and rubroglaucin, there were present in the light petroleum extracts of species in groups II and III other much less coloured, if not indeed colourless, extractives which were not examined further. Hence, in view of our more recent findings on rubroglaucin, it obviously became of interest to re-examine the species in groups II and III in more detail than was previously possible. This has now been done and the results obtained form the major portion of the present communication.

The following species, representing all those in groups II and III, except *A. lovaniensis* Biourge which has become atypical, together with certain additional strains of *A. ruber*, were grown on the same synthetic medium and under the same cultural conditions as were employed previously: Group II, *A. oriolus* Biourge, *A. albidus* Spegazzini; Group III, six different strains of *A. ruber* (Spieckermann & Bremer) Thom & Church, *A. ferrugineus* Fuckel, *A. glaucus* mut. *alba* Blochwitz, *A. umbrosus* Bainier & Sartory, *A. mongolicus* Biourge, *A. echinulatus* (Delacroix) Thom & Church, *A. herbariorum* series *minor* (Mangin) Thom & Church, *A. mutabilis* Bainier & Sartory and *A. repandus* Bainier & Sartory. The dried mycelium was exhaustively extracted with light petroleum and the extract, after removal of fat, was repeatedly and very carefully fractionated from different solvents.

It may be said at once that the apparent differences between groups II and III previously suggested now largely disappear. All the species, with the possible exception of *A. echinulatus* where the evidence is doubtful, gave flavoglaucin in larger or smaller amounts. Hence the view is confirmed that this pigment is specific for and constantly present in all members of the *A. glaucus* series. On the other hand auroglaucin has now been detected in three species in group III, i.e. *A. ruber* (in two strains out of six), *A. glaucus* mut. *alba* and *A. ferrugineus* and confirmed in both members of group II, i.e. *A. oriolus* and *A. albidus*. Hence, in view of the difficulty of detecting small amounts of auroglaucin in the presence of relatively large amounts of the other colouring matters, we now regard the isolation of auroglaucin as being not sufficiently certain to justify, in itself, the separation of groups II and III. The essential homogeneity of groups II and III is, moreover, seen in the results of the examination of their hydroxyanthraquinone content. In no species examined was any evidence forthcoming of the presence of a *pure* substance having the properties of rubroglaucin though in many cases fractions, which subsequently proved to be mixtures, were isolated approximating in colour reactions, M.P. etc. to those previously recorded for rubroglaucin. In all the species examined, physcion

(4:5-dihydroxy-7-methoxy-2-methylantraquinone) was detected and was identified in some cases by direct comparison with a sample prepared from the lichen *Xanthoria parietina* (L.) and in others by direct comparison of their diacetates. With the single exception of *A. mutabilis*, erythroglaucin was also isolated from all the species examined.

In addition to physcion and erythroglaucin we have also isolated two other substances from five of the mould species examined, i.e. *A. oriolus* in group II, *A. mongolicus*, *A. mutabilis*, *A. herbariorum* and two strains of *A. ruber* in group III. These substances, which are of some biological interest, are both reduction products of physcion and will be referred to as physcion anthranol A, m.p. 260°, and physcion anthranol B, m.p. 181–2°. We have also prepared physcion anthranol B by reduction of physcion and have converted both physcion anthranol A and physcion anthranol B into physcion by oxidation. The probable relation of the two anthranols to physcion (structure I) is given in structures II and III though it is not possible to say which of these structures is to be referred to anthranol A and which to anthranol B.

The occurrence of physcion and the two anthranols in the mycelium of the above-mentioned moulds adds one more example to the growing list of similar substances, reported from these laboratories as metabolic products of the lower fungi, which suggest that these substances probably play a part as oxidation-reduction systems in the vital processes of the moulds which produce them.

Physcion is widely distributed in nature in plants of very different types. It has been known for many years, formerly under the name parietin, as one of the so-called lichen acids, occurring especially in several species of *Xanthoria* and *Placodium*. It was isolated in small amounts by Raistrick *et al.* [1937] from crude flavoglaucin prepared from *A. glaucus* Link, a species which was included by Gould & Raistrick [1934] in group I. As long ago as 1894 it was observed by Perkin & Hummel, and described by them as emodin monomethyl ether, in the root-bark of *Ventilago madraspatana* Gärtn., a large climbing shrub common in Southern India and Ceylon. Of even greater interest, however, from the point

of view of the present communication, is the fact that Perkin & Hummel isolated, in addition to physcion, two anthranols A and B, which melted respectively at 260 and 173°, both of which they converted by oxidation into physcion. Although we have not been able to compare directly our two anthranols, melting respectively at 260 and 181–2°, there seems to be little doubt that the mould anthranols are identical with those from *Ventilago madraspatana*. So far as we are aware physcion anthranol A, M.P. 260°, has not been reported from any other sources, but physcion anthranol B, M.P. 181–2°, has been described by several other workers, notably by Hesse [1912; 1917] who isolated it (M.P. 181°, 184°) from chrysarobin ("Goa powder" of commerce), a crystalline exudation from the wood of various species of *Andira* especially *Andira araroba* Ag. The same worker [1895] also obtained it (M.P. 180–2°) by the reduction of physcion.

Erythroglauzin, $C_{16}H_{12}O_6$, deep red plates, M.P. 205–6°, is a monomethyl ether of a tetrahydroxymethylanthraquinone. It contains a methyl group and gives a triacetate but is not the methyl ether of cynodontin, itself a tetrahydroxymethylanthraquinone, and present in the mycelium of the mould *Helminthosporium cynodontis* Marignoni [Raistrick *et al.* 1933], since its trimethyl ether is not identical with the tetramethyl ether of cynodontin.

EXPERIMENTAL

History of cultures used

The following species were used in the experimental work described below:

Catalogue no.	Species	Source
(1) No. 6	<i>A. ruber</i> (Spieckermann & Bremer) Thom & Church	Isolated by Mr G. Smith in 1926 from mildewed cotton cloth
(2) No. 6 (Gould)	" "	Previous strain, maintained in culture in America by B. S. Gould from 1934 onwards and returned here, 1938
(3) No. 23	" "	Isolated by Mr G. Smith in 1926 from cotton yarn
(4) A. 20	" "	Received from Centraalbureau voor Schimmelcultures, Baarn, 1937. Miss Church's strain
(5) A. 22	" "	Received from Centraalbureau voor Schimmelcultures, Baarn, 1937. Pollacci (1931) strain
(6) A. 47	" "	Received from Mr L. D. Galloway, 1937. Isolated in India
(7) H.A. 5	" "	Isolated by Mr G. Smith in 1938 from infected hops
(8) Ac. 39	<i>A. ferrugineus</i> Fuckel	Received from Baarn, 1923
(9) A. 16	<i>A. glaucus</i> mut. <i>alba</i> Blochwitz	Received from Baarn, 1933
(10) A. 18	<i>A. umbrosus</i> Bainier & Sartory	Received from Baarn, 1933
(11) A. 36	<i>A. mongolicus</i> Biourge	Received from Prof. Ph. Biourge, Louvain, 1933
(12) A. 28	<i>A. echinulatus</i> (Delacroix) Thom and Church	Received from Prof. Ph. Biourge, Louvain, 1933
(13) No. 96	<i>A. herbariorum</i> series <i>minor</i> (Mangin) Thom & Church	Received by Mr G. Smith in 1927 from Shirley Institute
(14) A. 40	<i>A. mutabilis</i> Bainier & Sartory	Received from Dr Charles Thom in 1933
(15) A. 17	<i>A. repandus</i> Bainier & Sartory	Received from Baarn, 1933
(16) A. 30	<i>A. oriolus</i> Biourge	Received from Prof. Ph. Biourge, 1933
(17) A. 35	<i>A. albidus</i> Spegazzini	Received from Prof. Ph. Biourge, 1933

With the exception of cultures 3, 4, 5, 6 and 7 all these cultures had been examined previously by Gould & Raistrick [1934].

Cultural conditions

Two synthetic media, previously used by Gould & Raistrick, were employed throughout this work.

Medium A. Glucose, 1750 g.; diammonium tartrate, 75.6 g.; KH_2PO_4 , 35.0 g.; KCl, 17.5 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 17.5 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.35 g.; distilled water, 35 l.

Medium B. As for medium A except that 378 g. of diammonium tartrate was used in place of 75.6 g.

In order to avoid decomposition the ammonium tartrate was sterilized separately, the details being given in Gould & Raistrick's paper [1934, p. 1643]. With all species examined 100 one-litre conical flasks, each containing 350 ml. of sterile medium, were inoculated with a spore suspension of the chosen organism washed off beer-wort agar slopes, 4 flasks being sown with one slope. The flasks were incubated at 24° for periods of 28 to 100 days, until metabolism of the glucose present had become very slow. At the end of this time the medium was covered with a felt of mycelium varying in colour from greyish-brown with some species, through rust colour to a bright reddish-brown in others. The mycelium was then separated by filtration, washed carefully with distilled water, pressed, dried in a vacuum oven at 35–40° and ground in a coffee mill.

Extraction of the mycelium and fractionation of the colouring matters

Six different substances—flavoglaucin, auroglaucin, erythroglauclin, physcion and the physcion anthranols A and B—may be present in the mycelium under investigation, and since the total number of colouring matters present in any one species, and also their relative proportions, vary from species to species, it is obviously not possible to give the exact details of the methods of fractionation followed in every case. The general procedure, modified in some details to suit individual cases, was as follows. The ground mycelium was in all cases extracted exhaustively with light petroleum (B.P. 40–50°) in a Soxhlet apparatus until the extract became colourless. The time required for complete extraction varied from 8 to 22 hr. The light petroleum extracts were held at 0° until separation was complete and the solid separating was filtered, washed with cold light petroleum and dried. The filtrate and washings were combined, evaporated and further crops of solid material were united with the first crop. The final mother liquor, on evaporation, gave varying amounts of fat. The total crude pigment obtained varied from a minimum of 0.48 g. with *A. ruber* Cat. No. 23 to a maximum of 31.0 g. with *A. mongolicus*.

The combined crude colouring matters were now ground with cold methanol in which flavoglaucin is readily soluble and auroglaucin moderately soluble, while erythroglauclin, physcion and the physcion anthranols are only very slightly soluble; 8–10 ml. of methanol per g. of crude colouring matter were used. The undissolved material, usually bright red in colour, was separated by filtration (for further treatment see later) and the filtrate and washings, containing flavoglaucin and auroglaucin, evaporated to dryness in air. The dry residue was now extracted with boiling light petroleum (B.P. 50–60°) in which flavoglaucin is much more readily soluble than auroglaucin. The undissolved residue consisting of crude auroglaucin was purified by crystallization from ethyl acetate. Flavoglaucin was recovered from the light petroleum solution and was purified by crystallization from light petroleum (B.P. 40–50°) and 75% alcohol.

The crude red pigment obtained on Medium A varied in weight from 0.06 g. with *A. ferrugineus* to 10.42 g. with *A. oriolus* per 100 flasks, and in m.p. from 142 to 155° in the case of *A. repandus* to 182–7° in the case of *A. ferrugineus*. It may contain two or more of the substances erythroglaucon, physcion and the physcion anthranols A and B and was subjected to prolonged fractional crystallization. Ethyl acetate was used almost exclusively for the separation and purification of erythroglaucon and physcion, benzene and toluene being used for the purification of the anthranols. Erythroglaucon and physcion were separated by making use of the facts that physcion is much more soluble in warm ethyl acetate than is erythroglaucon, and that while erythroglaucon usually crystallizes in characteristic flat plates, physcion generally separates in slender needles. Actual details of each crystallization vary from fraction to fraction, but the general principle of the method used is as follows: the solution of the red pigments in hot ethyl acetate was allowed to cool very slowly and the type of crystal separating was followed by examination with a powerful hand lens. The first crystals to appear were usually flat plates (erythroglaucon) and these were separated by filtration of the hot solution as quickly as possible so as to minimize admixture with needle-shaped crystals (physcion). The mother liquors, on cooling to room temperature, deposited a mixture of erythroglaucon and physcion and the filtrate from these on concentration gave mainly physcion. At every stage the m.p., colour reaction with cold conc. H_2SO_4 , and crystalline form were determined. Consideration of each of these tests decided which fractions should be combined and the fractional crystallization was then repeated. The process is a long and tedious one but leads eventually to pure specimens of erythroglaucon and physcion. A mixture of about 30% erythroglaucon and 70% physcion is very difficult to separate into its constituents, melts at about 180°, looks homogeneous and gives an intense Tyrian purple colour with conc. H_2SO_4 . Such a mixture corresponds closely to the rubroglaucon of Gould & Raistrick [1934].

The fractionation of the crude red colouring matters is rendered very much more difficult if physcion anthranols A and B are present. Physcion anthranol A forms pale yellow needles, m.p. 260°, is much less soluble in organic solvents than any of the other colouring matters and hence can be fairly readily isolated. Physcion anthranol B, m.p. 181–2°, on the other hand, has physical properties which are almost identical with those of physcion. Hence it is only isolated from a mixture with physcion by a long and tedious fractional crystallization from ethyl acetate and benzene. So difficult is this separation that our specimens of physcion anthranol B have still a pale pink colour, whereas specimens of physcion anthranol B, prepared by the reduction of physcion with zinc dust in glacial acetic acid are yellow in colour, but a mixture of the two preparations shows no depression in m.p. The pinkish colour of specimens of mould physcion anthranol B also persists after fractional sublimation in a high vacuum at 130°.

Composition of the mixed colouring matters isolated from the different species

The experimental results obtained with all the species examined are given in Table I in which all weights are expressed as g. per 100 flasks of culture medium. The temperature of incubation was 24° throughout. It should be noted that the pigments described are only those which can be extracted with light petroleum, b.p. 40–50°. Many species, after complete extraction with light petroleum, are still highly coloured and the composition of these light petroleum-insoluble colouring matters will form the subject of a future communication. All species

Table I

Exp. no.	Species used and catalogue no.	Medium	Incu- bation period days	% re- sidual glucose	My- celium g.	Total pig- ment g.	Crude flavo- glucan g.	Auro- glucan g.	Total crude red pig- ment g.	M.P. crude red pig- ment ° C.	Pure ery- thro- glucan g.	Mainly ery- thro- glucan g.	Pure phy- sion g.	Mainly phy- sion g.
1	<i>A. ruber</i> (No. 6)	A	28	2.60	113	7.73	6.58	0.10	1.05	167-72	0.14	0.04	0.29	0.15
2	<i>A. ruber</i> (No. 6)	A	40	1.62	113	4.32	3.77	—	0.55	173-8	0.06	—	0.18	0.04
3	<i>A. ruber</i> (No. 6) (Gould)	A	41	1.86	124	5.85	4.80	—	1.05	166-71	0.12	—	0.33	0.16
4	<i>A. ruber</i> (No. 6)	B	41	0.20	157	7.71	7.05	—	0.66	170-85	0.08	0.15	0.10	0.02
5	<i>A. ruber</i> (No. 6)	A	100	1.84	123	7.35	6.37	0.020	0.96	177-81	0.065	0.195	0.41	—
6	<i>A. ruber</i> (No. 23)	A	42	2.57	100	0.48	0.27	—	0.21	176-80	—	0.08	Trace	—
7	<i>A. ruber</i> (A 20)	A	37	3.10	42	3.38	3.28	—	0.10	177-81	0.025	0.025	0.02	—
8	<i>A. ruber</i> (A 22)	A	39	3.10	126	3.59	2.36	—	1.23	160-5	0.32	—	0.015	0.04
9	<i>A. ruber</i> (A 47)	A	43	3.00	109	5.43	5.12	—	0.31	165-8	0.07	—	0.022	0.04
10	<i>A. ruber</i> (H.A. 5)	A	37	1.76	133	10.35	8.92	0.20	1.23	170-5	0.10	0.04	0.44	—
11	<i>A. ferrugineus</i> (Ac 39)	A	48	2.15	90	3.84	3.76	0.02	0.06	182-7	0.04	—	—	0.01
12	<i>A. glaucus</i> mut. <i>alba</i> (A16)	A	51	3.10	117	11.6	9.64	0.70	1.26	160-4	0.012	—	0.10	0.09
13	<i>A. umbrosus</i> (A 18)	A	45	2.20	108	3.35	3.22	—	0.13	180-6	0.006	—	0.007	—
14	<i>A. mongolicus</i> (A 36)	A	75	0.72	209	19.0	16.70	—	2.30	142-8	0.30	—	0.414	—
15	<i>A. mongolicus</i> (A 36)	B	96	0.076	298	31.0	27.16	—	3.84	171-7	0.075	0.075	1.96	0.61
16	<i>A. echinulatus</i> (A 28)	A	44	2.95	141	0.91	—	—	0.84	163-8	0.21	—	0.11	—
17	<i>A. herbariorum</i> (No. 96)	A	47	1.75	130	1.75	0.85	—	0.90	160-4	0.03	—	0.02	0.05
18	<i>A. mutabilis</i> (A 40)	A	64	0.75	148	6.26	0.76	—	5.50	160-5	—	—	0.44	0.33
19	<i>A. mutabilis</i> (A 40)	B	84	0.075	120	1.31	0.53	—	0.01	202-5	—	—	0.01	—
20	<i>A. repandus</i> (A. 17)	A	35	1.70	119	11.34	11.08	—	0.26	142-55	0.11	—	0.02	0.02
21	<i>A. oriolus</i> (A 30)	A	53	1.74	210	28.28	17.85	Trace	10.42	160-6	0.21	0.05	—	0.10
22	<i>A. albidus</i> (A 35)	A	70	2.43	157	20.77	19.45	1.20	0.12	180-1	0.022	0.01	0.02	0.01

were grown on culture medium A, which was the medium used by Gould & Raistrick [1934] in their original investigation of the red colouring matters. In certain additional instances (see col. 3) culture medium B was also used (for composition of media A and B see p. 1295).

The following conclusions may be drawn from the results given in Table I.

(1) *Flavoglaucin* (see col. 8). Flavoglaucin was isolated from all the species examined with the exception of *A. echinulatus*, thus confirming the view expressed by Gould & Raistrick [1934] that this colouring matter may be regarded as specific for species in the *A. glaucus* series. In almost all cases flavoglaucin constituted 80% or more of the total colouring matters extractable with light petroleum. Considerable amounts of flavoglaucin were isolated from almost all species examined and in some instances, e.g. *A. mongolicus*, *A. glaucus* mut. *alba*, *A. repandus*, *A. oriolus* and *A. albidus*, amounted to about 10% or more of the weight of the dry mycelium. The m.p. of the crude flavoglaucin isolated was 80–90° but was raised on purification to that previously recorded for pure flavoglaucin, i.e. 104–5°. Flavoglaucin formation appears to be enhanced on medium B in comparison with medium A (see exps. 2 and 4 for *A. ruber* Cat. No. 6 grown under otherwise identical cultural conditions and exps. 14 and 15 for *A. mongolicus*). This observation is in agreement with similar findings for *A. novus* made by Gould & Raistrick [1934].

(2) *Auroglaucin* (see col. 9). Auroglaucin was isolated, not only from *A. oriolus* and *A. albidus*, as was previously reported by Gould & Raistrick [1934], but also from *A. ruber* Cat. No. 6, *A. ruber* Cat. No. H.A. 5, *A. glaucus* mut. *alba* and *A. ferrugineus*, though in all cases the amounts isolated were small. Hence the view tentatively expressed by Gould & Raistrick that *A. oriolus* and *A. albidus* could be separated from the species in the *A. glaucus* series giving red pigments, because of their ability to produce auroglaucin, must now be abandoned. The auroglaucin isolated was identified in all cases by its colour, crystalline form, m.p. 153° not depressed in any instance on admixture with an authentic specimen, and in one case, that from *A. ruber* Cat. No. H.A. 5, by analysis. (Found: C, 76.10; 76.12; H, 7.42, 7.34%. Calc. for $C_{19}H_{22}O_3$, C, 76.45; H, 7.44%.)

(3) *Erythroglauicin* (see cols. 12 and 13). Erythroglauicin was isolated from all the species examined with the single exception of *A. mutabilis*. This species was grown on both media A and B, but only small amounts of hydroxyanthraquinone colouring matters were formed on either medium and consisted, so far as could be ascertained, almost exclusively of physcion and its anthranols on medium A and physcion alone on medium B. The amounts of erythroglauicin isolated were, in all cases, very small, reaching a maximum of 0.32 g. per 100 flasks with *A. ruber* Cat. No. A 22. The identity of the different specimens of erythroglauicin isolated was established in all cases by their colour reactions with conc. H_2SO_4 and with 2N NaOH and by their m.p. which varied according to purity from a minimum of 195–8° with the specimen from *A. repandus* to a maximum of 204–6° with that from *A. ruber* Cat. No. A 22. Almost all samples melted above 200° and none of them gave any depression on admixture with a specimen isolated from *A. ruber* Cat. No. 6, m.p. 199–201°. The general properties of erythroglauicin are described later (see p. 1300).

(4) *Physcion* (see cols. 14 and 15). Physcion was isolated from all the species examined, in amounts which while not generally large in themselves were usually greater than the amounts of erythroglauicin obtained from the corresponding species. Considerable quantities of pure physcion were isolated from *A. mongolicus* (1.96 g. from 100 flasks) when this species was grown on medium B. The

influence of the medium on the nature and amount of the colouring matters formed is particularly well shown by this species, since when it was grown on medium A only 0.41 g. of pure physcion was isolated. The different specimens of physcion obtained were identified by their colour reactions with conc. H_2SO_4 and with 2N NaOH and by their m.p. Almost all the samples melted in the range $200^\circ \pm 2^\circ$ and none of them gave any depression in m.p. on admixture with the specimen from *A. ruber* Cat. No. H.A. 5 (m.p. $199\text{--}200^\circ$). The general properties of mould physcion and the proof of its identity with lichen physcion are described later (see pp. 1300 and 1301).

(5) *Physcion anthranol A*, m.p. 260° . This anthranol, the properties of which are described later (see p. 1301), was isolated from the following species, grown in each case on medium A: (a) *A. ruber*, Cat. No. A 47; 0.005 g., m.p. $254\text{--}6^\circ$. (b) *A. ruber*, Cat. No. 22; 0.015 g., m.p. $253\text{--}7^\circ$. (c) *A. mongolicus*, 0.023 g., m.p. $253\text{--}5^\circ$. (d) *A. mutabilis*, 0.08 g., m.p. $253\text{--}7^\circ$. (e) *A. herbariorum*, 0.02 g., m.p. $253\text{--}7^\circ$. (f) *A. oriolus*, 0.05 g., m.p. $258\text{--}62^\circ$. None of the specimens (a) to (e) gave any depression in m.p. on admixture with specimen (f).

(6) *Physcion anthranol B*, m.p. $181\text{--}2^\circ$. This anthranol, the properties of which are described later (see p. 1301), was identified in each of the species giving physcion anthranol A, except *A. ruber* Cat. No. A 47, and was usually isolated in much larger amounts than physcion anthranol A. The medium in each case was medium A. (a) *A. ruber*, Cat. No. 6; a trace. (b) *A. ruber*, Cat. No. A 22; 0.065 g., m.p. $180\text{--}1^\circ$. (c) *A. mongolicus*; a trace. (d) *A. mutabilis*; 0.26 g., m.p. $181\text{--}2^\circ$. (e) *A. herbariorum*; 0.07 g., m.p. $180\text{--}1^\circ$. (f) *A. oriolus*; 2.44 g., m.p. $180\text{--}2^\circ$. None of the above specimens gave any depression in m.p. on admixture with a synthetic specimen of physcion anthranol B, m.p. 182° , made by the reduction of physcion. It is of interest that while both physcion anthranols A and B were detected in both *A. mongolicus* and *A. mutabilis* when grown on medium A, neither anthranol could be detected in either of these moulds when grown on medium B.

The total weight isolated of erythroglaucon, physcion and the two anthranols was in general only a relatively small fraction of the weight of crude red pigment fractionated. This is due in part to unavoidable experimental losses and to the presence of considerable amounts of resinous material which could not be crystallized. In one case, i.e. *A. mutabilis* on medium B, a colourless substance crystallizing from ethanol in small rosettes, m.p. $97\text{--}100^\circ$, was isolated. This substance, which gave a strongly positive Liebermann sterol reaction, was identified as ergosterol palmitate by direct comparison with an authentic specimen isolated from *P. brevi-compactum* Dierckx [Oxford & Raistrick, 1933].

Fractionation of crude red colouring matters prepared by Dr B. S. Gould in 1934. Certain of the fractions of crude red colouring matters prepared by Dr Gould in 1934 and described by Gould & Raistrick [1934] being available were fractionated by the methods described above and gave the following results:

(1) From *A. ruber* Cat. No. 6, wt. 0.63 g., 0.06 g. of erythroglaucon, 0.18 g. of physcion and 0.18 g. of a crude mixture of the two anthranols were isolated. No evidence was obtained of the presence of rubroglaucon.

(2) From *A. mongolicus*, wt. 0.07 g. 0.005 g. of erythroglaucon and 0.004 g. of physcion were isolated. The residues contained small amounts of anthranols as was indicated by the colour reaction with conc. H_2SO_4 .

(3) A mixture of pigments from *A. ferrugineus* and *A. repandus* recorded by Gould & Raistrick [1934, p. 1647] as melting at $193\text{--}5^\circ$. Wt. 0.01 g. This material was almost pure erythroglaucon and yielded 0.007 g. of erythroglaucon in a single crystallization.

General properties of the colouring matters

(A) *Erythroglaucin*. Erythroglaucin is dimorphic, and although it usually crystallizes in small, regular, square, deep red plates, it sometimes separates in short sharp-pointed red needles. The two forms have the same m.p. and show no depression on mixing. m.p. 205–6°. (Found: C, 63.85, 63.91; H, 3.98, 4.00; CH₃O, 10.0%. C₁₆H₁₂O₆ requires C, 63.98; H, 4.03; 1 CH₃O, 10.3%.) On oxidation with chromic acid (Kuhn-Roth method) evidence of one side-chain methyl group was obtained. (Found: 102.3, 95.7% of 1 CH₃COOH.) It is only slightly soluble in light petroleum, methanol, ethanol and ether, is moderately soluble in cold benzene, chloroform, acetone and ethyl acetate, but is readily soluble on heating. It gives an orange-red solution in organic solvents, the solution having a slight green fluorescence. It gives a pure deep blue colour in dilute solution in cold conc. H₂SO₄ and a purple solution with 2*N* NaOH which slowly gives a violet precipitate. An alcoholic solution gives the following reactions: (a) with aqueous NaOH or ammonia an intense red-violet colour which is destroyed on the addition of aqueous NaOCl. (b) With the following aqueous solutions; borax, purple; magnesium sulphate, pink; nickel chloride, cerise; ferric chloride, olive brown; copper sulphate, lilac precipitate; lead acetate, no change.

Triacetylerythroglaucin. Erythroglaucin (0.062 g.) was acetylated by the Peterson-West [1927] method by treatment at 37° for 16 hr. with 3 g. of a mixture of pyridine (5 parts) and acetic anhydride (1 part). Acetic anhydride equivalent to 6.1 ml. of *N*/10 NaOH was fixed. Theoretical for three acetylatable groups, 6.2 ml. The triacetyl-erythroglaucin separating during the estimation was crystallized from acetic acid in clusters of bright yellow rods (0.060 g.), m.p. 225°. (Found: C, 61.96, 61.82; H, 4.28, 4.09; CH₃O, 7.4, 7.2%. C₂₂H₁₈O₉ requires C, 61.95; H, 4.26; 1 CH₃O, 7.3%.)

Erythroglaucin trimethyl ether. A mixture of erythroglaucin (0.063 g.), K₂CO₃ (1 g.), acetone (20 ml.) and dimethyl sulphate (0.5 ml.) was refluxed for 2½ hr. K₂CO₃ (1 g.), acetone (10 ml.) and dimethyl sulphate (0.5 ml.) were now added and the mixture refluxed for a further 4 hr. The mixture was filtered, the acetone removed from the filtrate and the residue was washed with light petroleum and water. Wt. 0.058 g. It crystallized from a mixture of benzene and light petroleum (b.p. 80–100°) in yellow needles, m.p. 187–8°. (Found: C, 66.55, 66.55; H, 5.26, 5.36; CH₃O, 37.0%. C₁₉H₁₈O₆ requires C, 66.63; H, 5.30; 4 CH₃O, 36.3%.) The substance was quite insoluble in aqueous NaOH and gave a deep blue colour with cold conc. H₂SO₄. Erythroglaucin cannot be a monomethyl ether of cynodontin, a metabolic product of *Helminthosporium cynodontis* Marignoni [Raistrick *et al.* 1933] since erythroglaucin trimethyl ether is not identical with the *tetramethyl ether of cynodontin* which was prepared from cynodontin (0.1 g.) by the same method as is described above. Yield, 0.08 g. It was crystallized from benzene in orange needles, m.p. 233–4°, which are insoluble in aqueous NaOH and give a peacock blue colour with cold conc. H₂SO₄. (Found: C, 66.46, 66.51; H, 5.21, 5.35; CH₃O, 36.0%. C₁₉H₁₈O₆ requires C, 66.63; H, 5.30; 4 CH₃O, 36.3%.)

(B) *Physcion (emodin monomethyl ether, 4:5-dihydroxy-7-methoxy-2-methyl-anthraquinone)*. Physcion crystallizes when pure in long reddish-orange or brownish-orange needles, m.p. 203–4°, but appears to be dimorphic and tends, especially when slightly impure, to crystallize in large yellowish-orange lozenge-shaped plates. (Found: C, 67.61, 67.53; H, 4.20, 4.33; CH₃O, 10.6, 10.9%. Calc. for C₁₆H₁₂O₅, C, 67.57; H, 4.26; 1 CH₃O, 10.9%.) It is somewhat more soluble in organic solvents than is erythroglaucin. It is only slightly soluble in

light petroleum, somewhat more soluble in methanol, ethanol and ether, and readily crystallizes from ethyl acetate, glacial acetic acid, or a mixture of chloroform and ethanol. It gives a beautiful magenta colour in dilute solution in cold conc. H_2SO_4 , and since erythroglauca gives a pure blue colour with this reagent it is possible to estimate roughly by this means the proportions of physcion and erythroglauca present in mixtures of the two—a procedure which was used in their separation. Physcion gives a rose-pink solution with $2N$ NaOH from which a rose-pink precipitate slowly separates.

In order to establish the identity of the mould physcion a specimen of lichen physcion was prepared from an authentic sample of *Xanthoria parietina* (L.) (24 g.) kindly collected for us by Mr I. Mackenzie Lamb of the Natural History Museum, South Kensington, and by Dr W. Watson from a concrete post at Staplegrove, near Taunton, Somerset. The dried lichen was extracted with boiling light petroleum (B.P. $40-50^\circ$) and yielded 0.17 g. of crude product which on crystallization from glacial acetic acid gave pure physcion (0.1 g.), in yellowish-brown needles, M.P. $203-4^\circ$, not depressed on admixture with mould physcion. Since Raistrick *et al.* [1937] have already established, by direct comparison, the identity of mould physcion with Perkin & Hummel's [1894] emodin monomethyl ether from *Ventilago madraspatana* Gärtn. the identity of physcion from mould, lichen and climbing shrub follows.

Diacetylphyscion. A solution of mould physcion (0.02 g.) in acetic anhydride (1 ml.) containing a trace of conc. H_2SO_4 was boiled for a few minutes. The greenish-yellow solid obtained by pouring the solution into water was crystallized from ethanol. Fine yellow needles, M.P. $186-7^\circ$, unchanged on admixture with diacetylphyscion, M.P. $186-7^\circ$, prepared from lichen physcion. Perkin & Hummel [1894] give the M.P. as $185-6^\circ$.

(C) *Physcion anthranol A*, M.P. 260° (emodinanthranol monomethyl ether A). Physcion anthranol A was usually obtained from the mixture of mould colouring matters as colourless or very pale yellow needles which melt with decomposition at about 260° . (Found: C, 71.11, 71.01; H, 4.84, 4.89; CH_3O , 10.9, 11.3%. Calc. for $\text{C}_{16}\text{H}_{14}\text{O}_4$, C, 71.08; H, 5.22; $1\text{CH}_3\text{O}$, 11.5%.) It is almost insoluble in benzene, xylene and alcohol and because of its slight solubility it can be fairly readily isolated from mixtures of the other mould colouring matters. It dissolves in cold conc. H_2SO_4 with a golden-yellow colour which changes in about an hour to dark green at room temperature and very rapidly on heating. It is only slightly soluble in aqueous NaOH giving a yellow solution which is slowly oxidized in air to give a rose-pink precipitate of the sodium salt of physcion. It agrees closely in its analysis, M.P., solubilities and colour reactions with those recorded by Perkin & Hummel [1894, pp. 935, 936] for the anthranol A which they isolated from *Ventilago madraspatana*. Like the latter it also gives physcion on oxidation but we have not succeeded in obtaining physcion anthranol A by the reduction of physcion.

Oxidation of physcion anthranol A to physcion. Physcion anthranol A (0.02 g.) was oxidized with chromic acid in an exactly similar way to that described later in section (D) for the oxidation of physcion anthranol B. The crude oxidation product (0.015 g.) was purified by sublimation in a high vacuum at $130-40^\circ$ followed by crystallization from ethyl acetate. Yellowish brown needles, M.P. 203° , not depressed on admixture with lichen physcion from *Xanthoria parietina*.

(D) *Physcion anthranol B*, M.P. $181-2^\circ$ (emodinanthranol monomethyl ether B). Physcion anthranol B is dimorphic, being usually obtained from the mixture of mould colouring matters as fine hair-like needles, but also as larger lozenge-

shaped plates especially when in association with physcion. When isolated from the mixed mould colouring matters it is usually pale pink in colour, probably because of the presence of traces of physcion from which it is very difficult to separate. After several crystallizations the pinkish colour still remains and even persists after fractional sublimation in a high vacuum at 130°. It melts at 181–2°. (Found: C, 70.76, 70.71; H, 5.08, 5.20; CH_3O , 11.0, 11.45 %. Calc. for $\text{C}_{16}\text{H}_{14}\text{O}_4$, C, 71.08; H, 5.22; 1 CH_3O , 11.5 %.) It is slightly more soluble in most organic solvents than is physcion but is somewhat less soluble in benzene from which it is best purified. With cold conc. H_2SO_4 it gives a golden-yellow colour which changes in about an hour to deep green. This change is brought about rapidly on warming. It dissolves slowly and with difficulty in aqueous NaOH to give an orange-red solution.

Reduction of physcion to physcion anthranol B. Physcion (0.97 g., i.e. 0.43 g. from *A. mongolicus* and 0.54 g. from *A. mutabilis*) was dissolved in boiling glacial acetic acid (150 ml.) and zinc dust (5 g.) was added in small portions until the reduction was complete. The excess of zinc was separated and the acetic acid solution, on cooling, deposited 0.48 g. of pale yellow needles, m.p. 181–2°. The acetic acid mother liquors, on dilution with 3 vol. of water, gave a further 0.30 g. of crude anthranol which was crystallized from benzene, giving 0.14 g., m.p. 180–2°. A final crystallization from benzene raised the m.p. to 182–3° not depressed on admixture with physcion anthranol B isolated from the mixed mould colouring matters. Hesse [1895], who also prepared this anthranol by the reduction of physcion isolated from *Xanthoria parietina*, gives the m.p. as 180–2° while Perkin & Hummel [1894] give the m.p. of their anthranol B from *Ventilago madraspatana* as 173°.

Oxidation of physcion anthranol B to physcion. To a boiling solution of physcion anthranol B from *A. oriolus* (0.05 g.) in glacial acetic acid (1 ml.) were added 2.5 ml. of a boiling 1 % solution of chromic acid in the same solvent. The mixture was boiled for 2 min. and was then diluted with an equal volume of water. On cooling, crude physcion (0.029 g.) separated and was crystallized from ethyl acetate giving 0.02 g. of yellowish brown needles, m.p. 203°, not depressed on admixture with lichen physcion from *Xanthoria parietina*. The two specimens also gave the same magenta colour reaction with cold conc. H_2SO_4 . (Found: C, 67.42; H, 4.24 %. Calc. for $\text{C}_{16}\text{H}_{12}\text{O}_5$, C, 67.57; H, 4.26 %.)

SUMMARY

The colouring matters, extracted by light petroleum (b.p. 40–50°), and present in a number of species in the *Aspergillus glaucus* series, have been examined with special reference to the crude red colouring matters. Rubroglaucon, previously reported by Gould & Raistrick [1934] as occurring in certain species in the *A. glaucus* series, has now been shown to be a mixture of physcion (4:5-dihydroxy-7-methoxy-2-methylanthraquinone) and a hitherto undescribed colouring matter, *erythroglaucon*, which is the monomethyl ether of a tetrahydroxy-methylanthraquinone. In some of the species examined, erythroglaucon and physcion were accompanied by two reduction products of physcion, i.e. physcion anthranol A, m.p. 260° and physcion anthranol B, m.p. 181–2°. These anthranols were previously reported by Perkin & Hummel [1894] as being present, along with physcion, in the root-bark of the climbing shrub *Ventilago madraspatana*.

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