Studies in the Biochemistry of Micro-organisms

91. THE COLOURING MATTERS OF *PENICILLIUM ISLANDICUM* SOPP. PART 3. SKYRIN AND FLAVOSKYRIN

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In the first communication from this laboratory dealing with the colouring matters of *Penicillium islandicum* Sopp (Howard & Raistrick, 1949), it was shown that five out of the six strains of this species which were examined produced a hitherto undescribed colouring matter, islandicin, which was proved, by analytical methods, to be 1:4:5-trihydroxy-2-methylanthraquinone (I). This conclusion was confirmed by Joshi, Tilak & Venkataraman (1950) who prepared the compound by an unambiguous synthesis and showed that it was identical with natural islandicin. Re-examination of the sixth strain of this species (Howard & Raistrick, 1950), which bears the catalogue number N.R.R.L. 1175 and which is much more orange and less red in colour than the other five strains, failed to reveal any islandicin even when its mixed colouring matters were submitted to chromatographic analysis. It was shown however that the complex mixture of colouring matters present in the dried mycelium of this strain contained chrysophanol (chrysophanic acid) (II) and two other crystalline colouring matters which were not named or described at that time. It is the purpose of the present communication to describe the isolation and properties of these two substances, neither of which appears to have been described previously, and to discuss their structure and relationship to other fungal colouring matters. At the present time, a complete molecular structure cannot be assigned to either of them but so far as we are aware, no substances of similar properties have been described before and they appear to represent completely new chemical types:

When the dried and ground mycelium of the mould, a brown powder, was extracted with benzene at room temperature, a dark orange solution was obtained which contained numerous pigments. This solution was passed through columns of 'heavy' magnesium carbonate, and chrysophanol was readily isolated from the eluate. Development of the chromatograms with methanol produced *inter alia* an ochre-yellow band, from which was obtained a substance crystallizing as yellow needles, m.p. 215° (decomp.). We propose for it the name flavoskyrin* and shall describe it more fully later.

Further extraction of the mycelial powder with hot benzene in a Soxhlet apparatus removed a mixture of pigments similar in composition to that obtained with cold benzene, together with another substance which separated as a dark-brown powder on standing. This substance which when pure is dark-orange or yellow in colour, does not melt below 380°. We propose to call it skyrin. Further quantities of chrysophanol and flavoskyrin were isolated, as described above, from the hot benzene Soxhlet extracts after removal of crude skyrin, and further quantities of skyrin were obtained by subsequent exhaustive extraction of the mycelium with acetone. The amount of purified skyrin isolated was about 3% of the weight of the dried mould mycelium but

* Sopp (1912) isolated the type culture of *P. islandicum* from a mouldy specimen of skyr, a bacterially soured milk peculiar to Iceland. We have used the root 'skyr' to build up trivial names for the various new colouring matters of *P. islandicum*, qualifying it with Latin adjectives descriptive of the colour or appearance of the pigment concerned.

(I)
Islandicin

(II)
Chrysophanol

(III)
Emodin

the total amount present in it is undoubtedly much greater. The yields of flavoskyrin varied between 0.2 and 0.7 %.

SKYRIN

Skyrin, $C_{30}H_{18}O_{10}$, is dimorphic, crystallizing either as clusters of dark-orange, pointed rods or as hexagonal, yellow plates. Both forms darken but do not melt when heated to 380° . The molecule contains six hydroxyl groups which can be acetylated or methylated, two quinone groupings and two methyl groups attached to carbon. It contains no methoxyl groups. It is insoluble in 0.5N sodium bicarbonate but dissolves in N ammonia, N sodium carbonate and N sodium hydroxide to deep-purple coloured solutions. Skyrin exhibits very characteristic reactions with cold concentrated sulphuric acid, the two crystalline forms differing slightly in their behaviour towards this reagent. The orange crystals dissolve to a deep cherry-red solution, which immediately begins to turn brown and finally changes to a stable, emerald-green solution, the whole change taking about one minute. The yellow crystals dissolve to give the emerald-green solution immediately without the preliminary cherry-red colour. One of other of these two reactions with cold concentrated sulphuric acid is shown by all the derivatives of skyrin which have been prepared with the exception of the hexamethyl ether. The yellow form of the substance can be converted into the orange form by dissolving in alkali and acidifying, when orange material is precipitated which gives the red to green reaction in concentrated sulphuric acid. The green sulphuric acid solutions prepared from either form of skyrin yield material of the orange form when diluted with water. The two forms seem readily interconvertible in solution, but not in the solid phase.

The following functional derivatives of skyrin have been prepared. (a) Hexaacetylskyrin, $C_{48}H_{30}O_{16}$, i.e. $C_{30}H_{12}O_4(O.CO.CH_3)_6$, by heating with acetic anhydride and anhydrous sodium acetate. It forms thin yellow needles, m.p. $295-296^\circ$, and is optically inactive in chloroform solution. It is insoluble in 0.5N sodium bicarbonate and in N sodium carbonate, and is slowly soluble, with hydrolysis to skyrin, in cold aqueous N sodium hydroxide. (b) Skyrin hexamethyl ether, $C_{38}H_{30}O_{10}$, i.e. $C_{30}H_{12}O_4(OCH_3)_6$, by heating with dimethyl sulphate and anhydrous potassium carbonate in acetone solution. It forms an orange micro-crystalline powder on sublimation in a high vacuum at $300-310^\circ$ and melts at $370-372^\circ$. It is insoluble in alkalis; its solution in cold concentrated sulphuric acid is a stable, red-purple colour, which is indistinguishable from that of a solution, in the same reagent, of emodin trimethyl ether (i.e. the trimethyl ether of III). (c) Skyrin pyridine salt, $C_{40}H_{28}O_{10}N_2$,

i.e. $C_{30}H_{18}O_{10} \cdot 2C_5H_5N$, by crystallization from pyridine. It forms bright red, rhombohedral crystals which blacken without melting on heating.

Skyrin forms compounds with methyl and ethyl alcohols, either (a) on long standing in the alcoholic solution or, more rapidly, (b) by boiling a solution of hexaacetylskyrin in the alcohol containing concentrated sulphuric acid. Dimethylskylin, $C_{32}H_{22}O_{10}$, i.e. $C_{30}H_{16}O_8(OCH_3)_2$, prepared by method (b) forms orange-yellow plates which do not melt below 360° . Diethylskylin, $C_{34}H_{26}O_{10}$, i.e. $C_{30}H_{16}O_8(OC_2H_5)_2$, prepared by both methods (a) and (b) forms yellow plates from dioxan which do not melt below 360° . Unlike skyrin itself, both derivatives are insoluble in N sodium carbonate. Both dissolve in cold aqueous N sodium hydroxide to orange solutions which slowly become purple probably due to hydrolysis to skyrin. Both dissolve in cold concentrated sulphuric acid to give a green solution immediately. The nature of these two substances is not absolutely clear and must be left unsettled for the present. Despite their method of preparation they cannot be dimethyl or diethyl esters since skyrin does not contain any carboxyl groups. Both substances have some properties which suggest that they may be β -methyl ethers of a polyhydroxyanthraquinone, e.g. their insolubility in N sodium carbonate, although skyrin is readily soluble in this reagent. Typical β -methyl ethers of substituted anthraquinones, e.g. physcion, 4:5-dihydroxy-7-methoxy-2-methylanthraquinone, i.e. 7-methyl ether of (III), cannot however be prepared from the parent substance, except in extremely minute yields, by either method (a) or (b) above and are not hydrolysed to the parent substance by cold aqueous N sodium hydroxide.

Oxidation with chromic acid of hexaacetylskyrin in acetic anhydride-acetic acid solution results in the conversion of the two C-methyl groups into two COOH groups and the formation of the dibasic hexaacetylskyric acid, $C_{42}H_{26}O_{20}$, i.e. $C_{30}H_8O_8(O.CO.CH_3)_6$ as yellow, oblique-ended rods, m.p. $231-232^\circ$ (decomp.). Skyric acid, $C_{30}H_{14}O_{14} \cdot 3H_2O$, was prepared by alkaline hydrolysis of hexaacetylskyric acid and was obtained as bright orange-red rods which do not melt below 370° . Skyric acid, like skyrin, exists in two forms which show the same characteristic differences in their reactions with cold concentrated sulphuric acid. When a methanolic solution of hexaacetylskyric acid, containing concentrated sulphuric acid, was boiled, the dimethyl ester of skyric acid (dimethyl skyrate), $C_{38}H_{18}O_{14}$, i.e. $C_{28}H_{12}O_{10}(COOCH_3)_2$, was obtained as yellow, pointed rods which do not melt below 370° .

Our clearest insight into the molecular structure of skyrin was provided by the surprising discovery that when a cold alkaline aqueous solution of skyrin

is treated with sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) it rapidly undergoes reductive fission with more than a 90% quantitative formation of two molecules of *Frangula* emodin (III) according to the equation $\text{C}_{30}\text{H}_{18}\text{O}_{10} + 2\text{H} \rightarrow 2\text{C}_{15}\text{H}_{10}\text{O}_5$. This observation was reported by one of us in the Bakerian Lecture of the Royal Society (Raistrick, 1949). It was found later that skyrin acid on similar treatment is converted, though not quite so smoothly, into emodic acid (IV):

(IV)

Emodic acid

This experimental evidence is explained most logically by postulating that the molecule of skyrin consists of two emodin molecules linked together in such a way that both quinone groupings are intact and the six hydroxyl groups and two *C*-methyl groups are free. This inevitably means

that the two molecules of emodin are joined in skyrin by a carbon-carbon linkage, and that this linkage is broken almost quantitatively by treatment with sodium dithionite in cold, alkaline aqueous solution, an assumption that seemed highly improbable at the time when the experimental observation was made and reported on. However, Stoll, Becker & Helfenstein (1950), in one of a series of communications on sennosides A and B, the active glycosides of the laxative senna drugs of commerce, showed that these substances and their corresponding aglycones, the sennidins A and B, undergo reductive cleavage by catalytic hydrogenation in the presence of palladium at room temperature or, even better, by treatment of the esters of the glycosides or the salts of the aglycones with sodium dithionite.

In a review article by Stoll & Becker (1950) on 'Sennosides A and B, the Active Principles of Senna', the evidence, including synthetic evidence, is assembled on which they advance structure (V) for the stereoisomeric sennosides A and B and structure (VI) for the product of reductive fission which, as will be seen is derived from the substituted anthraquinone, rhein (VII).

(V)

Sennosides A and B

(VI)

8-Glucosido-1:8-dihydroxyanthrone-3-carboxylic acid

(VII)

Rhein

(VIII)

(IX)

It is clear from this evidence that, under certain conditions, the carbon-carbon linkage present in the substituted dianthrones, the sennosides A and B, can be readily broken by sodium dithionite. Assuming, as seems probable, that a carbon-carbon linkage in a substituted dianthraquinone can be broken by the same reagent, the problem of the constitution of skyrin resolves itself now into the difficult decision as to which of the free positions 1, 3, 6, 8 in each of the two molecules of emodin are joined together in the skyrin molecule. We have no experimental evidence available to decide this point so that, of the possible structures for skyrin, (VIII) and (IX) are advanced merely to indicate the type of structure which we believe skyrin to possess.

Either of these structures offers a satisfactory explanation of many of the properties of skyrin which we have recorded. Thus, the initial red colour of a solution of skyrin in cold concentrated sulphuric acid is very similar to that given by emodin itself, and it may well be that the final emerald-green colour of such skyrin solutions results from interaction between hydroxyl groups on the two emodin nuclei. In the case of the hexamethyl ether of skyrin, where such interaction is impossible, the sulphuric acid solution is indistinguishable in colour from that given by the trimethyl ether of emodin. The presence of two β -hydroxyl groups in the molecule of skyrin, one in each emodin moiety, explains the ready solubility of skyrin in aqueous N sodium carbonate, and also the formation of a pyridine salt containing two molecules of pyridine. The two β -hydroxyl groups also seem to be involved in the formation of the dimethyl and diethyl derivatives of skyrin described on p. 62, since these compounds are insoluble in aqueous N sodium carbonate. Finally, the preparations of hexa-acetylskyric acid and of dimethyl skyrate are exactly analogous to the oxidation of triacetyl-emodin to triacetylemodic acid by Fischer & Gross (1911) and the conversion of the latter substance into methyl emodate by Anslow, Breen & Raistrick (1940). Skyric acid, in which the two CH_3 groups present in skyrin are oxidized to two COOH groups, may thus be considered to be the emodic acid analogue of skyrin.

A number of mould colouring matters, having high melting points, are almost certainly polyhydroxydianthraquinones and some of them are clearly derivatives of emodin. Penicillipsin, from the mould *Penicillopsis clavariaeformis* Solms-Laubach (Oxford & Raistrick, 1940), has the empirical formula $\text{C}_{30}\text{H}_{24}\text{O}_8$ and melts at about 330° . On thermal decomposition it yields emodin anthranol, and on oxidation with nitric acid it gives tetranitroemodin. Aurofusarin, from *Fusarium culmorum* (W. G. Smith) Sacc. (Ashley, Hobbs & Raistrick, 1937), has the empirical formula

$\text{C}_{30}\text{H}_{20}\text{O}_{12}$ and does not melt below 360° . Little is known of its constitution except that it contains two methoxyl groups, and that its general properties are those of a polyhydroxydianthraquinone.

FLAVOSKYRIN

Flavoskyrin, the less abundant of the two colouring matters described in this communication, crystallizes readily from dioxan and acetic acid or from acetone and water, forming light-yellow needles, m.p. 215° (decomp.). Its molecular formula is probably $\text{C}_{16}\text{H}_{12}\text{O}_6$, though this is suggested somewhat tentatively since it has not yet been possible to prepare a crystalline derivative for confirmation. Flavoskyrin is insoluble in 0.5 N sodium bicarbonate but in N sodium carbonate and in N sodium hydroxide it dissolves to a yellow solution, the colour of which is similar to that of a solution of the free substance in organic solvents. A bright-yellow, uniform, stable band is formed when a solution is passed through a column of magnesium carbonate. The colour of the substance is not discharged either by sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) or by zinc and acetic acid.

When heated to its melting point flavoskyrin decomposes almost quantitatively into chrysophanol (II) and water according to the equation $\text{C}_{16}\text{H}_{12}\text{O}_6 \rightarrow \text{C}_{15}\text{H}_{10}\text{O}_4 + \text{H}_2\text{O}$. Chrysophanol is also formed, but in slightly lower yield, when flavoskyrin is dissolved in cold concentrated sulphuric acid; the solution is at first bright orange and changes during 2–3 hr. to a purple-red, as decomposition proceeds. Flavoskyrin was acetylated with acetic anhydride and pyridine at room temperature and was methylated with dimethyl sulphate and potassium carbonate in boiling acetone. Neither of the crude products so obtained could be crystallized.

In the present state of our knowledge speculation regarding the structure of flavoskyrin would be unprofitable. We believe however that, from its properties recorded above, of the many possibilities, it is improbable that flavoskyrin is either a substituted naphthaquinone, benzoylbenzoic acid or anthrahydroquinone. It is hoped to communicate further experimental evidence leading to a solution of the problem at a later date.

CHRYSOPHANOL

The facts that flavoskyrin is readily converted into chrysophanol and that both these substances have been isolated from the mycelium of *P. islandicum*, strain N.R.R.L. 1175, suggest the possibility that chrysophanol may be an artifact arising from flavoskyrin during the process of isolation, e.g. (a) during drying of the mycelium *in vacuo* at 40 – 42° . This possibility was disproved as follows. Fresh mycelium, without drying, was disintegrated at room temperature in a high-speed macerater in the

presence of benzene. Chrysophanol was found in the benzene extract. (b) During Soxhlet extraction of the dried mycelium with boiling benzene or boiling light petroleum, b.p. 40–60°. This possibility was disproved by the observation that chrysophanol was present in a benzene extract of the dried mycelium made at room temperature. (c) During chromatography on magnesium carbonate columns of the various solvent extracts. This was disproved by chromatographing pure flavoskyrin. A single, yellow band was obtained on magnesium carbonate, and prolonged washing with either benzene or methanol did not lead to the formation of a pink band below it as would happen if chrysophanol were being formed.

We consider therefore that at least part of the chrysophanol extracted from the mycelium is a true metabolic product of *P. islandicum*, strain N.R.R.L. 1175.

EXPERIMENTAL

Culture

The strain of *Penicillium islandicum* Sopp used in this work was received in December 1947 from the Thom-Raper collection from Dr Kenneth B. Raper, Northern Regional Research Laboratory (N.R.R.L.), U.S. Department of Agriculture, Peoria, Illinois, U.S.A., and bears his catalogue number N.R.R.L. 1175. It is the same strain as that from which chrysophanol was obtained by Howard & Raistrick (1950). Mature cultures of this strain are much more orange and less red in colour than a number of other strains of *P. islandicum* received from Dr Raper.

Cultural conditions

Culture medium. Czapek-Dox solution. Glucose, 50.0 g.; NaNO_3 , 2.0 g.; KH_2PO_4 , 1.0 g.; KCl , 0.5 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g.; distilled water, 1 l. Batches of 100 one-litre conical flasks, each containing 350 ml. of Czapek-Dox solution, were sterilized and inoculated with *P. islandicum*, strain N.R.R.L. 1175. Details of inoculation, incubation and harvesting of the mycelium were the same as those previously described (Howard & Raistrick, 1950). After 20–24 days' incubation at 24° the glucose content of the medium had fallen to 0.3% or less and the pH was about 4.1. The yield of dry mycelium was about 3.2 g./flask.

Isolation of the pigments

The dried and ground mycelium from a batch of 100 flasks, 320 g., was stirred with benzene (3.2 l.) at room temperature during 2 working days. The extract was separated by filtration from the mycelium which was then submitted to a second similar extraction. Tests on the twice-extracted mycelium showed it to contain only a negligible amount of chrysophanol.

The dark-orange benzene extracts were chromatographed on columns of heavy MgCO_3 . About 250 ml. of the first extract or 750 ml. of the second extract were passed through each column, 20 × 2.5 cm., followed by 100 ml. of fresh benzene. The mixture of pigments split into several bands which were, from top to bottom: dark-orange, narrow orange-red, broad yellow, narrow brown, pale purple and pale salmon-pink. The two extracts gave similar chromato-

grams, except that the second gave a much narrower yellow band. On washing with benzene the two lowest bands were eluted, the salmon-pink band as a yellow solution and the purple band as an orange solution. These two eluates were collected together and the isolation of chrysophanol from them is described on p. 64.

After the washing with benzene the columns were scraped out from below, leaving in place the two upper bands, and fresh MgCO_3 was packed into the spaces so made. Washing with methanol (100 ml.) developed the chromatogram into four bands which were from top downwards: (i) a narrow, greyish purple band, (ii) a broad ochre-yellow band, (iii) a pale purple band, and (iv) a bright yellow band. Bands (i), (iii) and (iv) were discarded. The broad ochre-yellow band (ii) was separated mechanically and, while still moist with methanol, was added with stirring to ice-cold, aqueous 2N-HCl. The brown suspension was extracted twice with large volumes of ether. *Flavoskyrin* (0.45 g.) separated as large yellow needles when the light brown-yellow ethereal solutions were concentrated.

The mycelium, after extraction with benzene in the cold as described above, was re-extracted with the same solvent in a Soxhlet for 5 hr., the solvent being changed at the end of each hour. The five orange-brown solutions so obtained were set aside for a few days and the reddish brown solid that had precipitated was collected as fraction A (4.91 g.) which contained *skyrin* and a colourless substance. The benzene filtrate from fraction A was chromatographed in the same way as is described above for the cold benzene extracts and gave a similar series of bands on the columns. The ochre-yellow bands, treated as described above, yielded flavoskyrin (1.70 g.). Chrysophanol was also recovered from the yellow eluates from these columns (see p. 64).

The benzene-extracted mycelium was now dried and the remainder of the colouring matters present in it were removed by extraction in a Soxhlet with two lots of acetone (5 hr., 7 hr.). The first extract was an intense-brown colour, the second rather lighter. The two solutions were evaporated to 500 and 250 ml. respectively, and the dirty, amorphous material which separated was removed (5.4 g.). The combined brown filtrates, evaporated to dryness *in vacuo*, yielded a very sticky, brown solid as fraction B (24.3 g.), consisting mainly of *skyrin*.

Purification of crude *skyrin*

Neither of the two crude fractions of *skyrin* (fractions A and B) could be purified by direct crystallization but were conveniently purified through the acetate. Thus fraction A (1 part) was added slowly to a mixture of acetic anhydride (10 parts by vol.) and fused sodium acetate (2 parts) at 100°. The greenish brown mixture was maintained at this temperature for 2 hr. with frequent shaking and was then cooled, poured into ice-water with stirring and gave crude *hexaacetylskyrin* as a pale yellow-green solid (1.3 parts). A portion of this (1.0 g.) crystallized from glacial acetic acid (8 ml.) as greenish yellow needles (0.63 g.) which on further purification melted at 293–295°.

Fraction B on acetylation in the same way as fraction A and with the same proportions of reagents gave an almost black solid on pouring into ice-water. This material contained a little fat which was separated from the dried, crude acetate by washing it with light petroleum (b.p. 40–60°). The washed crude acetate (26.2 g.) obtained from 24.3 g. of fraction B crystallized from glacial acetic acid (105 ml.) to

give hexaacetylskyrin as greenish yellow needles (6.7 g.), m.p. 292–294° after further purification. The acetates prepared from fractions A and B were identical in properties and gave no depression in melting point on admixture.

The acetate as prepared above had a slightly greenish yellow colour and still contained a trace of material which was readily soluble in cold aqueous $N-NaOH$. It was readily purified as follows. A portion (0.50 g.) was dissolved in $CHCl_3$ (50 ml.) and the slightly green-yellow solution was shaken with aqueous $N-NaOH$ (3×20 ml.). The dark to light purple extracts were discarded and the now orange-yellow $CHCl_3$ solution was washed with water, filtered and evaporated to 4 ml. Warm ethanol (25 ml.) was added with shaking causing the rapid formation of clusters of fine yellow needles of hexaacetylskyrin (0.40 g.), which appear pure yellow in bulk, m.p. 294–295° with darkening. Recrystallization from $CHCl_3$ -ethanol in a similar manner raised the melting point to a constant figure of 295–296°.

Pure hexaacetylskyrin (0.20 g.) was hydrolysed in a stream of N_2 by heating with aqueous $N-NaOH$ (20 ml.) for 1 hr. The purple solution was cooled, acidified with $2N-HCl$ (15 ml.) and extracted with ether (300 ml.). The dark-orange ethereal solution was washed with water, filtered and evaporated to 10 ml. Skyrin separated as clusters of reddish orange, pointed rods (0.11 g.), melting point above 380°. (Found, on material dried in a high vacuum at 150°: C, 66.7, 66.7; H, 3.5, 3.4; $C-CH_3$, 7.6. $C_{30}H_{18}O_{10}$ requires C, 66.9; H, 3.4; $2C-CH_3$, 5.6%.)

General properties of skyrin

Skyrin as prepared above slowly sublimes, with decomposition, in a high vacuum at 300–320°. Its ethanolic solution gives an intense brown-red colour with $FeCl_3$, which is almost unchanged on addition of water. It dissolves in cold conc. H_2SO_4 to give an initially deep cherry-red solution. Within a few seconds of solution taking place the colour becomes browner and finally clears to a stable emerald green, the whole change taking about 1 min. Skyrin is slightly soluble in dioxan and acetone and very slightly soluble in ether, ethanol, $CHCl_3$ and glacial acetic acid. It is insoluble in 0.5N- $NaHCO_3$ but dissolves readily in $N-NH_3$, $N-Na_2CO_3$ and $N-NaOH$ giving deep-purple solutions which are stable in air. When portions of an ethereal solution of skyrin were shaken with equal volumes of 0.2M borate or phosphate buffers the following changes were observed. The aqueous layer was colourless at pH 8.2 or less, very pale purple at pH 8.5 and strong purple at pH 9.0. At pH 10.0 or above the ether layer was quite colourless. An acetone solution of skyrin, passed through a column of MgO , gives a single reddish purple band which spreads slightly and becomes bluer when washed with methanol.

Skyrin is not readily crystallized from any of the solvents tested and the preparation of pure crystalline material is made more difficult by the fact that skyrin occurs in two crystalline forms which give somewhat different reactions with cold conc. H_2SO_4 . It is unusual for either form to separate from a solvent unaccompanied by at least traces of the other form. It has already been noted that amorphous skyrin, obtained by hydrolysis of its acetate, when crystallized from ether gives reddish orange, pointed rods. These crystals, as well as amorphous skyrin, when dissolved in cold conc. H_2SO_4 give a solution which is initially deep cherry red changing in about 1 min. to a stable emerald green. On the other hand, when skyrin is crystallized by evaporating a hot

saturated solution in either ethanol (ochre-yellow, pointed rods) or acetone (brownish yellow, elongated hexagonal plates) the resulting crystals in each case give an immediate emerald-green solution in cold conc. H_2SO_4 .

The two forms of skyrin are readily interconvertible. Thus skyrin obtained by crystallization from ether and giving the red to green reaction with cold conc. H_2SO_4 , when crystallized from acetone was obtained as yellow plates giving the immediate emerald-green H_2SO_4 colour. Similarly, when the crystals obtained from acetone were dissolved in aqueous $N-NaOH$ and the purple solution was immediately acidified and extracted with ether, red-orange rods were obtained on evaporation of the ethereal solution. These crystals dissolved in cold conc. H_2SO_4 to a solution initially red and changing to green in 1 min.

Derivatives of skyrin

Hexaacetylskyrin. The preparation of pure hexaacetylskyrin, m.p. 295–296°, is described on p. 60. (Found, on material dried at 100° in a high vacuum: N, S, halogens and OCH_3 , nil; C, 63.4, 63.3; H, 3.9, 3.8; CH_3CO , 31.6, 31.8; mol.wt. (cryoscopic in camphor), 807, 814, 813; optical rotation, nil ($c=0.635$ in $CHCl_3$). $C_{42}H_{30}O_{16}$ requires C, 63.8; H, 3.8; $6CH_3CO$, 32.7%; mol.wt. 791.) Hexaacetylskyrin crystallizes as pale-yellow, pointed needles which are very readily soluble in $CHCl_3$, readily soluble in acetone, and slightly soluble in dioxan, benzene, ether and glacial acetic acid. It does not dissolve in 0.5N- $NaHCO_3$ or in $N-Na_2CO_3$ but dissolves slowly in cold aqueous $N-NaOH$ to a purple solution. Its solution in cold conc. H_2SO_4 is deep cherry red at first passing through brown to emerald green in approx. 1 min. Its ethanolic solution gives no colour with $FeCl_3$. It does not sublime in a high vacuum below 220°.

Hydrolysis of acetate and re-acetylation. Dried hexaacetylskyrin (0.9818 g.) was heated on the boiling-water bath for 3 hr. in a stream of N_2 with aqueous $NaOH$ (100 ml., 0.9964N). $N-H_2SO_4$ (110 ml.) was then added from a burette to the dark-purple solution and the flask was kept in the boiling water for 10 min. to coagulate the orange precipitate. When cool this was collected by filtration, washed and dried (0.653 g.; theor. 0.679 g.). The filtrate and washings were titrated to phenolphthalein with the standard $NaOH$ used for hydrolysis. 17.62 ml. $NaOH$ were required, corresponding to 31.6% CH_3CO in the substance. The acetic acid in this neutralized solution was determined by a distillation process; net titre, 7.21 ml. $N-NaOH \equiv CH_3CO$ 31.6%. A portion of the crude orange pigment (0.03 g.) was dissolved in acetic anhydride containing 2% v/v, conc. H_2SO_4 (1 ml.) and heated to 100° for 5 min. The product recovered by pouring into water was crystallized twice from $CHCl_3$ and ethanol (0.5 + 10 ml.), yielding fine yellow needles (0.02 g.), m.p. 294–296° not depressed on admixture with hexaacetylskyrin, m.p. 295–296°.

Skyrin pyridine salt. Skyrin (0.30 g.) dissolved readily in hot pyridine (10 ml.) to a red-brown solution from which, on cooling, bright-red, rhomboidal crystals were deposited (0.31 g.). These were recrystallized from pyridine (10 ml.) to give skyrin pyridine salt (0.19 g.). (Found on material dried in a high vacuum at room temperature: C, 68.6; H, 4.5; N, 4.3. $C_{40}H_{28}O_{10}N_2$, i.e. $C_{30}H_{18}O_{10} \cdot 2C_5H_5N$, requires C, 69.0; H, 4.1; N, 4.0%.) The substance blackens without melting when heated. Its reactions with alkalis are the same as those of the parent pigment and in cold conc. H_2SO_4 it gives a red solution, changing to green.

Dimethylskyrin. Hexaacetylskyrin (0.50 g.) was boiled under reflux for 2 hr. with methanol (100 ml.) and conc. H_2SO_4 (3 ml.). A greenish yellow solid began to separate after 20 min., and at the end of the reaction period the mixture was cooled and the precipitate was collected (0.37 g.). A portion of this (0.17 g.) was crystallized from dioxan (70 ml.) to give *dimethylskyrin* as orange-yellow, rhomboidal plates (0.09 g.) which blacken on heating but do not melt below 360° . (Found on material dried in a high vacuum at 150° : C, 67.3; H, 4.1; OCH_3 , 11.1. $\text{C}_{32}\text{H}_{21}\text{O}_{10}$ requires C, 67.8; H, 3.9; 2OCH_3 , 11.0%.) Dimethylskyrin crystallizes unchanged from pyridine but is partially hydrolysed in hot glacial acetic acid. It is readily soluble in CHCl_3 but is almost insoluble in methanol, acetone and ethyl acetate. It is insoluble in cold aqueous $\text{N-Na}_2\text{CO}_3$, but dissolves readily in aqueous N-NaOH to an orange solution which quickly passes through red and brown to purple. Material recovered from this purple solution dissolves in $\text{N-Na}_2\text{CO}_3$ and conc. H_2SO_4 to give solutions identical in colour with those of skyrin. Dimethylskyrin dissolves in cold conc. H_2SO_4 to give a green solution immediately.

Diethylskyrin. Hexaacetylskyrin (1.0 g.) was boiled under reflux for 2 hr. with ethanol (150 ml.) containing conc. H_2SO_4 (4.5 ml.). The solid dissolved to a dark-orange solution from which a light-brown powder precipitated on further heating. This solid was collected (0.73 g.), treated with charcoal in CHCl_3 solution and precipitated by the addition of ethanol (5 vol.). The product was crystallized from dioxan (70 ml.) giving *diethylskyrin* (0.49 g.) as yellow rhomboidal plates appearing slightly greenish in bulk. (Found on material dried in a high vacuum at 160 – 170° : C, 68.3, 68.3; H, 4.4, 4.5; OC_2H_5 , 15.2, 15.35. $\text{C}_{34}\text{H}_{26}\text{O}_{10}$ requires C, 68.7; H, 4.4; $2\text{OC}_2\text{H}_5$, 15.2%.) On heating, diethylskyrin blackens in the neighbourhood of 300° , but does not melt below 360° . It is similar in general properties and colour reactions to the methyl analogue described above. Diethylskyrin was also formed when a solution of skyrin (0.27 g.) in boiling ethanol (300 ml.) was cooled and left in the refrigerator for 2 weeks.

Hexamethylskyrin. A mixture of skyrin (0.37 g.), dry acetone (100 ml.), dimethylsulphate (3.7 ml.) and anhydrous K_2CO_3 (7.4 g.) was boiled under reflux for 5 hr. The mixture was concentrated and poured into water (1 l.) containing 2N-HCl (100 ml.). The amorphous, orange solid which separated (0.42 g.) was dissolved in CHCl_3 and freed from traces of incompletely methylated material by shaking with dilute NaOH . The remaining alkali-insoluble material could not be crystallized but was purified by sublimation in a high vacuum at 300 – 310° to give *hexamethylskyrin* as a micro-crystalline orange powder, m.p. 370 – 372° . (Found: C, 68.8, 68.9; H, 4.8, 4.85; OCH_3 , 28.2; C-CH_3 , 3.3. $\text{C}_{36}\text{H}_{30}\text{O}_{10}$ requires C, 69.5; H, 4.9; 6OCH_3 , 29.9; 2C-CH_3 , 4.8%.) Demethylation of the substance in a Zeisel estimation proved to be very difficult. Hexamethylskyrin is sparingly soluble in most solvents but very readily dissolves to an orange solution in CHCl_3 . It is insoluble in alkalis but in cold conc. H_2SO_4 it dissolves to a stable red-purple solution, indistinguishable in colour from that given by trimethyl-emodin.

Oxidation of skyrin. Isolation of skyric acid

A solution of hexaacetylskyrin (2.0 g.) in glacial acetic acid (40 ml.) and acetic anhydride (40 ml.) was maintained at 57 – 58° . A solution of CrO_3 (4.0 g.) in water (3.2 ml.) and glacial acetic acid (40 ml.) was added in small portions

during 0.5 hr., in which time the solution became green-brown. After heating for a further 3 hr. at 66 – 68° the pure green solution was cooled and poured into cold water (1.5 l.) with stirring. The pale green-yellow precipitate (1.5 g.) was separated by filtration after stirring for 2 hr. When the original method of Fischer & Gross (1911) was used, i.e. pouring the reaction mixture into hot water and allowing to stand overnight, the yield from 2.0 g. starting material was 1.24 g.

The crude product was freed from a trace of incompletely oxidized material by shaking a solution of it in CHCl_3 (250 ml.) with 0.25 N-NaHCO_3 (250, 100 ml.). The extracts were quickly acidified, the orange precipitate was collected (1.27 g.) and re-acetylated by heating with acetic anhydride (6 ml.) containing conc. H_2SO_4 (two drops) at 100° for 5 min. Yield 1.38 g.

A portion of the re-acetylated material (0.61 g.) was crystallized three times from methanol (40, 62, 55 ml.), yielding *hexaacetylskyric acid* (0.35 g.) as yellow oblique-ended rods m.p. 231 – 232° with effervescence and blackening. (Found on material dried in a high vacuum at 100° : C, 59.0; H, 3.1. $\text{C}_{42}\text{H}_{26}\text{O}_{20}$ requires C, 59.3; H, 3.1%.) Crystallization from glacial acetic acid was found to cause slight hydrolysis, leading to an orange-yellow product, m.p. 194 – 200° . Hexaacetylskyric acid dissolves in conc. H_2SO_4 to an initially deep cherry-red solution which changes during about 4 min. through brown to green. It dissolves slowly in 0.5 N-NaHCO_3 to an orange solution changing to red in a few hours. It dissolves readily in $\text{N-Na}_2\text{CO}_3$, the colour changing from orange to red in about 5 sec. and then to purple during several hours. The solution of the substance in N-NaOH is initially red and changes to purple in 1 min.

Hexaacetylskyric acid (0.73 g.) was hydrolysed under N_2 with aqueous N-NaOH (100 ml.) on the boiling-water bath for 1 hr. 2N-HCl (75 ml.) was added to the purple solution and the mixture was heated again to coagulate the precipitate, which changed from orange to ochre-yellow during the heating. When separated, the crude skyric acid was obtained as an amorphous, ochre-yellow powder (0.57 g.) which did not melt below 370° . It was almost insoluble in nearly all organic solvents and could not be crystallized. It dissolved in 0.5 N-NaHCO_3 to a purple-red solution and in $\text{N-Na}_2\text{CO}_3$ and N-NaOH to a purple solution. It gave a green solution at once when dissolved in cold conc. H_2SO_4 .

Skyric acid was obtained crystalline as follows. Hexaacetylskyric acid (0.05 g.) was hydrolysed as described above and the cooled alkaline solution was acidified and extracted with ether (250 ml.). Bright orange-red rods and lozenge-shaped crystals (0.03 g.) which do not melt below 370° were obtained by evaporation of the ether extract (cf. the preparation of crystalline skyrin, p. 61). (Found on material dried in a high vacuum at 130° : C, 55.0; H, 3.7. $\text{C}_{30}\text{H}_{20}\text{O}_{17}$, i.e. $\text{C}_{30}\text{H}_{14}\text{O}_{14} \cdot 3\text{H}_2\text{O}$, requires C, 55.2; H, 3.1%.) This material gave colour reactions with alkalis which were identical with those given by the amorphous material prepared as described above, but in cold conc. H_2SO_4 it dissolved to a red solution which changed through brown to green in about 4 min.

Dimethyl skyrate. Hexaacetylskyric acid (0.50 g.) was boiled under reflux with methanol (25 ml.) containing 3%, v/v, H_2SO_4 . The substance dissolved and the yellow solution quickly turned orange. An orange crust, probably skyric acid, formed on the walls of the flask during the first hour. This slowly dissolved and the mixture became a lighter

colour as boiling continued. After boiling for 5 hr. the mixture was cooled and the amorphous yellow solid which separated was collected (0.30 g.). A portion of this (0.20 g.) was boiled with dioxan (20 ml.) and the cloudy solution clarified by filtration through kieselguhr. Addition of methanol (20 ml.) to the clear filtrate yielded *dimethyl skyrate* as yellow oblique-ended rods (0.09 g.) which blacken on heating above 200° but do not melt below 370°. (Found on material dried to constant weight in a high vacuum at 120°: C, 61.0; H, 3.4; OCH₃, 10.4. C₂₂H₁₈O₁₄ requires C, 61.3; H, 2.9; 2 OCH₃, 9.9%.) Dimethyl skyrate is insoluble in 0.5N-NaHCO₃ but dissolves in N-Na₂CO₃ or N-NaOH to orange solutions that quickly change through red to purple. It gives an immediate green colour when dissolved in cold conc. H₂SO₄.

*Reductive cleavage of skyrin. Isolation of
Frangula emodin*

Vacuum-dried amorphous skyrin (0.5918 g.), prepared by alkaline hydrolysis of hexaacetylskyrin, was dissolved in aqueous N-Na₂CO₃ (100 ml., accurately measured), and a solution of sodium dithionite, Na₂S₂O₄ (10 g.), in water (50 ml.) was added through a filter. After gentle shaking for 5 min. a volume of N-HCl equivalent to the Na₂CO₃ present was cautiously added. The orange solid thus precipitated was collected on a pad of kieselguhr, washed with water and dilute HCl, and dried. The product was separated by extraction of the kieselguhr in a Soxhlet, first with ether (30 hr.) and then with methanol (8 hr.). The dried ether extract (0.497 g.) was dissolved in the methanol extract and three crops of crystals (total weight 0.573 g.) were obtained by evaporation. Removal of the solvent from the final mother liquor left a sticky, brown solid (0.093 g.).

The crystalline material and the dried mother liquor were sublimed in a high vacuum at 175–185° giving orange sublimate, total weight 0.5357 g., equivalent to 90.5% of the starting material. The loss in weight on sublimation is approximately equivalent to 1 mol. methanol/1 mol. emodin. The sublimate was crystallized from CHCl₃ (300 ml.) and toluene and was resublimed in a high vacuum giving a product, m.p. 255–256°, not depressed on admixture with authentic *Frangula emodin*, m.p. 256°. (Found on sublimed material: C, 66.4; H, 3.7. Calc. for C₁₅H₁₀O₆: C, 66.7; H, 3.7%.)

A portion of the sublimate (0.26 g.) was methylated by boiling in acetone (60 ml.) with dimethyl sulphate (2.6 ml.) and anhydrous K₂CO₃ (5.2 g.). The methylated product was crystallized from ethanol (40 ml.) yielding glistening yellow needles (0.19 g.), m.p. 225°, either alone or mixed with authentic trimethylemodin, m.p. 225°. (Found on material dried in a high vacuum at 90°: C, 69.1, 69.3; H, 5.1, 5.3; OCH₃, 29.8, 29.7. Calc. for C₁₈H₁₄O₆: C, 69.2; H, 5.2; 3OCH₃, 29.8%.)

When a solution of emodin in N-Na₂CO₃ was treated with Na₂S₂O₄ the colour of the solution changed from red to orange and emodin could be isolated unchanged from this solution by extraction with CHCl₃.

*Reductive cleavage of skyric acid.
Isolation of emodic acid*

Vacuum-dried amorphous skyric acid (0.399 g.) was dissolved in N-Na₂CO₃ (100 ml.) by warming. To the cooled solution was added a filtered solution of Na₂S₂O₄ (7 g.) in

water (70 ml.). The colour changed from purple to dark orange. After 10 min. N-HCl (100 ml.) was cautiously added and the resulting brown-orange precipitate was collected on a pad of kieselguhr. When dry the product was extracted from the kieselguhr by boiling repeatedly with glacial acetic acid. The acetic acid solution was evaporated to dryness and sublimed in a high vacuum. The sublimate was shaken with CHCl₃ (150 ml.) and 0.25N-NaHCO₃ (350 ml.). The NaHCO₃-soluble fraction was recovered by acidification (0.272 g. = 68% of starting material). It crystallized from glacial acetic acid as long, dark-orange needles, m.p. 363–365° alone or mixed with authentic emodic acid, m.p. 363–365°. When dried in a high vacuum at 200°, the crystals lost 1 mol. acetic acid. (Found, loss on drying = 17.2%. Calc. for C₁₅H₈O₇, C₂H₄O₃ = 16.7%. Found on dried material: C, 60.5; H, 2.8. Calc. for C₁₅H₈O₇: C, 60.0; H, 2.7%.)

To confirm the identity of the product it was converted successively into its methyl ester and its methyl ester triacetate, following the procedure of Anslow *et al.* (1940). The methyl ester was thus obtained as fine, orange needles, m.p. 266–267° alone or mixed with authentic methyl emodate, m.p. 267–268°. The methyl ester triacetate was prepared as yellow needles, m.p. 188–189° alone or mixed with authentic methyl triacetylemodate of the same melting point. (Found on material dried in a high vacuum at 70°: C, 59.3; H, 3.7; OCH₃, 6.85. Calc. for C₂₂H₁₈O₁₀: C, 60.0; H, 3.7; 1 OCH₃, 7.05%.)

Flavoskyrin

The separation of flavoskyrin from mycelial extracts as orange- or green-yellow needles, m.p. about 200° (decomp.), has already been described (see p. 60). This crude material (0.20 g.) was purified by dissolving in warm dioxan (25 ml.). The solution was filtered, glacial acetic acid (25 ml.) was added and the dark-orange solution was concentrated *in vacuo* to 15 ml. Orange-yellow needles (0.13 g.) separated and were recrystallized by the same method giving *flavoskyrin* (0.10 g.) as yellow needles, m.p. 214–215° (decomp.). A portion of this material was further purified for analysis by crystallization from acetone plus water giving yellow needles, m.p. 215° (decomp.). (Found on material dried to constant weight in a high vacuum at 150°: C, 66.1, 66.2; H, 4.45, 4.5. C₁₅H₁₂O₆ requires C, 66.2; H, 4.4%.)

Flavoskyrin crystallizes as yellow needles, m.p. 215°, with vigorous effervescence, the residue setting to a dark-orange, partly crystalline solid. Flavoskyrin is fairly readily soluble in dioxan, less readily soluble in acetone, CHCl₃, ethanol, methanol or glacial acetic acid, and only slightly soluble in benzene and ether. Its solutions in organic solvents are bright yellow in colour.

Flavoskyrin is very slightly soluble in 0.5N-NaHCO₃ but dissolves readily in N-Na₂CO₃ and N-NaOH to yellow solutions which turn slightly more orange on standing. Portions of an ethereal solution were shaken with equal volumes of 0.2M borate or phosphate buffers. At pH 6.0 and below the aqueous layer was colourless; at pH 7.2 it was pale yellow and at pH 7.4 the colours of the aqueous and ether layers were of equal intensity; at pH 8.2 the ether layer was pale yellow and at pH 8.5 and above the flavoskyrin was wholly in the aqueous layer. Chromatography in acetone solution on MgCO₃ gives a single, uniform, bright-yellow band, the colour of which is stable over long periods, and which shows no dissociation into two bands on prolonged washing.

Flavoskyrin dissolves in cold conc. H_2SO_4 to an initially bright-orange solution which changes during 2 hr. to purple-red. Addition of FeCl_3 to an ethanolic solution of flavoskyrin produces an olive-brown colour. Flavoskyrin appears to be unaffected by $\text{Na}_2\text{S}_2\text{O}_4$ either in the solid state or dissolved in CHCl_3 or in aqueous Na_2CO_3 . No change in colour takes place when Zn dust is added to its solution in glacial acetic acid.

The molecular weight of flavoskyrin. In attempting to determine the molecular weight of flavoskyrin, the usual cryoscopic methods (camphor, dioxan) were found to be inapplicable, because of the thermal instability or the low solubility of the substance. An approximate value was obtained by the isothermal distillation method of Barger (1904), using azobenzene as standard and dioxan as solvent. Here again, however, the low solubility was a handicap, the molar concentrations attainable being only about one-quarter of the lowest used by Barger. Many observations suggest a value of about 350 for the molecular weight of flavoskyrin. (Theory for $\text{C}_{15}\text{H}_{10}\text{O}_5$, 272; for $\text{C}_{30}\text{H}_{20}\text{O}_{10}$, 544.)

Acetylation of flavoskyrin. Flavoskyrin was acetylated with pyridine and acetic anhydride (2:1, v/v) at room temperature for 3 days. The creamy orange acetylation product, m.p. 205–215°, could not be crystallized. It was insoluble in N-NaOH except on standing and did not sublime or appear to decompose when heated to 230° in a high vacuum.

Methylation of flavoskyrin. Treatment of flavoskyrin with dimethyl sulphate and K_2CO_3 in boiling acetone for 7 hr. yielded a yellow powder, m.p. 125–136°, which could not be purified. It was insoluble in N-NaOH and gave a stable red solution in cold conc. H_2SO_4 .

Decomposition of flavoskyrin. Isolation of chrysophanol

(a) *Thermal decomposition.* Dried flavoskyrin (47.9 mg.) was heated in a wide test tube in a stream of N_2 , freed from O_2 and CO_2 , and the issuing gas was led through 0.2N-Ba(OH) $_2$. Wet filter paper was wrapped round the upper parts of the tube while the contents of the tube were heated in an oil bath, first at 205° for 45 min. and then at 212° for 15 min. No CO_2 was evolved, but an orange sublimate had collected on the cooler upper part of the tube. The tube was now connected to a high vacuum pump and the decomposed residue was heated at 130°. By this means the decomposition product remaining in the residue was sublimed out alongside the sublimate formed during the reaction. (The equation $\text{C}_{15}\text{H}_{10}\text{O}_5 \rightarrow \text{C}_{15}\text{H}_{10}\text{O}_4 + \text{H}_2\text{O}$ requires $\text{C}_{15}\text{H}_{10}\text{O}_4$, 93.4; H_2O , 6.6%. Found: total weight of sublimate, 41.8 mg. = 87.3% of starting material; loss in weight during decomposition (H_2O), 3.2 mg. = 6.7%. The yield of sublimate is therefore 93.5% of the theoretical.) The sublimate was freed from a trace of Na_2CO_3 -soluble material and was then crystallized from ethanol (13 ml.) to give golden leaflets (0.04 g.), m.p. 193–194°, raised, by further crystallization from the same solvent, to 194–195° alone or mixed with authentic chrysophanol of the same melting point. (Found on substance dried in a high vacuum at 80°: C, 71.2; H, 4.0. Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_4$: C, 70.9; H, 4.0%.)

(b) *Decomposition by conc. H_2SO_4 .* Dried flavoskyrin (101.1 mg.) was dissolved in cold conc. H_2SO_4 (5 ml.) and the dark red-orange solution was held at room temperature for 4 hr. with occasional shaking. The colour changed during the first 2 hr. to an intense red-purple. At the end of the

reaction period the solution was rinsed into ice-water (150 ml.) and the bright orange-yellow precipitate was extracted into ether (3 $\times$ 200 ml.). The ethereal solution was shaken with 0.5N- Na_2CO_3 (2 $\times$ 50 ml.) to remove a little by-product, and the material remaining in the ether was sublimed in a high vacuum at 130°. (Yield of sublimate, m.p. 191–194°, 65.8 mg. = 69% of theoretical.) Crystallization of the sublimate from ethanol (21, 20 ml.) yielded chrysophanol as golden leaflets (44 mg.), m.p. 194–195° alone or mixed with authentic chrysophanol of the same melting point. (Found on material dried in a high vacuum at 70°: C, 71.1; H, 3.9. Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_4$: C, 70.9; H, 4.0%.) The ether-extracted dilute H_2SO_4 reaction mixture, which was pale yellow in colour becoming purple on making alkaline, obviously contained water-soluble material which probably consists of anthraquinonesulphonic acids formed as by-products during the decomposition.

Chrysophanol. Isolation from mould mycelium

(a) The chromatography of the benzene extracts of the dried mycelium of *P. islandicum*, N.R.R.L. 1175, both that obtained by extraction in the cold and that from hot extraction in a Soxhlet, has already been described (see p. 60). The coloured eluates obtained in each case contained chrysophanol which was isolated in the following manner. The eluate was shaken very gently with dilute aqueous NaOH and the extracts were quickly acidified. The regenerated pigments, now free from fat, were rechromatographed in benzene on MgCO_3 , giving a clean homogeneous lower band, salmon-pink in colour, from which almost pure chrysophanol was obtained by acidification.

The material from the cold benzene extracts (0.36 g.) was crystallized from ethanol (120 ml.) and then from benzene (10 ml.) as orange leaflets, m.p. 194–195° alone or mixed with authentic chrysophanol of the same melting point. (Found on material sublimed in a high vacuum at 130°: C, 70.7; H, 3.9. Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_4$: C, 70.9; H, 4.0%.)

The material from the hot Soxhlet benzene extracts (0.56 g.) was crystallized from benzene (14 ml.) and sublimed in a high vacuum at 130°. M.p. and mixed m.p. 194–195°. (Found: C, 71.3; H, 3.9. Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_4$: C, 70.9; H, 4.0%.)

(b) *P. islandicum*, strain N.R.R.L. 1175, was grown for 24 days on five 1 l. conical flasks each containing 350 ml. of Czapek-Dox solution. The mycelia were then separated from the culture fluid, washed with water and disintegrated in a Top Drive Macerater (Townson and Mercer, Croydon) for 10 min. with benzene (500 ml.). The benzene was separated by filtration and the pulp was extracted twice more with benzene in the macerater. The three dark-orange extracts were combined, washed, dried over Na_2SO_4 and passed through a column of heavy MgCO_3 (3.5 $\times$ 38 cm.). After washing with benzene the salmon-pink band was separated mechanically, dried in air, acidified with 2N-HCl, and the suspension was extracted with ether. Chrysophanol was separated from accompanying fat by shaking the ethereal solution with 0.5N-NaOH (2 $\times$ 50 ml.) and was then regenerated by acidification of the alkaline extract. The orange solid so obtained was sublimed in a high vacuum at 130° and the sublimate was crystallized from light petroleum, b.p. 80–100° (10 ml.), yielding orange leaflets (10 mg.), m.p. 192–194°, not depressed on admixture with authentic chrysophanol, m.p. 194–195°.

SUMMARY

1. Two hitherto undescribed colouring matters, skyrin and flavoskyrin, have been isolated from the mycelium of laboratory cultures of a morphologically typical but biochemically atypical strain of *Penicillium islandicum* Sopp.

2. Skyrin, $C_{30}H_{18}O_{10}$, crystallizes as clusters of orange-red rods or as yellow, hexagonal plates and does not melt below 380° . Several of its derivatives are described.

3. When an alkaline solution of skyrin is treated with sodium dithionite, $Na_2S_2O_4$, it undergoes reductive cleavage into two molecules of emodin, $C_{15}H_{10}O_5$, 4:5:7-trihydroxy-2-methylanthraquinone.

4. Flavoskyrin, $C_{15}H_{12}O_5$, crystallizes as yellow needles, m.p. 215° (decomp.).

5. Flavoskyrin loses a molecule of water to yield chrysophanol, $C_{15}H_{10}O_4$, 4:5-dihydroxy-2-methylanthraquinone, either by heating at its melting point or by the action of cold concentrated sulphuric acid.

6. The chrysophanol already reported as a metabolic product of this strain of *P. islandicum* has been shown not to be an artifact produced, during its isolation, by decomposition of flavoskyrin.

7. Possible structures for skyrin are discussed.

REFERENCES

- Anslow, W. K., Breen, J. & Raistrick, H. (1940). *Biochem. J.* **34**, 159.
 Ashley, J. N., Hobbs, B. C. & Raistrick, H. (1937). *Biochem. J.* **31**, 385.
 Barger, G. (1904). *J. chem. Soc.* **85**, 286.
 Fischer, O. & Gross, H. (1911). *J. prakt. chem.* (II), **84**, 369.
 Howard, B. H. & Raistrick, H. (1949). *Biochem. J.* **44**, 227.
 Howard, B. H. & Raistrick, H. (1950). *Biochem. J.* **46**, 49.
 Joshi, B. S., Tilak, B. D. & Venkataraman, K. (1950). *Proc. Indian Acad. Sci.* **32**, 348.
 Oxford, A. E. & Raistrick, H. (1940). *Biochem. J.* **34**, 790.
 Raistrick, H. (1949). *Proc. Roy. Soc. A*, **199**, 158.
 Sopp, O. J. O. (1912). *Skr. VidenskSelsk., Christ.* no. 11, 161.
 Stoll, A. & Becker, B. (1950). *Fortschr. Chem. org. Naturst.* **7**, 248.
 Stoll, A., Becker, B. & Helfenstein, A. (1950). *Helv. chim. acta*, **33**, 313.

Chemical Action of X-Rays on Nucleic Acids and Related Substances in Aqueous Systems

2. THE MECHANISM OF THE ACTION OF X-RAYS ON NUCLEIC ACIDS IN AQUEOUS SYSTEMS

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The nature of the chemical changes which occur on irradiation of nucleic acids in aqueous systems with X-rays have been described in Part 1 of this series (Scholes & Weiss, 1953*a*). Evidence was presented to show that these changes were the result of chemical reactions involving the free radicals produced by the absorption of the radiation by the water which is split according to the net process (Weiss, 1944, 1947*a, b*):

In these experiments, the detection and isolation of the products of irradiation were facilitated by employing fairly high doses of X-rays (approx. 1×10^6 r).

Previous investigations of the action of X-rays on deoxyribonucleic acid (DNA) solutions, using doses in the region 20 000–100 000r have been essentially concerned with the physical effects of the radiation, for example the decrease in viscosity

and of streaming birefringence (cf. Taylor, Greenstein & Hollaender, 1948). No chemical changes have been reported. It was therefore of interest, particularly from a biological standpoint, to investigate the nature of any chemical effects which may result by the use of similarly low, or even lower, doses of radiation. In fact, it was found that ammonia and inorganic phosphate were both formed when solutions of DNA and RNA (ribonucleic acid) were irradiated with doses of the order of 30 000r (Scholes & Weiss, 1950). Furthermore, quantitative determination of these products formed under different experimental conditions (e.g. in the presence of oxygen, of hydrogen and *in vacuo*) has yielded information about the nature of the radicals involved in the various processes. In the following, our detailed results are presented and the mechanism of the degradation induced by X-rays of the polynucleotides is discussed.