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Studies in the Biochemistry of Micro-Organisms

92. THE COLOURING MATTERS OF *PENICILLIUM ISLANDICUM* SOPP PART 4. IRIDOSKYRIN, RUBROSKYRIN AND ERYTHROSKYRINE

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In our first communication concerning the pigments of *Penicillium islandicum* Sopp (Howard & Raistrick 1949) it was shown that the dried mycelium of laboratory cultures of one strain of this species, no. N.R.R.L. 1036, contained about 20% of its weight of a complex mixture of colouring matters. One constituent of this mixture was isolated and identified as the hitherto undescribed islandicin (1:4:5-trihydroxy-2-methylanthraquinone, I). This substance was isolated from the mycelium of five out of six morphologically typical strains of *P. islandicum*, and the presence in the mycelium of several other colouring matters was indicated by a number of colour reactions. Three of these colouring matters have now been isolated in a pure crystalline state and it is the purpose of the present communication to describe, *inter alia*, their isolation, properties and reactions, and to discuss their relationship to other fungal colouring matters. None of them appears to have been described previously. Their chemical constitutions, at present uncertain, are under investigation in this laboratory.

When the dried and ground mycelium of strain N.R.R.L. 1036 was extracted for a long period in a percolator with boiling light petroleum, a bright red solid accumulated in the solvent. Islandicin was separated from this solid by sublimation in high vacuum at 180°. The unsublimed residue, on crystallization from benzene, gave small amounts of a substance, crystallizing as deep red plates or rods, m.p. 358–360°, for which we propose the name iridoskyrin because the crystals exhibit a striking green iridescence. Further extraction of the mycelial powder with chloroform removed a complex mixture of pigments, and when the extract was chromatographed on calcium carbonate, a further

small amount of iridoskyrin appeared in the eluate together with much islandicin. The chromatogram consisted of a number of bands of different colours. From one of them, a broad green band, there was obtained a substance crystallizing as dark red plates, m.p. 289–290° (decomp.), for which we propose the name rubroskyrin. The losses involved in the separation of these two colouring matters make it impossible to state with certainty the proportions present in the mould. It is estimated, however, that the dried mycelium of *P. islandicum* strain N.R.R.L. 1036 contains about 0.2% of iridoskyrin and about 2.5% of rubroskyrin.

An ether extract of the wet mycelium of the same strain of *P. islandicum*, N.R.R.L. 1036, was shaken out with aqueous sodium carbonate. Acidification of this alkaline extract yielded a dark red, amorphous powder which was fractionated by chromatographing an acetone solution on magnesium oxide. Development of the chromatogram with methanol gave an eluate containing the magnesium salt of a third colouring matter which was obtained as dark red micro-needles, m.p. 108–110°, for which we propose the name erythroskyrine. It was found later that the same substance constitutes practically the whole of the colouring matter present in the mycelium of a different strain, L.S.H.T.M. no. BB 233, of *P. islandicum*. It is estimated that the dried mycelium of this strain contains about 10% of erythroskyrine, while strain N.R.R.L. 1036 contains approximately 3.5%.

Iridoskyrin

Iridoskyrin, $C_{30}H_{18}O_{10}$, is dimorphic, crystallizing as thin rods or hexagonal plates of a deep red colour and exhibiting a green iridescence. It melts at

358–360° and sublimes unchanged at 260° in high vacuum. The molecule contains six hydroxyl groups which can be acetylated or methylated, probably two quinone groupings, two methyl groups attached to carbon and no methoxyl groups. Its solutions in organic solvents are orange-red in colour, appearing bluer in thin layers, and do not fluoresce in daylight. It is insoluble in 0.5N sodium bicarbonate and N sodium carbonate, but dissolves in aqueous N sodium hydroxide to a purple-blue solution, the colour of which rapidly fades on exposure to air. In cold, concentrated sulphuric acid it forms a stable, non-fluorescent, deep blue solution. It is not reduced in alkaline solution by sodium dithionite (cf. skyrin; Howard & Raistrick, 1954).

The following functional derivatives of iridoskyrin have been prepared. Hexaacetyliridoskyrin, $C_{42}H_{30}O_{16}$, i.e. $C_{30}H_{12}O_4(O.CO.CH_3)_6$, pale yellow, rectangular plates, m.p. 267–268°, and hexamethyliridoskyrin, $C_{36}H_{30}O_{10}$, i.e. $C_{30}H_{12}O_4(OCH_3)_6$, flat orange rods, m.p. 343–345°, insoluble in alkalis and forming a stable, blue solution in cold concentrated sulphuric acid.

Shortage of material has so far not permitted degradative experiments on iridoskyrin, but it is of interest to note that it is isomeric with skyrin, $C_{30}H_{18}O_{10}$, a colouring matter found in the mycelium of a biochemically atypical strain of *P. islandicum*, no. N.R.R.L. 1175 (Howard & Raistrick, 1954).

Both substances are polyhydroxyquinones of high melting point, possessing six hydroxyl groups, two quinone groupings and two methyl groups attached to carbon. The molecule of skyrin is considered to consist of two molecules of emodin (III) joined by a direct carbon-to-carbon link, for example as in (IV). The possibility must therefore be considered that the molecule of iridoskyrin is similarly derived, most probably from islandicin, for example as in (V). Unfortunately this possibility seems unlikely though not impossible for the following reasons. (i) Solutions in glacial acetic acid and in concentrated sulphuric acid of a compound of structure (V) might be expected to show the fluorescence in daylight typical of quinizarin (1:4-dihydroxyanthraquinone) derivatives (Raistrick, Robinson & Todd, 1934, p. 567); no such fluorescence is observed in solutions of iridoskyrin in these reagents. (ii) Solutions of skyrin in cold concentrated sulphuric acid show a change in colour on standing, most probably because of interaction of hydroxyl groups in the two emodin nuclei; no such change of colour is observed when iridoskyrin is dissolved in concentrated sulphuric acid. (iii) The most significant difference between the two substances is shown in their reactions with sodium dithionite ($Na_2S_2O_4$) in alkaline solution, when skyrin readily undergoes reductive cleavage yielding two molecules of emodin (III) whereas iridoskyrin can be recovered unchanged.

Rubroskyrin

Rubroskyrin crystallizes from ethanol as thin, rectangular plates, dark red in bulk, m.p. 289–290° (decomp.). On account of its instability it has not yet been possible to prepare a crystalline derivative and the suggested molecular formula, $C_{30}H_{22}O_{12}$, is based on analyses of the substance itself, on its relationship to iridoskyrin, $C_{30}H_{18}O_{10}$, and on its decomposition products. Solutions of rubroskyrin in organic solvents are red in colour and do not fluoresce in daylight. It is insoluble in 0.5N sodium bicarbonate but dissolves in N sodium carbonate and aqueous N sodium hydroxide to give emerald-green solutions, the colour of which fades on exposure to air. Rubroskyrin is thus the substance responsible for the green colour observed when portions of the mycelium of four out of the six strains of *P. islandicum* originally examined were immersed in alkalis (Howard & Raistrick, 1949). It dissolves in cold concentrated sulphuric acid to an initially red solution, the colour of which changes in a few hours to blue.

Rubroskyrin is reduced by zinc dust in glacial acetic acid and by sodium dithionite ($Na_2S_2O_4$) in alkaline solution. The product, 'reduced rubroskyrin' has so far not been obtained crystalline. Crude 'reduced rubroskyrin' is an amorphous, orange powder, m.p. 143–148° (decomp.). It dissolves in N sodium carbonate or in aqueous N sodium hydroxide to a red solution which turns green on shaking in air and from which rubroskyrin is precipitated on acidification. Thus, in both the ease and the manner of their interconvertibility, rubroskyrin and 'reduced rubroskyrin' behave like a quinone and the corresponding quinol.

Rubroskyrin is readily decomposed either (a) by the action of cold concentrated sulphuric acid or (b) by heating. The results obtained, while not providing conclusive evidence on the structure of rubroskyrin, establish the relationship of this substance both to iridoskyrin and to other mould colouring matters.

(a) A solution of rubroskyrin in cold concentrated sulphuric acid was kept overnight at room temperature and from the reaction products there was isolated 82% of crystalline iridoskyrin. The same basic change occurs when rubroskyrin is treated with acetic anhydride and sodium acetate at 100° since hexaacyliridoskyrin is formed in good yield.

(b) Rubroskyrin was heated at 290–300° in an atmosphere of nitrogen. The following yields of recognizable decomposition products were obtained: water, 6.4; carbon dioxide, 0.15; islandicin (I), 41.4; catenarin (II), 7.5; and iridoskyrin (possibly V) 31.9%; total = 87.3%. When rubroskyrin was

heated in high vacuum for 1 hr. at 270° islandicin (36%), catenarin (16%) and iridoskyrin (18%) were obtained. Hence the decomposition reactions of rubroskyrin may be represented by the following equations:

The decomposition of rubroskyrin by sulphuric acid would then be represented by equation (i) only, and the thermal decomposition by equations (i) and (ii) occurring simultaneously.

'Reduced rubroskyrin' is also readily decomposed by cold concentrated sulphuric acid—the solution initially green changing to purple-red on standing—or by heating. It yields the same three substances as are formed from rubroskyrin but the breakdown proceeds rather less smoothly and the total yield of recognizable products is smaller, owing no doubt, at least in part, to the uncertain purity of the starting material. The yields of islandicin, catenarin and iridoskyrin are 44, nil and 6%, respectively, when 'reduced rubroskyrin' is decomposed by concentrated sulphuric acid at room temperature, and 60, 4 and 10% when it is heated to 160°. It is, however, of interest to note the fundamentally different reactions of rubroskyrin and 'reduced rubroskyrin' in cold concentrated sulphuric acid. Thus, whereas rubroskyrin gives 82% of iridoskyrin but no islandicin, the products from 'reduced rubroskyrin' are 6% of iridoskyrin and 44% of islandicin.

The ready conversion of rubroskyrin, with the loss of water, into iridoskyrin, islandicin and catenarin links it with other mould colouring matters and is analogous to the conversion, again with loss of water by heating or by treatment with cold concentrated sulphuric acid, of flavoskyrin from *P. islandicum*, strain N.R.R.L. 1175 (Howard & Raistrick, 1954) into chrysophanol (VI) according the equation

Of the three conversion products of rubroskyrin—iridoskyrin, islandicin and catenarin—the first two are also metabolic products of strains of *P. islandicum*, while catenarin (II) is present in the mycelium of *Helminthosporium gramineum* Rabenhorst (Charles, Raistrick, Robinson & Todd, 1933), *H. catenarium* Drechsler and other species of *Helminthosporium* (Raistrick *et al.* 1934; Anslow & Raistrick, 1940, 1941) and *Aspergillus amstelodami* (Mangin) Thom & Church (Shibata & Natori, 1953).

Erythroskyrine

Erythroskyrine crystallizes from methanol or ethanol in dark red micro-crystals which effloresce on standing in air owing to loss of solvent of crystallization. It melts at 108–110° but does not sublime in high vacuum. It has not yet been possible to prepare any crystalline functional derivatives of erythroskyrine so that the molecular formula, $C_{24}H_{31}O_6N$, suggested for it is very tentatively advanced. It gives an iodine-brown ferric colour in ethanol. It dissolves in cold concentrated sulphuric acid to give a characteristic, intensely blue solution which is stable on standing. It is very sensitive to exposure to daylight in the solid state, quickly changing colour from dark red to an orange, amorphous substance which gives only a brown solution in cold concentrated sulphuric acid.

Erythroskyrine is a weak acid being soluble with difficulty in sodium bicarbonate but more readily soluble in sodium carbonate or aqueous sodium hydroxide. Many of its salts are soluble in organic solvents with the results that some of them may even be extracted from aqueous solution, e.g. with chloroform, and when solutions of erythroskyrine are chromatographed on magnesium oxide or magnesium carbonate the resulting deep yellow band may be eluted with methanol, not as the free colouring matter but as the magnesium derivative. The sodium derivative has been prepared as orange scales which analyse well for $C_{24}H_{30}O_6NNa$, $2H_2O$ and, after drying, for $C_{24}H_{30}O_6NNa$. This salt is readily soluble in water to give a neutral, orange solution which exhibits anti-bacterial activity against *Staphylococcus* species, corresponding to total inhibition of growth for 20 hr. at 37° of *Staph. albus* and *Staph. aureus* at a concentration of the sodium derivative of 1 part in 21 000.

It is also a weak base, being completely soluble in cold concentrated hydrochloric acid. This solution is however very unstable and undergoes deep-seated decomposition on boiling, part of the nitrogen in the molecule being released as a volatile base which has not yet been identified but is neither ammonia nor methylamine.

Erythroskyrine is a highly unsaturated substance, being rapidly reduced catalytically with palladium and hydrogen. Four moles of hydrogen are absorbed per mole of $C_{24}H_{31}O_6N$. The reduction product, a dark yellow oil which could not be crystallized, was methylated with diazomethane giving an oil which was oxidized with potassium permanganate. The resulting oxidation product crystallized in colourless, curved needles, m.p. 105–110°. It contains no nitrogen or methoxyl, titrates as a dibasic acid and has probably the molecular formula $C_{11}H_{20}O_4$, i.e. $C_9H_{18}(COOH)_2$. Its chemical constitution is under investigation.

During the last 6 years we have examined the colouring matters present in ten different, but morphologically authentic, strains of *P. islandicum* Sopp. Seven different colouring matters in all have been isolated in pure crystalline form. In those cases where isolation of the crystalline pigments proved impossible their presence or absence was determined by chromatography and/or colour reactions. The results obtained up to the present may be summarized as follows.

(a) Erythroskyrine was isolated from strains L.S.H.T.M. no. BB233 and N.R.R.L. 1036, and was identified in all the other eight strains.

(b) Islandicin (I), rubroskyrin and iridoskyrin were detected with certainty and were usually isolated in crystalline form from eight strains. These three pigments are almost certainly absent from strains N.R.R.L. 1175 and L.S.H.T.M. no. BB233. All are red in colour.

(c) Chrysophanol (VI), skyrin (IV) and flavoskyrin have been isolated in crystalline form from strain N.R.R.L. 1175; all three pigments are absent from strain L.S.H.T.M. no. BB233; chrysophanol and flavoskyrin are absent from strain N.R.R.L. 1036, except possibly in minute amounts, but a small amount of skyrin was isolated from it as the crystalline acetate. The three pigments are orange or yellow in colour.

The ten strains of *P. islandicum* examined, although all morphologically authentic, may therefore be subdivided into three groups.

Group A. Eight strains, all dark red in colour, which we regard as biochemically typical, all yield islandicin, rubroskyrin, iridoskyrin and erythroskyrine. Skyrin is present in at least one strain thus linking this group with group C.

Group B. One strain, L.S.H.T.M. no. BB233, bright orange in colour, yields only erythroskyrine.

Group C. One strain, N.R.R.L. 1175, is much more orange and less red in colour than the strains in group A. It yields chrysophanol, skyrin, flavoskyrin and erythroskyrine.

We regard groups B and C as being biochemically atypical.

Ignoring erythroskyrine for the present, the other six colouring matters fall into two distinct groups. In the first group are the three red substances, islandicin, iridoskyrin and rubroskyrin, the last two of which are structurally related to islandicin or to its 7-hydroxy derivative, catenarin. These three pigments are found in the biochemically typical strains of group A. In the second group are the three orange or yellow pigments, chrysophanol, flavoskyrin, and skyrin, the last two of which are structurally related to chrysophanol or to its 7-hydroxy derivative, emodin. These three pigments are found in the biochemically atypical strain, N.R.R.L. 1175 in group C. The biochemical

difference between the strains in group *A* on the one hand and group *C* on the other hand can therefore be most broadly stated as follows. Strains in group *A* are able to synthesize colouring matters derived from 1-hydroxy-2-methylantraquinone, whereas the colouring matters synthesized by the strain in group *C* are related to anthraquinones lacking the hydroxyl group in position 1.

EXPERIMENTAL

Culture

The strain of *Penicillium islandicum* Sopp used in this part of the work was received by us in 1943 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 the catalogue number N.R.R.L. 1036. It was received originally by Dr Charles Thom in 1922 from F. M. Putterill, Cape Town, South Africa, and is described in detail by Raper & Thom in their *Manual of the Penicillia* (1949, p. 623). It is the same strain as that used for the bulk preparation of islandicin, (Howard & Raistrick, 1949) and appears to be morphologically and biochemically typical of the species. The mycelium of a mature culture is a deep brownish red colour.

Cultural conditions

Batches of fifty or one-hundred 1 l. conical flasks, each containing 350 ml. of Czapek-Dox solution (glucose, 50.0 g.; NaNO_3 , 2.0 g.; KH_2PO_4 , 1.0 g.; KCl , 0.5 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g.; distilled water, 1 l.) were plugged with cotton wool, sterilized and inoculated with *P. islandicum* strain N.R.R.L. 1036. The flasks were incubated in the dark at 24° for 19–24 days, when the glucose content of the solution had fallen to 0.4% or less and the pH was about 4.2. The flasks were harvested in the usual way (see Howard & Raistrick, 1949, p. 230) and the culture filtrate was discarded. The yield of mycelium, dried *in vacuo* at 40°, was about 4.2 g. per flask.

Isolation of the colouring matters

Two methods have been used for the separation of rubroskyrin and iridoskyrin from the dried and ground mycelium of *P. islandicum* strain N.R.R.L. 1036.

A. Dried, powdered mycelium (201 g.) from fifty flasks was exhaustively extracted with CHCl_3 in a Soxhlet apparatus. The dark red extract was evaporated *in vacuo* and the residual solid was freed from fat by extraction with light petroleum. The material insoluble in light petroleum was re-dissolved in CHCl_3 and extracted with aqueous $\text{N-Na}_2\text{CO}_3$. After centrifugal separation the CHCl_3 solution was evaporated and yielded islandicin (6.19 g.). The Na_2CO_3 extracts were acidified. The resulting red precipitate was filtered off, washed with water and dried, giving a dark red solid (19.15 g.) which was finely ground. Portions of it were extracted for a short time with ether in a Soxhlet apparatus. This treatment removed much material, which was discarded, giving brown and purple bands on a column of MgCO_3 , together with a little rubroskyrin. Further extraction in the Soxhlet with acetone gave clean red solutions, which were passed through columns of CaCO_3 (British Drug Houses; 'precipitated'). A better separation of the bands

was obtained on this adsorbent than on heavy MgCO_3 . After adsorption, the columns were well washed with acetone; the brown and purple bands at the top were discarded; the clean green band below was separated mechanically, dried in air, and added slowly to aqueous 4N-HCl containing ice. The resulting red precipitate was removed by shaking three times with large volumes of ether. When washed, dried (Na_2SO_4) and concentrated, the ether deposited crude rubroskyrin as dark red rods, different fractions of which melted in the range 276–283°. Yield 2.0 g. from 8 g. crude Na_2CO_3 extract.

B. Dried powdered mycelium (417 g.) from 100 flasks was extracted in a percolator for 40 hr. with hot light petroleum, b.p. 40–60°. The extract, containing an orange-red solid in suspension, was filtered. Removal of the light petroleum from the pale orange filtrate yielded 'fat' (36 g.). The orange-red precipitate (4.2 g.) contained islandicin with a little iridoskyrin and other substances. This mixture was fractionated as follows. 1.0 g. portions of it were shaken with 0.5N- NaHCO_3 (300 ml.), kept overnight, and then filtered off and washed with water. The orange filtrates were discarded, and the remaining solids (0.7 g. in each case) were dried and heated in high vacuum at 180° for 1 hr. giving sublimates of almost pure islandicin (1.9 g.). The combined brownish residues were extracted with boiling benzene. On concentrating the extracts to 40 ml., crude iridoskyrin separated as red, iridescent, lozenge-shaped crystals (0.25 g.), m.p. 354–356°.

The light-petroleum-extracted mycelial powder was air dried and was re-extracted in the percolator with ether for 15 hr. The brown to orange-red extracts so obtained contained a little rubroskyrin, together with large amounts of colouring matters giving yellow, purple and red bands on a column of MgCO_3 . The yellow band yielded erythroskyrin (see p. 219). The other two bands have not been investigated further at present.

The mycelium was finally extracted in the percolator with six successive lots of CHCl_3 for 2, 2.5, 3.5, 5, 7 and 12 hr., respectively. All the CHCl_3 solutions were red and gave green extracts when shaken with N- Na_2CO_3 , the colours suggesting that the middle extracts were the purest. Extracts 1, 2, 3, 4 and 5 + 6, diluted with CHCl_3 to 7.7, 3.3, 1.8, 1.8 and 1.7 l., respectively, were passed through columns of CaCO_3 and each column was washed with fresh CHCl_3 . All the columns were similar in appearance having in order from top to bottom the following bands: (1) narrow purple; (2) broad brown-yellow; (3) narrow purple; (4) broad green; (5), (6) and (7) narrow red, yellow and purple; (8) broad pale purple; (9) broad salmon-pink. The broad green bands (4) were separated mechanically, acidified with ice-cold 4N-HCl and the liberated pigments were re-dissolved in CHCl_3 . This solution was re-passed through CaCO_3 columns and rubroskyrin was obtained from the resulting green bands by extraction with ether as described in method *A*. The yields and m.p.'s of the dark red, crystalline rubroskyrin obtained from the different extracts were as follows: 1, 0.47 g., 265–267°; 2, 0.37 g., 276–278°; 3, 0.48 g., 284–285°; 4, 0.22 g., 284–285°; 5 + 6, 0.15 g., impure and amorphous. Total weight of crude crystalline rubroskyrin, 1.54 g.

Bands (8) and (9), representing respectively iridoskyrin and islandicin, were eluted during washing of the CaCO_3 columns with CHCl_3 . The combined eluates were freed from CHCl_3 by evaporation and the sticky, dark red residue was heated in small portions in high vacuum at 180° giving

crystalline sublimes of islandicin. Iridoskyrin was most conveniently prepared from the islandicin-free residues (1.01 g.) via the acetate (see below).

Iridoskyrin

Purification and properties. The residues from the sublimations described in the previous paragraph were converted into the acetate as described below. The free pigment (0.42 g.) obtained by alkaline hydrolysis of the pure acetate was crystallized from benzene (140 ml.) giving pure *iridoskyrin*. Iridoskyrin is dimorphic and crystallizes from benzene either as deep red, thin rods or as hexagonal plates. The crystals have a green iridescence which is most marked with the larger hexagonal type. When heated, iridoskyrin begins to smoke at about 350° and melts to a purple-black liquid at 358–360°. When iridoskyrin is crystallized from benzene a variable amount of solvent is retained and cannot be removed by heating to 120° in high vacuum. Sublimation in high vacuum at 260° was employed to ensure solvent-free material for analysis. (Found on sublimed sample: N, halogen, S, OCH₃, nil; C, 66.9, 66.9, 66.9; H, 3.5, 3.5, 3.4; C-CH₃, 7.4. C₃₀H₁₈O₁₀ requires C, 66.9; H, 3.4; 2 C-CH₃, 5.6%.)

Iridoskyrin is readily soluble in CHCl₃, acetone and warm benzene, slightly soluble in ether and almost insoluble in ethanol. Its solutions in organic solvents are orange-red in colour, appearing much bluer in thin layers and not fluorescing in daylight. Its ethanolic solution gives with ethanolic FeCl₃ a deep violet colour indistinguishable from that given by salicylic acid. It dissolves to a stable, non-fluorescent, intensely blue solution in cold conc. H₂SO₄. It is insoluble in 0.5N-NaHCO₃, N-Na₂CO₃ and 0.01 N-NaOH, but dissolves in aqueous N-NaOH to a purple-blue solution, the colour of which rapidly fades on exposure to air. A stable, purple band is formed when a solution of iridoskyrin in acetone is passed through a column of MgCO₃ or MgO. The purple-blue solution of iridoskyrin in N-NaOH becomes brown-orange in colour on the addition of Na₂S₂O₄, but the pigment is recovered unchanged when the neutralized mixture is extracted with ether.

Derivatives of iridoskyrin

(a) *Hexaacetyliridoskyrin*. Crude iridoskyrin (1.01 g.; see above) was heated with acetic anhydride (10 ml.) and fused sodium acetate (2 g.) at 100° for 2 hr. The crude acetate (1.30 g.), recovered as an orange-yellow powder on pouring the cooled reaction mixture into ice water, was crystallized by diluting its solution in CHCl₃ (20 ml.) with ethanol (200 ml.). After two further similar crystallizations *hexaacetyliridoskyrin* was obtained as clusters of pale yellow, rectangular plates (0.75 g.), m.p. 267–268°. (Found on material dried in high vacuum at 160°: C, 63.6, 63.5; H, 4.0, 4.0; CH₃CO, 31.5; mol.wt., cryoscopic in camphor, 779, 795. C₄₈H₃₀O₁₆ requires C, 63.8; H, 3.8; 6CH₃CO, 32.7%; mol.wt. 791.)

Dried hexaacetyliridoskyrin (0.6164 g.) was hydrolysed by heating on the boiling water bath for 2 hr. in an atmosphere of O₂-free N₂ with aqueous NaOH (0.9644 N; 25.00 ml.). The dark blue solution was acidified by addition of N-H₂SO₄ (30.00 ml.) and the flask was then stoppered and held at 100° for a further 0.5 hr. to coagulate the ppt. The cooled mixture was filtered and the bright red solid was washed and dried (wt. 0.42 g.; theoretical 0.427 g.). When titrated to phenol-

phthalein with the NaOH used for hydrolysis the filtrate and washings required 10.77 ml., corresponding to 31.5% of CH₃CO in the acetate.

(b) *Hexamethyliridoskyrin*. Iridoskyrin (0.1 g., from benzene) was boiled under reflux with dry acetone (150 ml.), dimethyl sulphate (1.0 ml.) and freshly heated K₂CO₃ (1.0 g.). During 22 hr. boiling further quantities of dimethyl sulphate (2.0 ml.) and K₂CO₃ (5.5 g.) were added a little at a time. The orange mixture was then diluted with water, and acetone and dimethyl sulphate were removed by distillation under reduced pressure. The remaining suspension was extracted with CHCl₃, from which solution incompletely methylated material was removed by shaking with small amounts of N-NaOH. The dry, orange solid (0.09 g.), left on evaporation of the CHCl₃, was crystallized first from acetone and then twice from CHCl₃: ethanol (1.5 + 15 ml.) to give *hexamethyliridoskyrin* (0.06 g.) as flat, oblique-ended rods which are a deep, glistening orange colour in bulk, m.p. 343–345°. (Found on material sublimed in high vacuum at 300°: C, 69.4; H, 5.0; OCH₃, 30.0. C₃₆H₃₀O₁₀ requires C, 69.5; H, 4.9; 6OCH₃, 29.9%.) Hexamethyliridoskyrin is insoluble in alkalis and dissolves to a stable, blue solution in cold conc. H₂SO₄.

Rubroskyrin

Purification and properties. Rubroskyrin is not readily crystallized as it is not markedly more soluble in hot solvents than in cold. Crude crystalline rubroskyrin (0.23 g.) was boiled with ethanol (4 × 100 ml.) and the combined filtered extracts were evaporated *in vacuo* to 100 ml. The crystals that separated on standing were recrystallized in a similar manner to yield pure *rubroskyrin* (0.11 g.) as thin rectangular plates, dark red in bulk, m.p. 289–290° (decomp.). (Found on sample dried in high vacuum at 150°: N, halogen, S, OCH₃, nil; C, 62.5, 62.5; H, 4.0, 4.0. C₃₀H₂₂O₁₁ requires C, 62.7; H, 3.9%.)

Rubroskyrin behaves in a characteristic manner on heating; slight softening occurs at about 270° with the formation of an orange, crystalline sublimate; melting takes place at 289–290° and is accompanied by vigorous effervescence and blackening. Rubroskyrin is almost insoluble in light petroleum, ether and benzene, slightly soluble in CHCl₃, moderately soluble in ethyl acetate, glacial acetic acid, ethanol and dioxan, and fairly readily soluble in acetone. It is insoluble in 0.5N-NaHCO₃, but dissolves in N-Na₂CO₃ and N-NaOH to emerald-green solutions, the colour of which fades to a brownish yellow on exposure to air. Portions of an ethereal solution were shaken with equal volumes of 0.2M borate or phosphate buffers. At pH 7.4 and below, the aqueous layer was colourless; at pH 8.2 it was slightly green; at pH 8.5 the ether layer was pale pink and the aqueous layer a moderate green; at pH 9.0 the ether layer was very pale pink and at pH 10.0 and above it was quite colourless. The material crystallized as described above was chromatographically homogeneous, giving a uniform stable green band when an acetone solution was passed through a column of MgCO₃ or CaCO₃. A solution of rubroskyrin in cold conc. H₂SO₄ is red at first but changes during a few hours to an intense pure blue. Rubroskyrin gives a strong purplish brown ferric reaction in ethanol. The red colour of a solution of rubroskyrin in warm glacial acetic acid is rapidly changed to orange-yellow on the addition of Zn dust.

Attempts to prepare derivatives of rubroskyrin

(a) *Acetylation. Isolation of hexaacetyliridoskyrin.* Rubroskyrin (0.05 g.) was heated at 100° for 2 hr. with acetic anhydride (1 ml.) and fused sodium acetate (0.2 g.) during which time the colour of the solution changed from red to brown. The orange-brown solid which separated when the cooled mixture was shaken with ice was crystallized twice by dissolving in CHCl_3 (2 ml.) and adding ethanol (15 ml.). The acetate was thus obtained as clusters of pale yellow rectangular plates, m.p. 265–267°, not depressed on admixture with authentic hexaacetyliridoskyrin, m.p. 267–268°. It dissolved in cold conc. H_2SO_4 to an intensely blue solution, indistinguishable from that given by iridoskyrin in the same reagent.

(b) *Methylation.* Rubroskyrin (0.10 g.), dissolved in dry acetone (40 ml.), was refluxed for 14 hr. with dimethyl sulphate (2 ml.) and dried K_2CO_3 (4 g.). The orange-yellow solution was then concentrated and poured into water (300 ml.) containing 2N-HCl (60 ml.). The orange-yellow precipitate, after removal of a trace of NaOH-soluble material, dried to a gummy solid (0.06 g.) which could not be crystallized. It was insoluble in N-NaOH and gave an olive-green solution in cold conc. H_2SO_4 .

(c) *Reduction. Isolation of 'reduced rubroskyrin'.* Rubroskyrin (0.10 g.) was dissolved by shaking in cold N-NaOH (25 ml.), and to the green solution was added $\text{Na}_2\text{S}_2\text{O}_4$ (1.0 g.) dissolved in water (10 ml.) causing the colour to change immediately to red. N-HCl (25 ml.) was quickly added and the resulting orange precipitate was extracted into ether (3 × 50 ml.). The reduction product was obtained as an amorphous orange powder (0.10 g.) when the ether extracts were evaporated to dryness. It was readily soluble in organic solvents and appeared to decompose in solution. It could not be crystallized. Crude 'reduced rubroskyrin', m.p. 143–148° (decomp.), is insoluble in 0.5N- NaHCO_3 , but in N- Na_2CO_3 and N-NaOH it dissolves to a solution which is initially red and changes rapidly through brown to green. In cold conc. H_2SO_4 it gives initially a green solution, the colour of which changes during 1 hr. through brown to purple-red with a red fluorescence.

(d) *Oxidation of 'reduced rubroskyrin'. Regeneration of rubroskyrin.* Crude 'reduced rubroskyrin' (0.04 g.) was shaken in air with N-NaOH (5 ml.). The colour slowly became green during 15 min. when the mixture was acidified and extracted with ether. Typical crystals of rubroskyrin (0.01 g.), m.p. 286–288° (decomp.) separated on concentration. A mixture with authentic rubroskyrin, m.p. 288–289° (decomp.), melted at 287–289° (decomp.).

Decomposition of rubroskyrin

(a) *By H_2SO_4 .* Rubroskyrin (103 mg.) was dissolved in cold conc. H_2SO_4 (5 ml.) and the mixture was kept overnight in a stoppered tube at room temperature. The resulting deep-blue solution was poured into ice water (200 ml.) and the resulting red precipitate was extracted with ether (4 × 400 ml.) to give an orange-red solution which was freed from traces of Na_2CO_3 -soluble material, dried and concentrated. Two crops of crystals were thus obtained; the first (77 mg.), m.p. 356–358°, consisted of deep red hexagonal plates with a strong green iridescence and the second (7 mg.), m.p. 356–358°, of similar plate crystals with a few similarly coloured rods. (Yield of crystalline product 84 mg. = 82% of starting material.) This product was crystallized from benzene (18 ml.) from which it separated as hexagonal

plates and pointed rods (61 mg.), m.p. 358–360° alone or mixed with authentic iridoskyrin, m.p. 358–360°. (Found on material dried in high vacuum at 230°: N, halogen, S, OCH_3 , nil; C, 67.0, 66.9; H, 3.5, 3.5. $\text{C}_{30}\text{H}_{18}\text{O}_{10}$ requires C, 66.9; H, 3.4%.) The product also gave solutions in cold conc. H_2SO_4 and in aqueous N-NaOH which were identical in colour with those given by iridoskyrin in the same reagents.

For confirmation of identity, a portion of the product was acetylated with acetic anhydride and sodium acetate. The crude acetate crystallized from CHCl_3 (1 ml.):ethanol (10 ml.) as clusters of pale yellow plates, m.p. 266–268°, alone or mixed with hexaacetyliridoskyrin, m.p. 267–268°.

(b) *Thermal decomposition.* Rubroskyrin (151.3 mg., after drying in high vacuum at 130°) was heated in a stream of dry N_2 freed from O_2 and CO_2 . The N_2 and volatile products were then passed through a CaCl_2 tube and a bubbler containing 0.2M-Ba(OH) $_2$. The tube containing the rubroskyrin was immersed in a metal bath, heated first at 290° for 15 min. and, after 15 min. cooling, for a further 20 min. at 300°. The flow of N_2 was stopped during the two periods of heating in order to prevent solid reaction products from being carried over at the time of decomposition and was continued for 45 min. after the end of the second heating period. It was then found that the CaCl_2 tube had gained 9.66 mg. water and that 0.22 mg. CO_2 had been trapped in the Ba(OH) $_2$.

The solid products of the reaction (139.1 mg.) consisted of a bright orange-red sublimate and a dark residue. The whole of this material was dissolved in CHCl_3 , the last traces of the residue being brought into solution only by extraction in a Soxhlet. The combined CHCl_3 extracts were concentrated and diluted with ether. The material in solution was fractionated by shaking repeatedly with 0.2N- Na_2CO_3 . The purple alkaline extracts so obtained, together with the dark blue insoluble salt produced at the same time, were run into dilute acid, and the liberated products were extracted with ether. The ether solution was washed with water, evaporated to dryness and the residual Na_2CO_3 -soluble material was fractionally sublimed in high vacuum. The Na_2CO_3 -insoluble material remaining in the ether after Na_2CO_3 extraction was treated similarly.

The Na_2CO_3 -soluble material yielded an orange-red sublimate at 200° (11.28 mg.), but no other fraction sublimed below 320°. The sublimate crystallized from ethanol as long, narrow plates, glistening red in bulk, m.p. 244–246°, not depressed on admixture with authentic catenarin, m.p. 244–246°, isolated from *Helminthosporium tritici-vulgaris* (Raistrick *et al.* 1934). (Found on material dried in high vacuum at 110°: C, 62.7; H, 3.9. Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_8$: C, 62.9; H, 3.5%.) The product and authentic catenarin gave identical colours in the following tests: N- Na_2CO_3 , violet-red solution fading on exposure to air; glacial acetic acid, orange solution with green fluorescence; cold conc. H_2SO_4 , blue-violet solution.

The Na_2CO_3 -insoluble material yielded first an orange-red sublimate at 180° (62.59 mg.) and a deep red sublimate at 280° (48.27 mg.). The former sublimate crystallized from glacial acetic acid as glistening, maroon leaflets, m.p. 218° either alone or mixed with authentic islandicin, m.p. 218°, from *P. islandicum* strain N.R.R.L. 1036 (Howard & Raistrick, 1949). (Found: C, 66.8; H, 3.8. Calc. for $\text{C}_{14}\text{H}_{10}\text{O}_8$: C, 66.7; H, 3.7%.) The fraction subliming at 280° crystallized from benzene as clusters of pointed rods, dark red in colour with a green iridescence, m.p. 357–359° not depressed on admixture with authentic iridoskyrin, m.p. 358–360°. (Found on material dried in high vacuum at

210°: C, 67.1; H, 3.4. Calc. for $C_{30}H_{18}O_{10}$: C, 66.9; H, 3.4%.) The product and authentic iridoskyrin gave identical colours in the following tests: $N-Na_2CO_3$, insoluble; $N-NaOH$, purple-blue fading on exposure to air; glacial acetic acid, orange-red, much bluer in thin layers but without fluorescence; cold conc. H_2SO_4 , blue. A portion of the product was acetylated with acetic anhydride containing a little conc. H_2SO_4 . The resulting acetate, crystallized from $CHCl_3$ /ethanol (1:10, v/v) as pale yellow, rectangular plates, m.p. 266–267°, not depressed on admixture with authentic hexaacetyliridoskyrin, of the same m.p.

The residues from the sublimations of both the Na_2CO_3 -insoluble and the Na_2CO_3 -soluble material were dark purple and weighed about 8 mg. each.

Yields of recognizable decomposition products: water, 6.4; CO_2 , 0.15; islandicin, 41.4; catenarin, 7.5; iridoskyrin, 31.9%. Total = 87.3%.

In another experiment, rubroskyrin was heated in high vacuum for 1 hr. at 270°. The yields of islandicin, catenarin and iridoskyrin under these conditions were respectively 36, 16 and 18%.

Decomposition of 'reduced rubroskyrin'

(a) By H_2SO_4 . Crude, freshly prepared 'reduced rubroskyrin' (100 mg.) was dissolved in cold conc. H_2SO_4 (5 ml.), and the solution was kept for 1.5 hr. at room temperature with occasional shaking. The deep purple-red solution, which had a strong red fluorescence, was then rinsed into ice water (300 ml.) and the suspension was extracted repeatedly with ether. The mixture of substances in the ether was fractionated as described under thermal decomposition of rubroskyrin (p. 218), to yield the following fractions. The products were identified by m.p., mixed m.p. and colour reactions.

Fraction A, Na_2CO_3 -insoluble, subliming in high vacuum at 180°, weighing 44 mg., crystallized from glacial acetic acid to yield islandicin, m.p. 218°.

Fraction B, Na_2CO_3 -insoluble, not subliming in high vacuum at 180°, weighing 6 mg., was heated in high vacuum at 290° and the sublimate was crystallized from ether to give iridoskyrin deep red plates and rods m.p. 353–355°.

Fraction C, Na_2CO_3 -soluble, subliming in high vacuum at 180°, nil.

Fraction D, Na_2CO_3 -soluble, not subliming in high vacuum at 180°, weighing 9 mg., not investigated further. Yields of recognizable decomposition products: islandicin, 44; iridoskyrin, 6%. Total = 50%.

(b) Thermal decomposition. Crude, freshly prepared 'reduced rubroskyrin' (20 mg.) was heated *in vacuo* at 160° for 1 hr. The orange sublimate and dark brown residue were dissolved in $CHCl_3$ and fractionated as described under thermal decomposition of rubroskyrin (p. 218).

Four fractions were obtained and purified as above:

A. 12 mg.; islandicin, m.p. 218°.

B. 2.5 mg.; gave red plates and rods, iridoskyrin.

C. 0.8 mg.; crystallized from ethanol, catenarin.

D. 1 mg.; deep purple-red solid, not further investigated.

Yields of recognizable decomposition products: islandicin, 60; iridoskyrin, 12.5; catenarin, 4%. Total = 76.5%.

Erythroskyrine

The most suitable strain of *P. islandicum* for the preparation of this colouring matter is strain L.S.H.T.M. no. BB233 (see p. 221), since its colouring matter consists largely of erythroskyrine.

Eighty-four 1 l. flasks, each containing 350 ml. of Czapek-Dox solution, were sterilized, inoculated with *P. islandicum* strain L.S.H.T.M. no. BB 233 and incubated at 24° in the dark for 27–29 days. The resulting bright orange mycelium was separated from the culture filtrate, washed with water, broken into small pieces while still wet and immersed in a mixture of aqueous 2N-HCl (2 l.) and benzene (4 l.). The mixture was then stirred vigorously for 1 day. The deep red benzene extract was separated, washed with water, dried ($MgSO_4$) and evaporated *in vacuo* to 150 ml. The mycelium, still suspended in aqueous 2N-HCl, was re-extracted four times more with benzene (4 l. each time) and each extract was treated similarly to the first one. To each evaporated extract was now added light petroleum (2 vol.; b.p. 60–80°). A deep red precipitate was formed in each case and, after standing at room temperature overnight, was decanted through a filter. The gummy residue was triturated with more light petroleum and was thus readily granulated. It was finally separated by filtration, washed and dried *in vacuo*. All operations were carried out as far as possible in a subdued light. Yields: 1st extract, 9.75 g.; 2nd, 12.81 g.; 3rd, 7.08 g.; 4th, 3.47 g.; 5th, 1.30 g. Total, 34.41 g. crude erythroskyrine/84 flasks = 41 g./100 flasks, corresponding approximately to 10% of the dry weight of the mycelium.

Purification and properties. The above red precipitates were purified by chromatography. 0.5 g. Portions, dissolved in acetone (250 ml.), were passed through columns (10 × 2.5 cm.) of MgO (heavy) or $MgCO_3$ (heavy). The chromatogram, which consisted mainly of an orange-yellow band, was developed with methanol (500 ml.), giving rise to a yellow eluate which was evaporated to dryness *in vacuo*. The orange residue, consisting essentially of the magnesium salt of erythroskyrine, was decomposed with aqueous 2N-HCl in ice, and the liberated colouring matter was extracted into $CHCl_3$ which, on evaporation, left a dark red solid of free erythroskyrine. Treated in this way, 6.9 g. of dried methanol eluate gave 4.1 g. of erythroskyrine. The aqueous acid liquor was evaporated to dryness and ashed with conc. H_2SO_4 giving a white solid residue (2.3 g.) which consisted essentially of $MgSO_4$ since it contained no metals other than Mg. This weight 2.3 g., of $MgSO_4$ corresponds to an equivalent weight of 107 for erythroskyrine.

No completely satisfactory solvent was found for the crystallization of erythroskyrine, the most suitable being ethanol (4 ml./g.) or methanol 6–8 (ml./g.). Erythroskyrine separates as clusters of dark red micro-needles from ethanol and as dark red, rough, hexagonal tablets from methanol. Both types of crystals crumble to an amorphous powder on drying, even at room temperature in air, owing to loss of solvent of crystallization.

Erythroskyrine melts at 108–110° but does not sublime in high vacuum. It is readily soluble in acetone, $CHCl_3$, CCl_4 , methanol, ethanol, benzene, glacial acetic acid and pyridine, much less soluble in ether and light petroleum and almost insoluble in water. It dissolves in cold conc. H_2SO_4 to give a characteristic solution which is a very intense, almost pure blue colour in transmitted light but appears rather more violet by reflected light. It gives an iodine-brown ferric colour in ethanolic solution. (Found, on three different samples: C, 67.9, 67.8; 67.2, 67.4; H, 7.2, 7.4; 7.3; 7.4; N, 3.6, 3.7; 3.3; 3.2; OCH_3 , nil; mol.wt. in camphor (Rast), 470; in dioxan (Oxford), 444; equiv. via Mg salt (see above) 107. $C_{31}H_{27}O_6N$ requires C, 67.5; H, 7.3; N, 3.75%; mol.wt. 373; $C_{28}H_{21}O_6N$ requires C, 67.1; H, 7.3; N, 3.26%; mol.wt. 429.)

Erythroskyrine is very sensitive in the solid state to the action of diffused daylight, whereby it is converted into an orange amorphous substance which, in contrast to erythroskyrine, dissolves in NaOH to a brown solution, gives only a brown colour with cold conc. H_2SO_4 and is much more soluble in methanol and much less soluble in CCl_4 . Erythroskyrine is stable in the solid state if kept in the dark, and dilute solutions of it in CCl_4 remain stable on long exposure to diffused daylight in closed vessels. It therefore exhibits a type of light sensitivity which is quite distinct from that of penicillipsin (mycoporphyrin) (Oxford & Raistrick, 1940) which is stable in the dry state but is transformed, by oxidation, to 'irradiated oxypenicillipsin' by the action of light on its solution in certain organic solvents.

Erythroskyrine is a weak acid, being soluble with difficulty in dilute NaHCO_3 but more readily soluble in dilute Na_2CO_3 or aqueous NaOH. The solutions are a stable orange-yellow colour in each case. A striking property of the salts of erythroskyrine is their solubility in organic solvents. The solubility of the magnesium salt in methanol has already been mentioned. The sodium and potassium salts are soluble in CHCl_3 and benzene and less so in ether, and can even be removed from solutions in aqueous alkalis by shaking with these solvents.

The pigment is also a weak base, being completely and immediately soluble in cold conc. HCl (sp.gr. = 1.16) to a deep brown solution. If this solution is immediately diluted with much water the pigment is precipitated unchanged, as judged by the blue H_2SO_4 reaction. If, however, the solution is held at room temperature for some hours and then diluted with much water, the dirty grey precipitate so formed gives only a brown colour with conc. H_2SO_4 with no trace of blue or purple. When the conc. HCl solution was boiled for some hours, deep-seated decomposition took place with the formation of much black insoluble material, the filtrate from which contained a volatile base which was isolated as the hydrochloride by distilling the filtrate into dilute HCl, after making alkaline with 2N-NaOH. The base formed a benzoyl derivative, m.p. 82–85°, in a Schotten-Baumann reaction with benzoyl chloride; benzamide melts at 128°; a mixture of the benzoyl derivative of the base, m.p. 82–85°, and *N*-methylbenzamide, m.p. 80°, melted at 50–70°. Hence the base is neither ammonia nor methylamine.

Sodium derivative of erythroskyrine. A filtered solution of purified erythroskyrine (0.5 g.) in CCl_4 (100 ml.) was vigorously shaken with half-saturated aqueous NaHCO_3 (100 ml.). After standing for some hours, the CCl_4 layer, which contained the bulk of the Na derivative as a gelatinous mass inseparable by filtration or centrifuging, was run out on to a series of porous plates, all of which were then covered with clock glasses and kept overnight. The solid Na derivative remaining on the plates was scraped off, dried *in vacuo*, extracted thoroughly with boiling CCl_4 , and re-dried. It was then dissolved in boiling ethanol (10 ml.), filtered and the filtrate was evaporated to dryness *in vacuo*. The Na derivative was thus obtained as orange scales (0.1 g.). (Found, (a) on material dried as above: N, 2.9, 3.1; Na, 4.5, 4.4; alkoxy, nil; loss in wt. (water) on drying at 100° in high vacuum, 7.15. $\text{C}_{24}\text{H}_{30}\text{O}_6\text{NNa} \cdot 2\text{H}_2\text{O}$ requires N, 2.9; Na, 4.7; $2\text{H}_2\text{O}$, 7.4%; (b) on material dried to constant wt. at 100° in high vacuum: C (including carbon in the residue, assumed to be Na_2CO_3 , left after combustion), 63.9, 63.7; H, 7.8, 7.7; residue, Na_2CO_3 , left after combustion, 11.5. $\text{C}_{24}\text{H}_{30}\text{O}_6\text{NNa}$ requires C, 63.8; H, 6.7; residue, $\frac{1}{2}\text{Na}_2\text{CO}_3$, 11.7%.)

The Na derivative is readily and completely soluble in water to give a neutral solution and appears to be quite stable in the dark both in the solid state and in solution. On acidifying its dilute, orange aqueous solution with dilute HCl, a deep red colour is first obtained, followed almost immediately by the precipitation of erythroskyrine in amorphous flocks. Like erythroskyrine it gives the characteristic intense-blue colour with conc. H_2SO_4 , but this colour is no longer given by the Na derivative after exposure to daylight for some days. An aqueous solution of the Na derivative exhibits some anti-bacterial activity against *Staphylococcus* species. At a concentration of 1 part in 21 000 in 2% glucose broth at pH 7.2 it completely inhibits the growth of *Staph. albus* (N.C.T.C. no. 3256) and *Staph. aureus* (N.C.T.C. no. 3750) for 20 hr. at 37°, the initial inoculum being 500–1000 viable bacteria per ml.

Reduction and degradation of erythroskyrine

Neither an aqueous solution of the Na derivative of erythroskyrine nor an ethanolic solution of the free colouring matter appears to be reduced by $\text{Na}_2\text{S}_2\text{O}_4$, the colour changing in the latter case from red to an intense orange. Erythroskyrine appears to be reduced by Zn dust in warm glacial acetic acid, the colour changing from orange to a very pale yellow. It is quickly and smoothly reduced catalytically.

A solution of erythroskyrine (1 g.) in ethanol (50 ml.) was shaken in H_2 with a catalyst made from PdCl_2 (0.2 g.) and Norite charcoal (1 g.). H_2 (220 ml. at s.t.p.) was very rapidly absorbed. Theor. for absorption of 4 moles H_2 /mole of erythroskyrine, $\text{C}_{24}\text{H}_{31}\text{O}_6\text{N}$, (mol.wt. 429) = 208.8 ml. The charcoal catalyst was removed by filtration and the lemon-yellow filtrate, which gave a brownish red ferric colour, was freed from ethanol yielding a dark yellow oil (0.80 g.) which could not be obtained crystalline. It gave a very weak brown colour with cold conc. H_2SO_4 . The oil was re-dissolved in ethanol (15 ml.) and treated with a large excess of ethereal diazomethane. N_2 was rapidly evolved. On removal of the solvents a light brown oil remained but could not be crystallized. It was therefore oxidized, first at 50° and later at 100°, with a total of 50 ml. of 5% aqueous KMnO_4 added in small portions. MnO_2 was removed by filtration and washed with hot water. The filtrate and washings were neutralized to litmus with 2N-HCl, evaporated *in vacuo* to about 20 ml. and made strongly acid with 2N-HCl. The resulting amorphous, almost colourless solid (0.12 g.) was collected, dried and sublimed in a high vacuum at 120–130° to give a colourless, micro-crystalline, slightly oily sublimate (0.09 g.) which crystallized from boiling light petroleum (b.p. 80–100°) in colourless, curved needles (0.06 g.), m.p. 105–110°. It is very soluble in methanol, ethanol, benzene, CHCl_3 and glacial acetic acid and slightly soluble in light petroleum. (Found: C, 61.4, 61.4; H, 9.3, 9.3; N, nil; OCH_3 , nil; equiv. by titration, 128. $\text{C}_{11}\text{H}_{20}\text{O}_4$, $(\text{C}_9\text{H}_{18}(\text{COOH})_2$), requires C, 61.1; H, 9.3; equiv. titrating as a dibasic acid, 108.)

Identification of colouring matters present in ten strains of *Penicillium islandicum*

Cultures

The history of the ten strains of *P. islandicum* used in this part of the work is as follows:

1–6. Strains N.R.R.L. 1036, 1037, 1038, 1175, 1177 and 2115 were all received from Dr Kenneth B. Raper, Northern

Regional Research Laboratory (N.R.R.L.), U.S. Department of Agriculture, Peoria, Illinois, U.S.A. Full details of them are recorded by Howard & Raistrick (1949, p. 229).

7. Strain N.R.R.L. 2239 received from Dr Raper in June 1950. Isolated by him from African soil.

8. Strain, London School of Hygiene and Tropical Medicine, L.S.H.T.M., no. P. 250. Received in May 1950 from Mr W. Turner, British Leather Manufacturers' Research Association. Isolated from, and producing red stains on, leather. Identified by Mr G. Smith.

9. Strain L.S.H.T.M. no. BB233. Received in May 1940 from Dr G. R. Bisby, Commonwealth Mycological Institute, Kew. Identified by Mr G. Smith.

10. Strain L.S.H.T.M. no. SM420. Received in April 1951 from Mr John L. Yuill of York. Isolated by him as an aerial contaminant and identified by Dr C. Thom.

When grown on Czapek-Dox solution, mature cultures of strains N.R.R.L. 1036, 1037, 1038, 1177, 2115, 2239 and L.S.H.T.M. no. P. 250 were almost indistinguishable from each other. They all developed reddish drops on the surface of the mycelium which consisted mainly of reddish brown to brownish red growth with small areas of green growth, having a dark reddish brown to brownish red reverse. Strain L.S.H.T.M. no. SM420 was very similar to them, but not quite so highly pigmented. Strain L.S.H.T.M. no. BB233 was unusual in having a bright orange mycelium, while strain N.R.R.L. 1175 was much lighter in colour, being yellow to orange, with green areas, and with a yellow-to-brown reverse.

In general, the methods used for the identification of the colouring matters were those which have already been described, i.e. islandicin (Howard & Raistrick, 1949); chrysophanol (Howard & Raistrick, 1950); skyrin and flavoskyrin (Howard & Raistrick, 1954); rubroskyrin, iridoskyrin and erythroskyrine (present communication). In those cases where the method used or the source of material have been varied with advantage, details are given later. All the results obtained are recorded in Table 1.

Erythroskyrine. The almost complete absence of colouring matters, other than erythroskyrine, from strain L.S.H.T.M. no. BB233 was demonstrated as follows. The bright orange mycelium from a single flask of this strain, grown on Czapek-Dox medium, was separated from the culture fluid, washed, pressed between filter papers and extracted with boiling acetone (2×150 ml.). This treatment removed almost all the colouring matter from the mycelium. The deep orange extract was chromatographed on MgO. A very narrow, pale grey-green band was formed at the top and the rest of the column was occupied by a uniform yellow band, which was eluted almost completely by methanol. Removal of the solvent from the eluate gave a typical orange residue, having all the characteristic properties of the magnesium salt of erythroskyrine.

Islandicin. Strains N.R.R.L. 2239, L.S.H.T.M. no. P. 250 and L.S.H.T.M. no. SM420 gave, in addition to the normal mycelium, a highly pigmented, partly crystalline sediment in the culture fluid, which was collected by decanting the fluid through a filter. A CHCl_3 extract of this material was chromatographed on CaCO_3 . A number of coloured bands were formed, two of which were of particular interest. (a) A green band (see next paragraph). (b) The lowest pink band was eluted with CHCl_3 . Removal of the solvent gave glistening maroon leaflets melting in each case at 218° alone or mixed with authentic islandicin, m.p. 218° , obtained from strain N.R.R.L. 1036.

Table 1. *Distribution of pigments of Penicillium islandicum Sopp*

Pigment	Strain									
	N.R.R.L. 1036	N.R.R.L. 1037	N.R.R.L. 1038	N.R.R.L. 1175	N.R.R.L. 1177	N.R.R.L. 2115	N.R.R.L. 2239	L.S.H.T.M. no. P. 250	L.S.H.T.M. no. BB233	L.S.H.T.M. no. SM420
Erythroskyrine	+	+	+	+	+	+	+	+	+	+
Islandicin	+	+	+	-	+	+	+	+	-	+
Rubroskyrin	+	+	+	-	+	+	+	+	-	+
Iridoskyrin	+	+	+	-	+	+	+	+	-	+
Chrysophanol	-	0	0	+	0	0	0	0	-	0
Skyrin	+	0	0	+	0	0	0	0	-	0
Flavoskyrin	-	0	0	+	0	0	0	0	-	0

+ + + = Isolated in crystalline form; + = detected by chromatography and/or colour reactions; - = all tests negative; 0 = not investigated.

Rubroskyrin. The green bands referred to in the previous paragraph as well as a similar band from strain N.R.R.L. 1038 were treated according to procedure *B* (see p. 216) and in each case yielded dark red plates, m.p.'s and mixed m.p.'s 285–286° (decomp.) and having the colour reactions with $\text{N-Na}_2\text{CO}_3$ and cold conc. H_2SO_4 which are characteristic of rubroskyrin. In contrast, chromatography of Na_2CO_3 -soluble material from strain N.R.R.L. 1175 yielded only brown and purple bands. Indeed, none of the many chromatographic experiments carried out with this strain has shown a green band indicative of rubroskyrin.

Iridoskyrin. During chromatography on MgCO_3 of extracts of the mycelium of strain N.R.R.L. 1175, a purple band was noticed near the bottom of the column, immediately above the broad, salmon-pink chrysophanol band (Howard & Raistrick, 1950, p. 52; 1954, p. 60). It was suspected that this band might indicate the presence of iridoskyrin. However, while examination of the colouring matter contained in the band has not yielded the substance in crystalline form, it has shown conclusively that it is not iridoskyrin, but is probably a new colouring matter. Its solution in CHCl_3 is orange in colour and yields a sparingly soluble, violet salt when shaken with N-NaOH . A solution of the substance in cold conc. H_2SO_4 is crimson in colour.

Skyrin. Identification in strain N.R.R.L. 1036. 5.0 g. of the mixed Na_2CO_3 -soluble colouring matters from an ether extract of the wet mycelium of this strain were dissolved in CHCl_3 (500 ml.) and chromatographed on MgCO_3 . The column was washed with fresh CHCl_3 (500 ml.) and then with methanol (1 l.) to remove erythroskyrin. The purple band from the middle of the column was separated and dried. The resulting purple powder was well shaken with CHCl_3 (500 ml.) containing glacial acetic acid (2 ml.), and the orange extract was separated by filtration and evaporated to dryness. The residual orange-brown material was acetylated and purified as already described (Howard & Raistrick, 1954, p. 60). The crude acetate (0.1 g.), when purified, yielded pale yellow needles (0.02 g.), m.p. 292–294° not depressed on admixture with authentic hexaacetylskyrin, m.p. 295–296° from strain N.R.R.L. 1175. The product also exhibited reactions characteristic of hexaacetylskyrin with cold conc. H_2SO_4 and aqueous N-NaOH . The yields of crude and pure product are however only 4 and 2%, respectively, of the yields of crude and pure hexaacetylskyrin from the same weight of dried mycelium of strain N.R.R.L. 1175.

SUMMARY

1. Three hitherto undescribed mould colouring matters, iridoskyrin, rubroskyrin and erythro-

skyrine, have been isolated from strains of *Penicillium islandicum* Sopp.

2. Iridoskyrin, $\text{C}_{30}\text{H}_{18}\text{O}_{10}$, m.p. 358–360°, crystallizes as deep red rods or plates which have a green iridescence. A hexaacetate and a hexamethyl ether are described.

3. Rubroskyrin, $\text{C}_{30}\text{H}_{22}\text{O}_{12}$, crystallizes in dark red, rectangular plates, m.p. 289–290° (decomp.).

4. Treatment of rubroskyrin with concentrated sulphuric acid at room temperature results in the loss of two molecules of water and the formation of iridoskyrin in 82% yield.

5. Thermal decomposition of rubroskyrin gives, in good yield, a mixture of iridoskyrin and the previously described mould colouring matters, islandicin (1:4:5-trihydroxy-2-methylantraquinone) and catenarin (1:4:5:7-tetrahydroxy-2-methylantraquinone).

6. The relationships of iridoskyrin and rubroskyrin to other mould colouring matters are discussed.

7. Erythroskyrine, $\text{C}_{24}\text{H}_{31}\text{O}_6\text{N}$, crystallizes in dark red micro-needles, m.p. 108–110°. It is a highly unsaturated, light-labile substance which shows some anti-bacterial activity against *Staphylococcus* species.

8. The distribution of seven crystalline colouring matters in ten different strains of *P. islandicum* has been investigated and is described.

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