the acid was converted into the amide and that 22–24% was excreted unchanged in the urine. The fate of the remainder of the acid is as yet unknown.

Table 2. *Urinary excretion of the 2:5-acid and amide before and after oral administration of 500 mg. N-methyl-2-pyridone-5-carboxylic acid*

Subject no.	Compound determined	Urinary excretion (mg.) 24 hr. specimen before dose	48 hr. specimen after dose	Percentage of dose excreted in urine
1	Amide	11·0	21·1	0
	Acid	Nil	119	24
2	Amide	6·5	9·3	0
	Acid	Nil	109	22

Table 3. *Urinary excretion of the 2:5-acid and amide before and after oral administration of 500 mg. N-methyl-2-pyridone-5-carboxylamide*

Subject no.	Compound determined	Urinary excretion (mg.) 24 hr. specimen before dose	48 hr. specimen after dose	Percentage of dose excreted in urine
1	Amide	20·6	423	76
	Acid	Nil	Nil	0
2	Amide	9·3	404	77
	Acid	Nil	Nil	0

In a further experiment the urinary excretions of the 2:5-acid and amide by the same two subjects were determined before and after the ingestion of 500 mg. of the amide. The results (Table 3) show that none of the amide was converted into the acid and that 76–77% of the dose was excreted unchanged in the urine in 48 hr.

These results indicate that *N*-methyl-2-pyridone-5-carboxylic acid does not play a part in human nicotinic-acid metabolism.

SUMMARY

1. A method is described for the determination of *N*-methyl-2-pyridone-5-carboxylic acid in human urine, based on preliminary purification by adsorption on Lloyd's reagent followed by extraction with ether from solution in 10N-hydrochloric acid, and colorimetric determination after nitration and neutralization with sodium carbonate.

2. By means of this method it is shown that *N*-methyl-2-pyridone-5-carboxylic acid is not present in urine in any detectable amount normally or after the ingestion of nicotinamide.

3. It is also shown that none of a dose of the acid is converted in the body into the corresponding amide, and that none of a dose of the amide is converted into the acid.

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Studies in the Biochemistry of Micro-organisms

81. THE COLOURING MATTERS OF *PENICILLIUM ISLANDICUM* SOPP. PART 2. CHRYSOPHANIC ACID, 4:5-DIHYDROXY-2-METHYLANTHRAQUINONE

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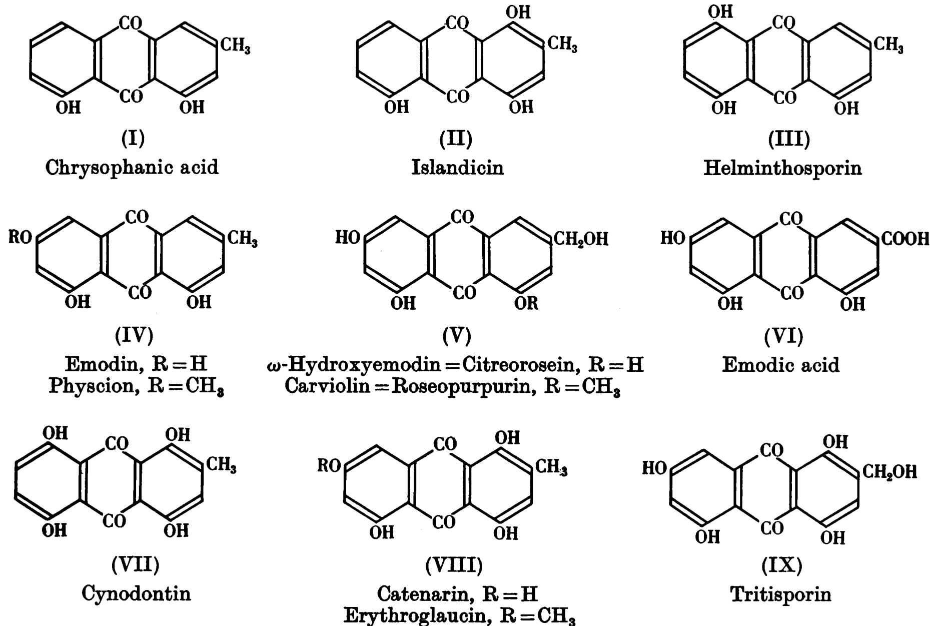
In a recent communication from this laboratory (Howard & Raistrick, 1949) an account was given of the isolation of the hitherto undescribed 1:4:5-trihydroxy-2-methylanthraquinone from the mycelium of five out of six different strains of *Penicillium islandicum* Sopp which were examined. This colouring matter has properties which are very similar to those described for funiculosin which Igaraci (1939) isolated from laboratory cultures of *P. funiculosum* Thom. We have tried to obtain a culture of the strain of *P. funiculosum* used by Igaraci and also a specimen of funiculosin itself, but have failed to do so. We have also failed to isolate or detect any pigment having properties similar to funiculosin or to 1:4:5-trihydroxy-2-methylanthraquinone from a number of different authentic strains of *P. funiculosum*, most of which were kindly supplied by Dr Kenneth B. Raper, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois, U.S.A. We propose, therefore, now to give the trivial name *islandicin* to the 1:4:5-trihydroxy-2-methylanthraquinone from *P. islandicum*.

The sixth strain of *P. islandicum* which we examined (strain N.R.R.L. 1175), although morphologically a typical strain of *P. islandicum*, appeared to be biochemically atypical, since no islandicin

could be detected in the complex mixture of colouring matters obtained by solvent extraction of its dried mycelium. The mixture of colouring matters of this strain has now been submitted to more exhaustive examination, particularly by chromatographic analysis, and three colouring matters have been isolated in a state of purity, two of which have not been previously described and constitute the greater part of the colouring matters of this strain. They will form the subject of future communications. It is of interest to note that, even by the rather searching method of chromatographic analysis, no clear evidence could be obtained of the presence of islandicin in this strain.

The amount of chrysophanic acid isolated is only about 0·3 % of the total weight of colouring matter present in *P. islandicum*, strain N.R.R.L. 1175, so that from the purely quantitative point of view it is of relatively minor importance. We are of the opinion however, that it is of great significance in relation to the general question of the biosynthesis of fungal metabolic products, a subject dealt with in more detail by one of us in the Bakerian Lecture of the Royal Society (Raistrick, 1949). It is the only dihydroxymethylanthraquinone and the simplest anthraquinone derivative yet described as a metabolic product of any species of fungus. Furthermore, all the anthraquinone derivatives, with the exception



(I) Chrysophanic acid

(II) Islandicin

(III) Helminthosporin

(IV) Emodin, R = H; Physcion, R = CH_3

(V) ω-Hydroxyemodin = Citreorosein, R = H; Carviolin = Roseopurpurin, R = CH_3

(VI) Emodic acid

(VII) Cynodontin

(VIII) Catenarin, R = H; Erythroglaucin, R = CH_3

(IX) Tritisporin

The third pigment was identified as chrysophanic acid (chrysophanol), 4:5-dihydroxy-2-methylanthraquinone. This was isolated from the vacuum-dried mycelium of *P. islandicum*, strain N.R.R.L. 1175, grown on Czapek-Dox medium, by extraction with light petroleum, b.p. 40–60°. The orange-yellow light petroleum extract, containing a fair amount of fat, was passed through columns of 'heavy' magnesium carbonate and gave finally characteristic salmon-pink bands which were separated by mechanical means, acidified with hydrochloric acid and extracted with ether. The ethereal solution was washed with aqueous N-sodium carbonate to remove traces of carbonate-soluble pigments. On removal of the solvent and trituration of the residue with small amounts of cold light petroleum, there remained 0·10 g. of almost pure crystalline chrysophanic acid, m.p. 192–193·5°, from 238 g. of dried mycelium.

of boletol from the higher fungus *Boletus satanas* Lenz (Kögl & Deijs, 1935), which have been reported as metabolic products of species of fungi are derivatives of chrysophanic acid. The inter-relationships in structure between twelve derivatives of anthraquinone which are known to be fungal metabolic products are illustrated by structures (I) to (IX), and their relationships to chrysophanic acid are summarized in Table 1. The molecular constitutions of all of them, except tritisporin, have been established with certainty, and most of them have been synthesized. The constitution suggested for tritisporin, though a very probable one, has not yet been proven.

Chrysophanic acid has been known for over a century as a plant colouring matter occurring especially in a number of species of *Rheum* and *Rumex*. It is present in Chinese rhubarb as the glycoside chrysophanein, $C_{21}H_{20}O_9$. Its molecular con-

stitution as 4:5-dihydroxy-2-methylanthraquinone was settled by an unambiguous synthesis by Naylor & Gardner (1931).

EXPERIMENTAL

Culture

We received the strain of *Penicillium islandicum* Sopp used in this work 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. It was received by Dr Raper originally in 1940 from Dr G. A. Ledingham, Ottawa, Canada, and bears Dr Raper's catalogue number N.R.R.L. 1175. 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.

Table 1. *Relationship to chrysophanic acid of other fungal metabolic products known to be derivatives of anthraquinone*

Metabolic product	Species of fungus	Structure	Relationship to chrysophanic acid	References
Chrysophanic acid	*Penicillium islandicum* Sopp (N.R.R.L. 1175)	(I)	—	Present paper
Islandicin	*P. islandicum*	(II)	1-Hydroxy derivative	Howard & Raistrick (1949)
Helminthosporin	*Helminthosporium gramineum* Rabenhorst (*a*); *H. catenarium* Drechsler (*b*) and (*c*)	(III)	8-Hydroxy derivative	(*a*) Charles, Raistrick, Robinson & Todd (1933) (*b*) Raistrick, Robinson & Todd (1934) (*c*) Raistrick, Robinson & Todd (1933*a*)
Emodin	*Cortinarius* (*Dermocybe*) *sanguineus* (Wulf.) Fries	(IV) R=H	7-Hydroxy derivative	Kögl & Postowsky (1925)
Physcion	*Aspergillus glaucus* series	(IV) $R=CH_3$	7-Methoxy derivative	Raistrick, Robinson & Todd (1937) Ashley, Raistrick & Richards (1939)
{ω-Hydroxyemodin (*a*) {Citreorosein (*b*)	(*a*) *P. cyclopium* Westling (*b*) *P. citreo-roseum* Dierckx	(V) R=H	7-Hydroxy derivative $CH_3 \rightarrow CH_2OH$	(*a*) Anslow, Breen & Raistrick (1940) (*b*) Posternak & Jacob (1940)
{Carviolin (*a*) {Roseopurpurin (*b*)	(*a*) *P. carmino-violaceum* Dierckx (*b*) *P. roseo-purpureum* Dierckx	(V) $R=CH_3$	7-Hydroxy-4-methoxy derivative $CH_3 \rightarrow CH_2OH$	(*a*) Hind (1940*a*, *b*) (*b*) Posternak (1940)
Emodic acid	*P. cyclopium* Westling	(VI)	7-Hydroxy derivative $CH_3 \rightarrow COOH$	Anslow *et al.* (1940)
Cynodontin	*H. cynodontis* Marignoni	(VII)	1:8 Dihydroxy derivative	Raistrick, Robinson & Todd (1933*b*); Anslow & Raistrick (1940*a*)
Catenarin	*H. catenarium* Drechsler	(VIII) R=H	1:7-Dihydroxy derivative	Raistrick *et al.* (1934) Anslow & Raistrick (1941)
Erythroglaucin	*A. glaucus* series	(VIII) $R=CH_3$	1-Hydroxy-7-methoxy derivative	Ashley *et al.* (1939) Anslow & Raistrick (1940*b*)
Tritisporin	*H. tritici-vulgaris* Nisikado	(IX)	1:7-Dihydroxy derivative $CH_3 \rightarrow CH_2OH$	Raistrick *et al.* (1934)

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.; $MgSO_4.7H_2O$, 0·5 g.; $FeSO_4.7H_2O$, 0·01 g.; distilled water 1 l.

Seventy-one 1 l. conical flasks plugged with cotton wool and each containing 350 ml. of Czapek-Dox solution were sterilized and inoculated with a spore suspension, in the same sterile medium, prepared from twenty-four mature test-tube cultures of *P. islandicum* strain N.R.R.L. 1175 grown on malt agar for 10 days at 24°. The flask cultures were incubated in the dark at 24°, and were harvested after 22 days' incubation. The orange-yellow culture fluid, pH 4·2, was separated by decantation from the deep orange mould mycelium which was washed in the flasks with cold distilled water. The mycelium was strained through butter muslin and squeezed as dry as possible in a tincture press. It was then thoroughly dried in a vacuum oven at 40–42° and ground to a fine powder in a coffee mill. Weight of dry ground mycelium, 238 g.

The ground mycelium was distributed equally between four large thimbles and extracted in four Soxhlets with light petroleum, b.p. 40–60°, on each of 5 successive days, fresh light petroleum being used each day. On standing overnight, all the five orange-coloured extracts, except the first, deposited a small amount of orange solid which was separated by filtration and discarded, since subsequent chromatography in acetone solution showed that it contained no chrysophanic acid. The clear orange-yellow light petroleum

solutions were separately passed with light suction through tightly packed columns of $MgCO_3$ (British Drug Houses Ltd., 'heavy') contained in glass tubes, each column being approximately 20 cm. long × 2·5 cm. diameter.

Chromatography of extracts

First extract (*5 hr. extraction*). This extract required seven columns, each holding about 200 ml. of extract. Most of the colouring matters were adsorbed in a narrow orange band at the top of the column. Two fainter bands, separated from the top band by a broad almost colourless zone, moved down the column as the extract was added, finally reaching the bottom of the column as an upper narrow faint purple band and a lower broad salmon-pink band. Each column was now washed with 200 ml. of freshly distilled light petroleum, b.p. 40–60°, by which means the salmon-pink band was eluted giving a yellow solution. This solution was now rechromatographed on two fresh columns and washed thoroughly with light petroleum giving finally a narrow pink band at the top of the column followed by a broad colourless band, then by a very narrow purple band and a broad homogeneous salmon-pink band, the remainder of the column being quite colourless. The two columns were drained free of light petroleum, corked and stored in the dark.

Second extract (*5 hr. extraction*). This extract was passed through two $MgCO_3$ columns giving a similar series of bands to the first extract. The salmon-pink band, however, was not very easily eluted since it was not completely removed after washing each column with 600 ml. of light petroleum. The resulting yellow eluate was passed through a single fresh column giving a broad uniform salmon-pink band at the top of the column which was not removed by washing with 200 ml. of light petroleum.

Third extract (*7 hr.*), *fourth extract* (*9 hr.*) *and fifth extract* (*7 hr.*). These extracts behaved similarly to each other giving, after washing with 200 ml. of light petroleum, a dark orange band at the top of the column followed successively by a narrow, very faint, purple band, a broad almost colourless band, a narrow very pale red band, a narrow purple band, a faint blue band and finally a broad homogeneous salmon-pink band.

Each of the light petroleum extracts, after passing through the columns, gave colourless filtrates which were evaporated to dryness leaving in each case a pale yellow oil. Weights: first extract, 3·39 g.; second extract, 1·01 g.; third + fourth + fifth extracts, 0·29 g. Total weight of 'fat', 4·69 g.

Recovery of colouring matters from columns

The salmon-pink bands from the five extracts were carefully scraped from the tubes, combined and acidified with 2N-HCl, the mixture being cooled by the addition of crushed ice. An orange solid floated to the surface and was extracted with ether. The orange ethereal solution was washed twice with water, three times with N-Na_2CO_3 solution and three times with water. The solution was then filtered and evaporated to dryness giving 0·52 g. of orange-yellow crystals still contaminated with 'fat'. This was washed with a little cold light petroleum, b.p. 40–60°, and gave an orange crystalline solid, weight 0·10 g., m.p. 192–193·5°. It was recrystallized from ethanol (30 ml.), yielding 69 mg. of shining golden plates, m.p. 194°. A portion of this material was further purified by sublimation in a high vacuum at 125–130°. The sublimate melted at 195–196°, and the mixture of it with authentic sublimed chrysophanic acid, m.p. 194–195°, melted at 194–195·5°. (Found on sublimed material: C, 70·4; H, 4·0. Calc. for $C_{15}H_{10}O_4$: C, 70·9; H, 4·0%.) The metabolic product and authentic chrysophanic acid gave identical reactions in the following tests; N-Na_2CO_3, insoluble; conc. NH_3 solution, slowly soluble in the cold giving a solution which was cherry red in bulk and magenta in thin layers; N-NaOH, readily and completely soluble in the cold, giving a red solution which became pale orange on standing, with the formation of a red precipitate; conc. H_2SO_4, an intense red colour but somewhat bluer in tone than the solution in NaOH.

The identity of the metabolic product with chrysophanic acid was confirmed by acetylation at 100° with acetic anhydride containing a trace of conc. H_2SO_4. The acetyl derivative, which separated on pouring the acetylation mixture into ice and water, was isolated, washed and dried, sublimed in a high vacuum at 150–160°, and the sublimate crystallized from benzene. It was thus obtained as pale yellow plates, m.p. 208·5–209° alone, or in admixture with an authentic specimen of diacetyl chrysophanic acid, m.p. 208·5–209°, kindly supplied by the Wellcome Research Institution and prepared originally by Dr F. B. Power.

The other bands on the columns were scraped out separately, acidified, and extracted with ether. On removal of the ether none of the pigments so obtained gave reactions typical of islandicin.

SUMMARY

1. The colouring matters present in the mycelium of a strain of *Penicillium islandicum* Sopp, which, though morphologically typical, appears to be biochemically atypical, have been examined.

2. One of these colouring matters has been identified as chrysophanic acid (chrysophanol), 4:5-dihydroxy-2-methylanthraquinone.

3. Eleven other fungal colouring matters, the molecular structures of which are known, are shown to be derivatives of chrysophanic acid.

4. 1:4:5-Trihydroxy-2-methylanthraquinone, previously isolated from five other strains of *P. islandicum* but absent from the present strain, has now been named *islandicin*.

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Changes in Water and Ion Metabolism and in Kidney Functions During the Development of Oedema in Rats Fed on Protein-deficient Diets

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It was assumed until recently that 'famine oedema' is due to a decrease in the colloid osmotic pressure of plasma as the result of an inadequate intake of dietary protein. Some doubts as to the validity of this conception were raised when cases of 'famine oedema' in human beings were described, in which protein concentration and colloid osmotic pressure of the plasma were found to be normal (Govaerts & Lequime, 1942; Keys, Taylor, Mickelsen & Henschel, 1946). Furthermore, in rats fed on a protein-deficient diet it could be shown that tissue oedema started at a time when the plasma protein concentration, and hence the plasma colloid osmotic pressure, were still normal (Dicker, 1948*b*); and secondly, that there was no clear correlation between the degree of hypoproteinaemia, the fall of the colloid osmotic pressure of plasma and the magnitude of the extracellular fluid phase of tissues when tissue oedema had fully developed (Dicker, 1948*b*). Finally, it was found that starved rats developed tissue oedema only when free access to water was allowed, and that the increase of the extracellular space was proportional to the amount of water drunk by the animal (Dicker, 1949*b*). It is not clear whether the retention of water demonstrated in the latter findings is of renal or extra-renal origin, or whether both factors apply. The present paper attempts to investigate the mechanism of the incipient oedema by correlating changes in water and ion excretion with changes in the extracellular fluid phase of tissues, and by analysing kidney functions by clearance methods.

METHODS

Experimental animals and standard diet (HH). Adult male albino rats weighing 270–350 g. were used. Control rats were given the following diet: casein 25·0, wheaten starch 40·0, hardened ground-nut oil 12·5, dried yeast 15·5, salt mixture (Hawk & Bergeim, 1942) 4·0, cod-liver oil 3·0%. This diet yielded approx. 30 kg.cal./10 g.

Protein-deficient diets. Four different types of diet were used; one yielded the same amount of calories as the standard diet, i.e. approx. 30·0 kg.cal./10 g. (diet *BB*), but contained only 0·5% casein, while the others were low both in protein content and in calorific values. Of the latter, two (*TT* and *FF*) had a water content of about 75%, while the other diet (*DD*) did not contain more water than diet *BB*, i.e. 15·0%.

(*a*) *Low-casein, high-carbohydrate diet* (BB). Casein 0·5, wheaten starch 80·0, hardened ground-nut oil 12·5, salt mixture 4·0 and cod liver oil 3·0%. In addition, each animal received a mixture containing 30 μg. aneurin, 50 μg. riboflavin, 1 mg. nicotinic acid, and 15 μg. of tocopheryl acetate; 10·0 g. of this food yieded approx. 30·0 kg.cal.

(*b*) *Vegetable low-protein diet* (TT). This diet was the same as that used by Dicker, Heller & Hewer (1946). It had the following composition: fresh turnips 81·5, wheaten starch 7·0, hardened ground-nut oil 3·5, salt mixture 4·0 and cod liver oil 4·0%. Vitamins B and E were added in the same amount as in diet *BB*. This diet yielded approx. 8·0 kg.cal./10 g.

(*c*) *Wet filter-paper diet* (FF). This diet was devised to avoid the use of turnips which introduces a factor difficult to control. It was made up as follows: filter paper 4·7, casein 0·8, wheaten starch 7·0, hardened ground-nut oil 3·5, salt mixture 4·0, cod liver oil 4·0%, and was made up to a semi-liquid paste by addition of 76·0% water. Vitamins B and E were added in the same amount as in diet *BB*. This diet is very similar to the vegetable low-protein diet (*TT*), especially with reference to its protein and water content, and had approximately the same calorific value as the diet *TT*.

(*d*) *Dry filter-paper, low-protein diet* (DD). Casein 1·5, wheaten starch 13·3, hardened ground-nut oil 8·0, salt mixture 5·3, cod liver oil 5·3, and finely ground filter paper 66·6%. Additions of vitamins B and E were provided in the same amounts as in diet *BB*.