26. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS

64. EMODIC ACID (4:5:7-TRIHYDROXYANTHRAQUINONE-2-CARBOXYLIC ACID) AND ω-HYDROXYEMODIN (4:5:7-TRIHYDROXY-2-(HYDROXYMETHYL)-ANTHRAQUINONE), METABOLIC PRODUCTS OF A STRAIN OF *PENICILLIUM CYCLOPIUM* WESTLING

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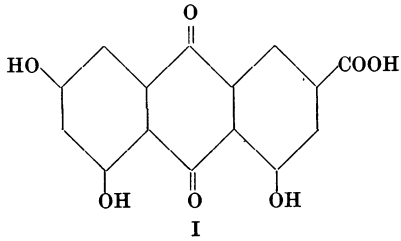
A NUMBER of polyhydroxyanthraquinones, all of which are derivatives of 2-methylanthraquinone, have been reported from these laboratories as metabolic products of different species of moulds. They are: helminthosporin, 4:5:8-trihydroxy-2-methylanthraquinone, from *Helminthosporium gramineum* Rabenhorst [Charles *et al.* 1933]; cynodontin, 1:4:5:8-tetrahydroxy-2-methylanthraquinone, from *H. cynodontis* Marignoni [Raistrick *et al.* 1933]; catenarin, a 1:5:8-trihydroxy-β-(hydroxymethyl)-anthraquinone, from *H. catenarium* Drechsler [Raistrick *et al.* 1934]; tritisporin, a 1:3:5:8-tetrahydroxy-β-(hydroxymethyl)-anthraquinone from *H. tritici-vulgaris* Nisikado [Raistrick *et al.* 1934]; physcion, 4:5-dihydroxy-7-methoxy-2-methylanthraquinone [Raistrick *et al.* 1937] and erythroglaucin, a monomethyl ether of a tetrahydroxymethylanthraquinone of at present undetermined molecular constitution, both of which were isolated from a large number of species in the *Aspergillus glaucus* series [Ashley *et al.* 1939]. The purpose of the present communication is to describe an investigation of the colouring matters present in the mycelium of a strain of *Penicillium cyclopium* Westling from which have been isolated two further derivatives of 2-methylanthraquinone, i.e. emodic acid, 4:5:7-trihydroxyanthraquinone-2-carboxylic acid and the hitherto undescribed *ω-hydroxyemodin, 4:5:7-trihydroxy-2-(hydroxymethyl)-anthraquinone.*

The strain of *P. cyclopium* used in this investigation (L.S.H.T.M. Cat. No. P 214), while otherwise morphologically indistinguishable from three other strains in our collection, differs from them in giving, under certain well defined conditions, a bright orange reverse. This colouring matter readily dissolves on treating the mould with cold dilute sodium carbonate solution to give an intensely crimson extract. Methods are described for the isolation of the colouring matter and for its fractionation into its constituents, emodic acid and ω-hydroxyemodin, which are present in approximately equal proportions and amount together to about 1·5% of the dry weight of the mould.

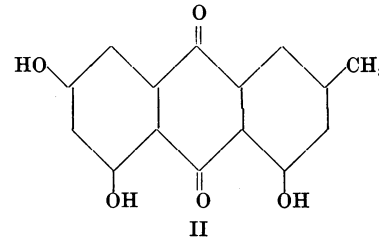
Emodic acid (I), $C_{15}H_8O_7$, M.P. 363–365°, forms orange needles which are readily soluble in cold dilute sodium bicarbonate solution. It gives a *monomethyl ester* and a triacetyl derivative which itself gives a monomethyl ester. The

identity of the mould emodic acid was placed beyond all doubt by direct comparison of the acid, its *methyl ester*, triacetate and *triacetyl methyl ester* with the corresponding derivatives of synthetic emodic acid prepared by the oxidation of the triacetate of *Frangula*-emodin with chromic acid in acetic anhydride-glacial acetic acid solution according to the method of Fischer & Gross [1911]. The constitution of emodic acid (I) as 4:5:7-trihydroxyanthraquinone-2-carboxylic acid follows from that of *Frangula*-emodin (II), 4:5:7-trihydroxy-2-methylanthraquinone, which has been settled by synthesis by Eder & Widmer [1923], Eder & Hauser [1925] and Jacobson & Adams [1924]. Although emodic acid has been prepared from *Frangula*-emodin by purely chemical means by a number of workers [Fischer & Gross, 1911; Eder & Hauser, 1925; Asahina & Fuzikawa, 1935], it has not been reported previously, so far as we are aware, from any natural source.

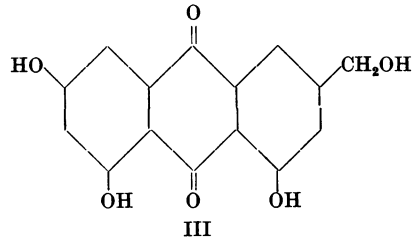
ω-Hydroxyemodin (III), $C_{15}H_{10}O_6$, forms dull orange needles, M.P. 288°. It is insoluble in sodium bicarbonate solution but, as is usual with hydroxyanthraquinones having a β-hydroxyl group, it readily dissolves in cold dilute sodium carbonate solution. It forms a *tetraacetate*, M.P. 190–191°, which on oxidation with chromic acid in acetic anhydride-glacial acetic acid solution gives triacetylemodic acid, identified by its conversion into free emodic acid, its methyl ester and triacetyl methyl ester. On reduction with hydriodic acid and red phosphorus in glacial acetic acid solution by the method used by Oesterle [1911] for the reduction of aloe-emodin (4:5-dihydroxy-2-(hydroxymethyl)-anthraquinone) to chrysophanic acid (4:5-dihydroxy-2-methylanthraquinone), ω-hydroxyemodin (III) is converted into *Frangula*-emodin (II). These facts can only be explained logically by assuming that ω-hydroxyemodin only differs from emodic acid (I) and *Frangula*-emodin (II) in having a CH_2OH group in the same position as the COOH group of emodic acid and as the CH_3 group of *Frangula*-emodin, and that it has, in fact, the molecular constitution 4:5:7-trihydroxy-2-(hydroxymethyl)-anthraquinone (III).



I



II



III

It is of some interest to note that *Frangula*-emodin (4:5:7-trihydroxy-2-methylanthraquinone), ω-hydroxyemodin (4:5:7-trihydroxy-2-(hydroxymethyl)-anthraquinone) and emodic acid (4:5:7-trihydroxyanthraquinone-2-carboxylic acid), all of which have now been obtained from natural sources, bear exactly the same relationship to each other as do the naturally occurring substances,

chrysophanic acid (4:5-dihydroxy-2-methylanthraquinone), aloe-emodin (4:5-dihydroxy-2-(hydroxymethyl)-anthraquinone) and rhein (4:5-dihydroxyanthraquinone-2-carboxylic acid).

While this investigation was in progress our attention was called to a recent communication [Posternak, 1939] on the pigments of *P. rubrum* Grasberger-Stoll and of *P. citreo-roseum* Dierckx. This paper gives a short preliminary account of the isolation of a pigment from *P. citreo-roseum* which has the formula $C_{15}H_{10}O_6$, melts at 273°, gives a tetraacetate, M.P. 187°, and from its general behaviour was believed by Posternak to be a polyhydroxyquinone. We were struck by the resemblance between this pigment and our ω-hydroxyemodin, and through the courtesy of Dr Posternak, whom we wish to thank, have exchanged specimens of the two pigments and of their acetates. Direct comparison of these specimens by both Dr Posternak and ourselves leaves no doubt that ω-hydroxyemodin from *P. cyclopium* and Dr Posternak's unnamed pigment from *P. citreo-roseum* are one and the same substance.

Experimental

Culture

The culture used throughout this work was isolated here in October 1938 by Dr T. Richards from mouldy gelatin. It bears Dr Richard's catalogue No. B 521 and the L.S.H.T.M. Cat. No. P 214.

We are indebted to our colleague, Mr G. Smith, for the following description of the organism. On Czapek agar (with 3% sucrose) colonies grow rapidly, spreading widely, blue-green turning duller with age, with fairly broad white margin, surface almost velvety but showing distinct granulation especially near the edges; drops large, colourless; reverse becoming faint brown from the centre outwards, becoming indistinctly zonate with age.

On wort agar colonies are slightly bluer but usually otherwise similar. On thin layers of medium, and with cultures exposed to daylight, the reverse colour is yellowish and occasionally bright yellow.

Under the microscope colonies show distinct fasciculation but no definite coremia. Conidiophores are faintly roughened, 3·2–4μ in diameter; penicilli compact, 3 times verticillate, normally with 2–3 unequal rami; conidia globose, smooth, 3μ in diameter.

The morphological findings correspond very closely with the diagnosis of *P. cyclopium* Westling given by Thom [1930], and, in culture on Czapek agar, P 214 is morphologically indistinguishable from *P. cyclopium*, L.S.H.T.M. Cat. No. P 123, obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland in 1931 and originally contributed to Baarn by Dr Charles Thom. A culture of P 214 was sent to Dr Thom who reported "It has been examined several times and morphologically your identification (as *P. cyclopium* Westling) is satisfactory."

Cultural conditions

The culture medium used throughout was a Raulin-Thom solution of the following composition: glucose, 75 g.; tartaric acid, 4·0 g.; ammonium tartrate, 4·0 g.; $(NH_4)_2HPO_4$, 0·6 g.; K_2CO_3, 0·6 g.; $MgCO_3$, 0·4 g.; $(NH_4)_2SO_4$, 0·25 g.; $ZnSO_4$, $7H_2O$, 0·07 g.; $FeSO_4$, $7H_2O$, 0·07 g.; distilled water to 1500 ml. This medium was distributed in 350 ml. amounts in batches of 100 one-litre conical flasks plugged with cotton wool, sterilized and sown with a spore suspension of *P. cyclopium*, P 214, grown for 1–2 weeks on beer-wort agar.

It was found that, when the flasks were incubated at 24° in the dark, pigment formation was very erratic, the growth obtained being often white with little evidence of colour in the reverse. Exposure to light appears to be essential for maximum pigment production and the optimum conditions proved to be to incubate the flasks in the open laboratory at an average temperature of 20–21°. Under these conditions about 5 times as much colouring matter was isolated as was obtained from duplicate flasks incubated at 24° in the dark.

Under the optimum conditions described the mould grew quickly and after 5 days the whole surface of the culture solution was covered with a thickly sporing, bright blue-green felt having an intensely yellow or orange reverse. The metabolism solution also gradually became yellow in colour but the bulk of the colouring matter remained in the mycelium. On shaking the mycelium with dilute aqueous sodium carbonate the yellow-orange reverse changes to a brilliant crimson colour and much of the pigment dissolves out giving a crimson solution.

The production of the orange-yellow colouring matter, at any rate in appreciable amounts, appears to be peculiar to the strain of *P. cyclopium*, Cat. No. P 214. This strain, together with three other authentic strains of *P. cyclopium*, were grown side by side on Raulin-Thom solution at room temperature in daylight. The other strains used were:

(*a*) *P. cyclopium*, L.S.H.T.M. Cat. No. P 123. Obtained from Baarn in 1931.

(*b*) *P. cyclopium*, L.S.H.T.M. Cat. No. B.B 143. Isolated by Mr G. Smith from rotting tulip bulbs in 1935.

(*c*) *P. cyclopium*, L.S.H.T.M. Cat. No. H.F. Isolated by Mr G. Smith from Kentish hops in 1936.

After 14 days' growth the mycelium and metabolism solution, in the case of P 214, had become bright yellow in colour. None of the other strains showed this bright yellow reverse but instead varied in colour from pale buff to purplish-brown with the metabolism solutions pale dirty yellow to brown. On treatment with dilute aqueous sodium carbonate there was in each case only a slight reddening which was not comparable with the intense crimson given by P 214. Extraction of the mycelia with dilute HCl and ether gave faintly yellow ethereal solutions which turned brown on shaking with aqueous sodium carbonate, whereas P 214 gave an intense crimson colour in the carbonate solution.

Isolation of the crude colouring matters

At the end of the incubation period the mycelium from each batch of 100 flasks was separated from the metabolism solution, which was discarded, by straining through butter muslin. The total mycelium was divided into two approximately equal parts. Each part was placed in a 10 l. bottle together with 5 l. of ether and 2·5 l. of 2*N* HCl. The contents of each bottle were vigorously stirred for 1 working day with a mechanical stirrer and were then set aside overnight. The ether-acid layer was drained from the mycelium, which was washed with about 1 l. of fresh ether. The combined ether extract and washings were separated from the acid layer, filtered, washed with water and dried over anhydrous $MgSO_4$. The golden-yellow ethereal solution was evaporated to 100–150 ml. and set aside for 2–3 hr. The golden-brown solid which separated was filtered and washed with a little ether, giving Fraction I consisting of a dry, golden-brown powder which did not melt completely below 310°. The ether mother liquors were evaporated to dryness giving a somewhat sticky residue which was extracted with boiling light petroleum (20–30 ml., B.P. 50–60°), filtered and dried giving Fraction II, which consisted of a dry brown powder melting with much decomposition and blackening between 290° and 310°.

The method of extraction described gives complete extraction of all colouring matters and the extracted mycelium no longer gives a colour with dilute sodium carbonate. It is much superior to other methods tried in preliminary experiments, e.g. extraction of the dried mycelium with ether in a Soxhlet apparatus or extraction of the undried mycelium with dilute sodium carbonate and acidification of the extract.

The experimental details of five representative batches, all of which were grown in the open laboratory, follow. In all cases all flasks were covered with a thick, crinkled, green, heavily sporing mycelium with a striking golden reverse. Average temperature, 20–21°. (Limits during night and day, 17–23°.)

Incubation period in days	16	19	18	17	18
Residual sugar by polarimeter, %	0·71	0·52	0·42	0·44	0·41
Yields of Fraction I, g.	5·4	5·1	5·5	4·6	5·3
Yields of Fraction II, g.	1·2	1·1	0·5	1·0	0·6

Fractionation of the crude colouring matters

Fraction I of the crude colouring matters consists essentially of a mixture, in about equal amounts, of emodic acid (4:5:7-trihydroxyanthraquinone-2-carboxylic acid) and the hitherto undescribed *ω-hydroxyemodin* (*4:5:7-trihydroxy-2-(hydroxymethyl)-anthraquinone*). Fraction II is a much cruder product containing a bigger proportion of emodic acid. In the earlier work these two substances were separated from Fraction I by a very laborious series of fractionations, first from ethanol and then from glacial acetic acid. A much simpler and more satisfactory method of separation depends on the fractionation of their acetates and regeneration of the parent substances by alkaline hydrolysis of the purified acetates.

A typical example of this method was carried out as follows: The mixture of crude colouring matters (2·0 g.) was suspended in acetic anhydride (20 ml.) and conc. H_2SO_4 (0·4 ml.) was added drop by drop. The pigment quickly dissolved to give a yellowish-brown solution which was warmed on the water bath for a few moments and was then boiled under a reflux condenser for 2 min. The cooled mixture was poured into ice-water. The brown oil which separated quickly crystallized and was filtered, washed and dried (2·8 g.). This material was dissolved in boiling ethanol (180 ml.), norite (0·5 g.) was added and the mixture was filtered and cooled. A woolly mass of pale yellow needles of almost pure *tetraacetyl-ω-hydroxyemodin* very quickly separated (1·06 g.) which, on recrystallization from ethanol, had a constant M.P. of 190–191°. The ethanol filtrate was evaporated to 40 ml. and, on standing, slowly deposited brownish-yellow irregular plates (1·12 g.) which, on recrystallization from glacial acetic acid (with norite) gave bright canary-yellow prisms or plates of triacetylemodic acid, M.P. 218–219°. A further small amount of crude triacetylemodic acid was obtained from the evaporated ethanol mother liquors.

The total yields obtained from five batches of 100 flasks per batch were: (*a*) Fraction I of crude colouring matters, 25·9 g., giving 34·7 g. of crude mixed acetates, from which were isolated 11·5 g. of tetraacetyl-ω-hydroxyemodin and 13·8 g. of triacetylemodic acid. (*b*) Fraction II of crude colouring matters, 4·4 g., giving 5·5 g. of crude mixed acetates from which were isolated 1·0 g. of tetraacetyl-ω-hydroxyemodin and 2·1 g. of triacetylemodic acid.

(A) *ω-Hydroxyemodin* (*4:5:7-trihydroxy-2-(hydroxymethyl)-anthraquinone*)

(1) *Preparation of ω-hydroxyemodin from its acetate.* Tetraacetyl-ω-hydroxyemodin (3 g., M.P. 190°) was heated with aqueous 2*N* NaOH (150 ml.) on a boiling water bath for 2 hr. in an atmosphere of nitrogen. The acetate quickly dissolved,

giving finally an intensely reddish-purple solution which, at the end of the hydrolysis period, was acidified with 2*N* HCl (225 ml.). The acidified mixture was heated for 30 min. to coagulate the gelatinous orange-yellow precipitate which was then separated by filtration, washed free from chlorides and dried. The dark brown solid (1·97 g.) was crystallized from methanol (300–400 ml.) with norite giving beautifully crystalline, dull orange, slender rods or needles (1·5 g.), M.P. 287–288°. The M.P. was raised to 288° (constant) by further recrystallization from methanol. (Found: on material dried to constant weight in a high vacuum at 120°, loss in wt. 7·0 %. C, 62·78, 62·61; H, 3·57, 3·72; N, nil; CH_3O, nil. $C_{15}H_{10}O_6$ requires C, 62·91; H, 3·52 %.)

ω-Hydroxyemodin may also be prepared from its acetate by boiling with methanol containing 3 % by volume of concentrated H_2SO_4.

ω-Hydroxyemodin is insoluble in cold 2 % aqueous $NaHCO_3$; readily soluble in cold *N* Na_2CO_3 and in *N* NaOH, the solution being in each case red in the bulk with a bluish shade in thin layers; with cold conc. H_2SO_4 it gives a reddish-orange colour in bulk with an eosin-like shade in thin layers. Emodin (4:5:7-trihydroxy-2-methylanthraquinone) behaves similarly with the above reagents and gives colours with them which are almost indistinguishable from those given by ω-hydroxyemodin.

(2) *Tetraacetyl-ω-hydroxyemodin* (*4:5:7:2′-tetraacetoxy-2-methylanthraquinone*). This acetate, whether prepared by fractionation of the crude mixed acetates as described above or by direct acetylation of ω-hydroxyemodin prepared by fractionation of the mixed colouring matters themselves, forms very characteristic pale yellow, soft, woolly needles, M.P. 190–191°, which are quite insoluble in either cold 2*N* Na_2CO_3 or in 2*N* NaOH. (Found: C, 60·81, 60·83; H, 3·98, 4·03; CH_3CO, 35·2, 35·0 %. $C_{23}H_{18}O_{10}$, i.e. $C_{15}H_6O_2$ $(O.CO.CH_3)_4$ requires C, 60·77; H, 4·00; $4CH_3CO$, 37·9 %.)

(3) *ω-Hydroxyemodin monomethyl ether* (*4:5-dihydroxy-7-methoxy-2-(hydroxymethyl)-anthraquinone*). A mixture of ω-hydroxyemodin (0·54 g.), methyl iodide (0·9 g.) and sodium methoxide (0·14 g. of sodium dissolved in 10 ml. of methanol) was heated in a sealed tube for 4 hr. in a boiling water bath. The initially intensely purple solution changed to a brownish orange in colour and deposited, on cooling, 0·38 g. of crystalline material which was crystallized from ethanol in pale dull orange plates which are paler and duller in colour than the parent substance. M.P. 229–231°. (Found: C, 63·93; H, 4·09; CH_3O, 10·0 %. $C_{16}H_{12}O_6$ requires C, 63·98; H, 4·03; $1CH_3O$, 10·3 %.) The substance is quite insoluble in cold 2 % aqueous Na_2CO_3 but dissolves in *N* NaOH and conc. H_2SO_4 giving solutions which are indistinguishable in colour from those given by the parent substance. Its constitution as the 7-methyl ether of ω-hydroxyemodin follows from its insolubility in dilute Na_2CO_3 and from its method of preparation which is that used for the methylation of β-hydroxy groups in polyhydroxyanthraquinones, α-hydroxy groups not being methylated under these conditions (cf. the conversion of *Frangula*-emodin into its 7-methyl ether, physcion [Jowett & Potter, 1903; Tutin & Clewer, 1910]).

(4) *Oxidation of ω-hydroxyemodin to emodic acid* (*4:5:7-trihydroxyanthraquinone-2-carboxylic acid*). Tetraacetyl-ω-hydroxyemodin was oxidized to triacetylemodic acid by the method described by Fischer & Gross [1911] for the oxidation of triacetylemodin to triacetylemodic acid. Tetraacetyl-ω-hydroxyemodin (2 g.) was dissolved at 50–60° in a mixture of glacial acetic acid (40 ml.) and acetic anhydride (40 ml.). The temperature was maintained at 50–60° while a mixture of chromic anhydride (4 g.) dissolved in water (3·2 ml.) and glacial acetic acid (40 ml.) was gradually added over 30 min. with constant

shaking. The bath was then raised to 65–70° and kept at this temperature for 3 hr. when a pure deep green colour had developed. The solution was now poured into hot water (1200 ml.) and left in the cold room overnight. The yellow crystalline product which had separated was filtered, washed and dried. Wt. 1·44 g., M.P. 218–219°. The product is almost pure triacetylemodic acid since a specimen of this substance made by oxidizing triacetylemodin by the same method (see below) melted at the same temperature, as did a mixture of the two acetates. Contrary to what might be expected on theoretical grounds tetra-acetyl-ω-hydroxyemodin appears to be rather more difficult to oxidize with chromic acid than is triacetylemodin.

The oxidation acetate (1·44 g.), without further purification, was hydrolysed by heating with 2*N* NaOH (75 ml.) on a boiling water bath for 2 hr. in an atmosphere of nitrogen. The deep reddish-purple solution was then acidified with 2*N* HCl (100 ml.), heated for 30 min. more to coagulate the orange gelatinous precipitate, filtered, washed free from chlorides and dried. Wt. 0·91 g. This dark brown amorphous solid was crystallized twice from glacial acetic acid (about 300 ml.) from which it separates, with very little loss, in shining orange needles. The product, emodic acid, (4:5:7-trihydroxyanthraquinone-2-carboxylic acid) behaves in a characteristic manner on heating. No change is obvious up to 340°, but at about 350° the substance begins to smoke and finally melts with decomposition at about 363–365°. The same behaviour was seen on heating synthetic emodic acid prepared from emodin, and mould emodic acid (see below) and with mixtures of either of these specimens with the oxidation product. (Found, on material dried in a boiling xylene bath: Loss in wt., 15·9 %. Theoretical for loss of 1 mol. of acetic acid, 16·7 %. C, 59·95, 59·86; H, 2·64, 2·77 %. Calculated for $C_{15}H_8O_7$, C, 59·99; H, 2·69 %.)

In order to avoid any dubiety as to the identity of the oxidation product with synthetic emodic acid, arising out of their high M.P., the *methyl ester* and *triacetyl-methyl* ester were prepared.

(5) *Methyl ester of the oxidation product* (*methyl emodate*). For method of preparation see p. 167. 0·7 g. of the oxidation product gave 0·57 g. of crude methyl emodate, M.P. 266–267°, which, on recrystallization from methanol, gave reddish-orange needles (0·53 g.), M.P. 268–269°, alone or in admixture with the methyl ester of synthetic emodic acid. (Found: C, 60·65; H, 3·38; CH_3O, 10·15, 9·7 %. $C_{16}H_{10}O_7$ requires C, 61·13; H, 3·21; $1CH_3O$, 9·9 %.)

(6) *Triacetate of the methyl ester of the oxidation product* (*methyl triacetyl emodate*). For method of preparation see p. 167. 0·3 g. of the above methyl ester gave 0·3 g. of recrystallized triacetate. Fine, pale yellow needles, *ex* glacial acetic acid, M.P. 188°. A mixture with the triacetyl methyl ester of synthetic emodic acid, M.P. 188–189°, melted at 188–189°. (Found: C, 60·24, 59·98; H, 3·82, 3·98; CH_3O, 7·4, 7·2; CH_3CO, 29·6, 29·3 %. $C_{22}H_{16}O_{10}$ requires C, 59·98; H, 3·66; $1CH_3O$, 7·05; $3CH_3CO$, 29·3 %.)

(7) *Reduction of ω-hydroxyemodin to* Frangula-*emodin* (*4:5:7-trihydroxy-2-methylanthraquinone*). A mixture of ω-hydroxyemodin (1 g.), glacial acetic acid (20 ml.), hydriodic acid (4 ml. sp. gr. 1·7) and red phosphorus (1 g.) was boiled under reflux for 4 hr. The reaction mixture was cooled, poured into water (200 ml.) and the pale brown colour of the solution was discharged by the addition of a slight excess of sodium bisulphite. After standing overnight the purple-brown precipitate was filtered, washed and dried. It was extracted with boiling acetic acid (about 200 ml.) and from the extract there was obtained a total of 0·72 g. of sand-coloured material which appeared under the microscope as almost colourless flat rectangular plates which, on heating, showed no change up to

250°, began to darken at 250–255°, and decomposed completely with intense blackening at 255–258°. (Jacobson & Adams [1924] give a decomposition point of 250–258° for emodin anthranol, 4:5:7-trihydroxy-2-methylanthranol, and Tutin & Clewer [1912] about 255°.)

The anthranol (0·71 g.) was dissolved in boiling glacial acetic acid (200 ml.), the solution cooled to 60°, and to it was quickly added a solution of chromium trioxide (0·75 g.) in water (10 ml.) and glacial acetic acid (10 ml.). The dark-brown reaction mixture was maintained at 60° for 30 min., and was then evaporated *in vacuo* to 25 ml. On standing, emodin (0·46 g.) separated in cinnamon-coloured rods, M.P. 255–256°. On recrystallization from toluene (norite), pure emodin was obtained in dull orange needles, M.P. 256–257°, not depressed on admixture with an authentic specimen of *Frangula*-emodin. (Found on material dried in a boiling xylene bath: C, 66·69, 66·74; H, 3·84, 3·61 %. Calc. for $C_{15}H_{10}O_5$, C, 66·64; H, 3·73 %.)

The identity of the reduction product was confirmed by conversion into the triacetate, yellow needles, *ex* ethanol, M.P. 198°, alone or in admixture with triacetylemodin.

(B) *Mould emodic acid (4:5:7-trihydroxyanthraquinone-2-carboxylic acid)*

(1) *Preparation of mould emodic acid from its acetate.* The acetate (3·0 g.) having the higher M.P. 218–219°, and obtained as the more soluble fraction of the mixture of acetates from the acetylation of the crude colouring matters, was heated with aqueous 2*N* NaOH (150 ml.) on a boiling water bath for 2 hr. in an atmosphere of nitrogen. The intensely reddish-purple hydrolysis solution was acidified with 2*N* HCl (210 ml.) and heated for 30 min. more to coagulate the orange-yellow gelatinous precipitate which was filtered, washed free from chlorides and dried (1·98 g.). This material was crystallized twice from glacial acetic acid (400–450 ml.), with norite, giving a mass of glistening orange needles, which show no change on heating up to 340°, smoke at about 350° and melt with decomposition at 362–363°. The M.P. was not depressed on admixture either with synthetic emodic acid from emodin or with the hydroxyanthraquinone, M.P. 360°, prepared by direct fractional crystallization from glacial acetic acid of the crude mixture of colouring matters. (Found: (*a*) on material dried to constant wt. in a high vacuum at 110°, loss in wt. 12·7 %. C, 56·60, 56·48; H, 3·20, 3·19 %. CH_3O, nil. $C_{15}H_8O_7, H_2O$ ($C_{15}H_{10}O_8$) requires C, 56·59; H, 3·17 %. (*b*) On material dried in a boiling nitrobenzene bath at 210°, loss in wt. 15·9 %. C, 59·78, 59·67; H, 2·85, 2·84 %. Calc. for $C_{15}H_8O_7$, C, 59·99; H, 2·69 %.)

Hydrolysis of the acetate (0·1 g.), M.P. 218–219°, with methanol (5 ml.) containing 3 % by volume of conc. H_2SO_4 for 2 hr. led to the formation, not of emodic acid, but of its methyl ester (0·06 g.), which crystallized from chloroform in glistening orange rectangular leaflets, M.P. 268°. (Found: C, 60·77, 60·77; H, 3·24, 3·17; CH_3O, 9·75 %. $C_{16}H_{10}O_7$ requires C, 61·13; H, 3·21; $1CH_3O$, 9·9 %.)

Mould emodic acid is readily soluble in cold 2 % aqueous $NaHCO_3$ giving a reddish-orange solution. It readily dissolves in *N* Na_2CO_3 and in *N* NaOH giving in each case a solution which is cherry-red in bulk with a blue shade in thin layers. With cold conc. H_2SO_4 it gives a reddish-orange colour when seen in bulk, with an eosin-like shade in thin layers, this colour being indistinguishable from that given by ω-hydroxyemodin with conc. H_2SO_4. Synthetic emodic acid from emodin and emodic acid from the oxidation of ω-hydroxyemodin behave similarly with the above reagents and give colours with them which are indistinguishable from each other.

(2) *The triacetate of mould emodic acid.* This acetate, whether prepared by the direct acetylation of mould emodic acid with acetic anhydride and conc. H_2SO_4 or by fractionation, as previously described, of the mixed acetates from the crude mould colouring matters, forms bright canary-yellow blunt-ended prisms from glacial acetic acid, which give a deep red solution in cold dilute Na_2CO_3. Its M.P., which varies somewhat with the rate of heating, is 218–219° and is not depressed on admixture with triacetylemodic acid obtained either by the oxidation of triacetyl emodin (see below) or by the oxidation of tetraacetyl-ω-hydroxyemodin (see p. 165). (Found: on material dried to constant wt. at 110° in a high vacuum, loss in wt. 12·80%; loss of 1 mol. acetic acid requires 12·35%. C, 58·75; H, 3·56; CH_3CO, 28·7%. Calc. for $C_{21}H_{14}O_{10}$, C, 59·14; H, 3·31. $3CH_3CO$, 30·3%.)

(3) *The methyl ester of mould emodic acid.* For method of preparation see below. 0·72 g. of mould emodic acid gave 0·49 g. of the methyl ester which on recrystallizing from methanol gave shining orange-red needles (0·39 g.), M.P. 270°. A mixture with synthetic methyl emodate (M.P. 268–269°) melted at 268–269°. (Found: CH_3O, 10·45%. $C_{16}H_{10}O_7$ requires for $1CH_3O$, 9·9%.)

(4) *The triacetate of the methyl ester of mould emodic acid.* For method of preparation see below. 0·2 g. of the above methyl ester gave 0·26 g. of acetate, which on crystallization from glacial acetic acid gave fine, pale yellow needles, M.P. 187–188°, alone or in admixture with the triacetyl methyl ester of synthetic emodic acid. (Found: C, 59·51; H, 3·57; CH_3O, 7·35%. $C_{22}H_{16}O_{10}$ requires C, 59·98; H, 3·66; $1CH_3O$, 7·05%.)

(C) *Synthetic emodic acid (4:5:7-trihydroxyanthraquinone-2-carboxylic acid) and its derivatives*

Emodin (4:5:7-trihydroxy-2-methylanthraquinone, M.P. 255°, supplied by Schering-Kahlbaum A.G. Berlin) (5 g.) was acetylated by heating at 60–70° for 30 min. with acetic anhydride (50 ml.) and conc. H_2SO_4 (1 ml. added drop by drop). The crude triacetylemodin obtained by pouring the acetylation mixture into ice-water was dried (7·0 g., M.P. 193–195°) and oxidized, according to the method of Fischer & Gross [1911] with chromic acid in a mixture of acetic anhydride and glacial acetic acid. Yield of crude triacetylemodic acid, 5·97 g., M.P. 218–219°. This almost pure product was hydrolysed with aqueous 2*N* NaOH to emodic acid which was crystallized from glacial acetic acid. Yield of recrystallized emodic acid, 4·11 g. Orange needles, M.P. 364–365° (decomp.) after smoking at about 350°. The details, modified for quantities, of the oxidation and hydrolysis are the same as those described for the oxidation of tetraacetyl-ω-hydroxyemodin and the hydrolysis of the resulting triacetylemodic acid (see p. 164).

Methyl emodate. A mixture of synthetic emodic acid (1 g.), methanol (100 ml.) and conc. H_2SO_4 (3 ml.) was refluxed for 3·5 hr. The emodic acid slowly dissolved (1·5 hr.) and, on standing, the esterification mixture deposited crystals of almost pure ester (0·84 g.), which were recrystallized from methanol. Glistening reddish-orange needles, M.P. 268°. (Found: C, 60·87, 61·00; H, 3·22, 3·40; CH_3O, 9·4, 9·3%. $C_{16}H_{10}O_7$ requires C, 61·13; H, 3·21; $1CH_3O$, 9·9%.)

The ester is insoluble in cold 2% aqueous $NaHCO_3$ but readily dissolves in cold *N* Na_2CO_3 and in cold conc. H_2SO_4 giving colours with these reagents which are indistinguishable from those given by emodic acid itself.

Triacetylemodic acid methyl ester. Methyl emodate (0·8 g.) was suspended in acetic anhydride (10 ml.) and conc. H_2SO_4 (5 drops) was added. On warming the mixture gently on the water bath the ester dissolved, giving a clear pale yellow

solution from which, on cooling, the acetate quickly separated in pale yellow needles (0·81 g.) which were filtered, washed with glacial acetic acid and recrystallized from this solvent. A further 0·19 g. of the acetate was obtained on pouring the acetic anhydride mother liquors into water. This acetate, which is very suitable for the identification of emodic acid, crystallizes from glacial acetic acid in fine, pale yellow needles which melt sharply at 188–189°, the melt resetting on cooling. (Found: C, 60·08, 60·30; H, 3·57, 3·78; CH_3O, 6·9, 7·0; CH_3CO, 28·3, 28·0 %. $C_{22}H_{16}O_{10}$ requires C, 59·98; H, 3·66; $1CH_3O$, 7·05; $3CH_3CO$, 29·3 %.)

SUMMARY

Emodic acid (4:5:7-trihydroxyanthraquinone-2-carboxylic acid) and the hitherto undescribed *ω-hydroxyemodin* (*4:5:7-trihydroxy-2-(hydroxymethyl)-anthraquinone*) have been isolated from the mycelium of a strain of *Penicillium cyclopium* Westling grown in daylight at 20–21° on Raulin-Thom solution.

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