the appropriate amino acids or, in common with the greater part of the depot fat of fishes, derived from direct assimilation from the diet (cf. Shorland, 1955).

SUMMARY

The liver oil of the New Zealand school shark (*Galeorhinus australis* Macleay) has been shown to contain traces (0.1–0.2%) of 13-methyltetradecanoic, (+)-12-methyltetradecanoic, 15-methylhexadecanoic and (+)-14-methylhexadecanoic acids.

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Studies in the Biochemistry of Micro-organisms

98. TRITISPORIN, AN ANTHRAQUINONE COLOURING MATTER OF *HELMINTHOSPORIUM TRITICI-VULGARIS* NISIKADO: STRUCTURE AND SYNTHESIS*

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Helminthosporium tritici-vulgaris Nisikado is a micro-fungus which is pathogenic to wheat (*Triticum vulgare* Vill.), on the leaves of which it produces a yellow-spot disease which occurs fairly frequently in Japan. Nisikado (1929*a, b*) first isolated the fungus from this source and described it. In the course of an extensive investigation on the production of hydroxyanthraquinones by species of *Helminthosporium*, Raistrick, Robinson & Todd (1934) showed that the dried mycelium of laboratory cultures of *H. tritici-vulgaris*, grown on Czapek–Dox solution, contains three such substances, i.e. catenarin, helminthosporin and tritisporin.

Catenarin, $C_{15}H_{10}O_6$, was also isolated from other species of *Helminthosporium*, e.g. *H. gramineum* Rabenhorst by Charles, Raistrick, Robinson & Todd (1933) and *H. catenarium* Drechsler—hence its name—and *H. velutinum* Link by Raistrick *et al.* (1934), who presented evidence which indicated that catenarin is a β -hydroxymethyl-1:4:5-trihydroxyanthraquinone with the β -hydroxymethyl group in position 2, 3 or 6, position 6 being preferred. On this basis catenarin would be 1:4:5-trihydroxy-6-

hydroxymethylanthraquinone (structure I). Later it was proved by Anslow & Raistrick (1940) by analytical methods that catenarin has a CH_3 - and not a $CH_2.OH$ - side chain and has in fact structure (II), and this conclusion was confirmed by the synthesis of catenarin by Anslow & Raistrick (1941).

Tritisporin, $C_{15}H_{10}O_7$, of which only a small amount was available for investigation at the time, was believed by Raistrick *et al.* (1934) to be a derivative of 1:4:5:7-tetrahydroxyanthraquinone, and they suggested that either structure (III) or (IV) should be attributed to tritisporin, which was

* Part 97: Raistrick & Rudman (1956).

known to form a pentaacetate insoluble in sodium carbonate, and hence cannot contain a carboxyl group.

Additional supplies of crude tritisporin from *H. tritici-vulgaris* having recently become available, we have reopened the question of the constitution of this interesting substance. Investigation of a specimen of tritisporin which was rigorously purified through its acetate showed that (i) the ultraviolet absorption spectrum of tritisporin is practically identical with that of catenarin; (ii) although tritisporin gives 2-methylanthracene on zinc-dust distillation, it does not contain a $C-CH_3$ grouping, as was proved by a Kuhn-Roth estimation.

It therefore seemed probable that the $C-C$ grouping in the 2-position is a $C-CH_2.OH$ grouping. Hence the possibility emerged that tritisporin is indeed ω -hydroxycatenarin of structure (III), and we have now been able to prove by synthesis that this deduction is correct.

The starting point of the synthesis is catenarin (II). Natural catenarin acetate (V, $R=H$) from *H. catenarium* was submitted to the Wohl-Ziegler reaction (Djerassi, 1948). A mixture of it, *N*-bromosuccinimide, benzoyl peroxide and carbon tetrachloride was refluxed for 24 hr. and from the rather complex mixture of reaction products there was isolated a crude bromoacetate, yellow prisms, m.p. 199–200°, partial hydrolysis of the tetraacetate having occurred. A solution of the bromoacetate in acetic anhydride was then debrominated by heating with anhydrous sodium acetate (or silver acetate), giving ω -acetoxycatenarin tetraacetate (V, $R=O.CO.CH_3$), fine pale-yellow needles, m.p. 217°, not depressed on admixture with natural tritisporin pentaacetate, m.p. 217°, from *H. tritici-vulgaris*. Hydrolysis of the synthetic acetate with methanolic sulphuric acid gave 1:4:5:7-tetrahydroxy-2-hydroxymethylanthraquinone (III) identical in all respects with natural tritisporin from *H. tritici-vulgaris*.

Since catenarin has already been synthesized (Anslow & Raistrick, 1941), that synthesis and the present work constitute a total synthesis of tritisporin.

The Wohl-Ziegler reaction has been used on at least two previous occasions for the synthesis of polyhydroxyanthraquinones of natural origin containing a β -hydroxymethyl group. By this means Neelakantan & Seshadri (1954) synthesized teloschistin (4:5-dihydroxy-2-hydroxymethyl-7-meth-

oxyanthraquinone), which occurs in the Indian lichen *Teloschistes flavicans*, from physcion (4:5-dihydroxy-7-methoxy-2-methylanthraquinone), a constituent of the same lichen and also of *Xanthoria parietina* and of species of moulds in the *Aspergillus glaucus* series (Raistrick, Robinson & Todd, 1937) and of *Penicillium herquei* (Galarraga, Neill & Raistrick, 1955). Joshi, Parkash & Venkataraman (1955) have similarly converted rubiadin (1:3-dihydroxy-2-methylanthraquinone) into lucidin (1:3-dihydroxy-2-hydroxymethylanthraquinone), which Briggs & Nicholls (1949, 1953) isolated from the dried bark of the New Zealand tree *Coprosma lucida*.

Raistrick *et al.* (1934) described the preparation of a hydroxycatenarin by the oxidation of catenarin (II) with manganese dioxide and concentrated sulphuric acid. They assigned structure (VI) to it in the mistaken belief that catenarin has structure (I), so that it is now clear that structure (VI) must be revised. We have repeated the preparation of hydroxycatenarin and have confirmed the experimental findings of Raistrick *et al.* (1934), and in particular its resemblance in appearance and many of its colour reactions to cynodontin (1:4:5:8-tetrahydroxy-2-methylanthraquinone). In addition, we have shown that hydroxycatenarin contains a $C-CH_3$ group and that its ultraviolet absorption spectrum indicates the presence of four α -hydroxyl groups, from which it follows that hydroxycatenarin has almost certainly structure (VII) and is therefore 8-hydroxycatenarin (1:4:5:7:8-pentahydroxy-2-methylanthraquinone).

This conclusion, which involves the assumption that a nuclear hydroxyl group is introduced *para* to a pre-existing α -hydroxyl group, i.e. in this case in position 8, is supported by the previously established fact that cynodontin (1:4:5:8-tetrahydroxy-2-methylanthraquinone) is formed by the oxidation with manganese dioxide and concentrated sulphuric acid of both helminthosporin (4:5:8-trihydroxy-2-methylanthraquinone; Raistrick, Robinson & Todd, 1933) and of islandicin (1:4:5-trihydroxy-2-methylanthraquinone; Howard & Raistrick, 1949).

Finally, as would be expected of a compound of structure (VII) which is a derivative of alizarin (1:2-dihydroxyanthraquinone), 8-hydroxycatenarin is a powerful mordant dyestuff and dyes white wool mordanted with chromium, aluminium or tin strong violet-blue, violet, or deep bluish pink respectively.

EXPERIMENTAL

Culture

The culture used in the preparation of tritisporin was a strain of *Helminthosporium tritici-vulgaris* Nisikado obtained in 1933 from the Centraalbureau voor Schimmelcultures, Baarn, Holland. It was received by them in 1929, as strain number 285, from Nisikado, who had isolated it from diseased wheat (*Triticum vulgare* Vill.). It bears the London School of Hygiene and Tropical Medicine catalogue number Ag 141.

Cultural conditions and characteristics

100 conical (11.) 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, 1000 ml.; pH adjusted to 5.5 by the addition of N-NaOH , 12.5 ml./35 l.), were sterilized at 100° for 1 hr. on each of three consecutive days. The flasks were then inoculated with part of a spore suspension in sterile distilled water of *H. tritici-vulgaris* prepared from thirty-three cherry agar slopes aged 16 days. The flasks were incubated at 24° in the dark and harvested after 43 days' incubation. The appearance of the cultures at the end of the incubation period was uniform. The upper surface of the mycelium was pale buff to brown and the reverse dark brown to chocolate coloured. The culture filtrate was deep purple brown and stained white muslin blue.

Isolation of crude colouring matters

At the end of the incubation period the culture fluid (residual glucose by polarimeter, 0.81%) was separated from the mycelium by decantation through muslin and was discarded. The mycelium was washed with water, pressed as dry as possible in a tincture press and dried thoroughly in a vacuum oven at $40\text{--}45^\circ$ (wt. of dried mycelium, 237 g.). It was then ground to a fine powder in a coffee mill and was defatted in a Soxhlet extractor for 1 day with light petroleum (b.p. $40\text{--}50^\circ$). The extracted mycelium was air-dried and re-extracted with boiling dioxan in a percolator for about 10 hr. Dioxan is a much more efficient solvent for the extraction of crude polyhydroxyanthraquinones than is CHCl_3 as used previously by Raistrick *et al.* (1934), but it also extracts other materials, notably mannitol. Hence the dioxan extract was evaporated *in vacuo* and the residue was dried (48.2 g.) and ground with much water to remove mannitol and other water-soluble material. The residue was then re-dried (36.0 g.), re-extracted with light petroleum (b.p. $40\text{--}50^\circ$) and finally dried in air, giving the main fraction of crude hydroxyanthraquinone colouring matters (23.45 g.), m.p. $215\text{--}229^\circ$.

This light-petroleum extract was combined with the initial extract with the same solvent, evaporated to about 150 ml. and held at 0° overnight. The bright-red solid which separated was collected by filtration (1.21 g.), m.p. $175\text{--}192^\circ$, and, on removal of the solvent from the filtrate, there remained crude 'fat' (42.2 g.).

Fractionation of the crude colouring matter

The crude colouring matter consists of a mixture of about 3 parts of catenarin to 1 part of tritisporin, together with a very small proportion of helminthosporin, the m.p. of the three colouring matters being 246° , 278° and 226° re-

spectively. The isolation of tritisporin in a pure state, which proved tedious, was carried out as follows. The crude mixture (23.5 g.) was extracted with isopropyl ether in a Soxhlet apparatus for several days and the crystalline fractions which separated from the dark-red fluorescent extracts were collected daily with the following results: fractions 1+2, 7.5 g., m.p. $220\text{--}230^\circ$; fractions 3+4, 5.7 g., m.p. $230\text{--}245^\circ$; fractions 5+6, 3.6 g., m.p. $245\text{--}255^\circ$; fraction 7 from the mother liquors, 2.6 g., m.p. $220\text{--}230^\circ$; dark-brown residue, 4.1 g. The combined fractions 3+4 were re-extracted for one working day with isopropyl ether. There separated from the cooled extract 2.3 g. of colouring matter, m.p. $230\text{--}240^\circ$, and there remained in the Soxhlet thimble 3.2 g. of colouring matter, m.p. $250\text{--}255^\circ$. This material, together with fractions 5 and 6, was then acetylated by refluxing for 10 min. with acetic anhydride (10 ml./g.) and conc. H_2SO_4 (3 drops/g.). The crude yellowish green solid acetate obtained on pouring the cooled acetylation mixture into ice water was collected and dried (about 1.5 g./g.). It was repeatedly crystallized to constant m.p. from ethyl acetate, giving finally tritisporin pentaacetate as fine, pale-yellow needles, m.p. 217° . (Raistrick *et al.* 1934, give m.p. 215° .) (Found: C, 58.55, 58.6; H, 3.97, 3.95; $\text{CH}_3 \cdot \text{CO}$, 43.7. Calc. for $\text{C}_{25}\text{H}_{20}\text{O}_{12}$, i.e. $\text{C}_{15}\text{H}_5\text{O}_2(\text{O} \cdot \text{CO} \cdot \text{CH}_3)_5$: C, 58.6; H, 3.93; $5\text{CH}_3 \cdot \text{CO}$, 42.0%.)

Hydrolysis of pure natural tritisporin pentaacetate

A solution of pure natural tritisporin pentaacetate (0.52 g.) in methanol (50 ml.) containing 3% (v/v) of conc. H_2SO_4 was boiled under reflux for 1.5 hr. The solid dissolved to an orange-red solution after 5–6 min. and tritisporin began to separate as red bunches of needles after 30 min. The crystalline tritisporin was collected, washed with ice-cold methanol and dried (0.26 g.; Calc. 0.31 g.), m.p. $257\text{--}260^\circ$.

The product (0.25 g.) was purified for analysis by crystallization from dioxan (20 ml.), giving tritisporin as brick-red bunches of prisms, m.p. $277\text{--}278^\circ$, containing solvent of crystallization. (Found: loss on drying in high vacuum at 130° , 12.55%. $\text{C}_{15}\text{H}_{10}\text{O}_7 \cdot \frac{1}{2}\text{C}_4\text{H}_8\text{O}_2$ requires 12.7%. Found on dried material: C, 59.6, 59.6; H, 3.51, 3.39; $\text{C} \cdot \text{CH}_3$, nil. Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_7$: C, 59.6; H, 3.33%.) Salient details of the ultraviolet absorption spectrum of natural tritisporin are given in Table 1.

Synthesis of tritisporin

The starting point of the synthesis was catenarin, which was isolated from the previously unused crude CHCl_3 extracts of the dried mycelium of *Helminthosporium catenarium* Drechsler (Raistrick *et al.* 1934, p. 564). These extracts consisted essentially of a mixture of catenarin and helminthosporin; since catenarin is readily soluble in cold dilute aqueous Na_2CO_3 and helminthosporin does not dissolve in this reagent, the two pigments were readily separated from each other. Parts of fractions 1, 2, 3, 5, 7 of the CHCl_3 extracts were weighed and stirred vigorously at room temperature for 0.5 hr. with 2% aqueous Na_2CO_3 (250 ml./g. of extract). The mixture was then filtered without delay from undissolved helminthosporin. The deep-purple filtrate was acidified with 2N-HCl and heated to flocculate the amorphous orange precipitate, which was collected, washed, dried and crystallized from ethanol, giving pure catenarin (15.0 g. from 20.7 g. of CHCl_3 extract) as red plates melting sharply

at 246°. This material was then acetylated in the usual way with acetic anhydride and a little conc. H_2SO_4 . The crude acetate so obtained was crystallized from ethyl acetate, giving catenarin tetraacetate as lemon-yellow rods, m.p. 234–235°.

A mixture of catenarin acetate (1.0 g.), *N*-bromosuccinimide (0.7 g.), benzoyl peroxide (0.1 g.) and CCl_4 (350 ml.) was refluxed for 24 hr. All reagents were carefully dried and the *N*-bromosuccinimide was freshly recrystallized from hot water. The CCl_4 was removed by distillation and the yellow residue was washed repeatedly with hot water to remove succinimide and unchanged *N*-bromosuccinimide. The undissolved residue was collected and dried. Catenarin acetate (5 g.) gave a total of 6.3 g. of this residue, which was crystallized from ethyl acetate (200 ml.), giving the crude bromoacetate (3.05 g.) as yellow prisms, m.p. 199–200°. A mixture of this bromoacetate (1.75 g.), fused sodium acetate (3.5 g.) and acetic anhydride (35 ml.) was refluxed for 1.5 hr. The de-brominated acetate which was recovered by treatment of the cooled acetylation mixture with ice water was collected and dried (1.59 g.). It was then purified by crystallization from ethyl acetate + charcoal, giving synthetic tritisporin pentaacetate (0.43 g. from 3.75 g. of bromoacetate together with 0.83 g. of somewhat lower m.p.). The acetate, which does not dissolve in cold dilute Na_2CO_3 solution, forms very fine, pale-yellow needles, m.p. 217° alone or in admixture with tritisporin pentaacetate of the same m.p. from *H. tritici-vulgaris*. (Found: C, 58.6, 58.7; H, 4.03, 3.99; CH_3CO , 41.3. Calc. for $\text{C}_{25}\text{H}_{20}\text{O}_{12}$, i.e. $\text{C}_{15}\text{H}_5\text{O}_2(\text{O.CO.CH}_3)_5$, C, 58.6; H, 3.93; $5\text{CH}_3\text{CO}$, 42.0%.) De-bromination of the bromoacetate (1.0 g.) was also effected, though somewhat less efficiently, by refluxing a mixture of it with silver acetate (0.8 g.) and acetic anhydride (10 ml.) for 6 hr. The solid material recovered after treating the acetylation mixture with ice water and drying was extracted with cold CHCl_3 and filtered from AgBr . The CHCl_3 solution was evaporated to dryness *in vacuo* and the residue was crystallized from ethyl acetate + charcoal, giving tritisporin pentaacetate (0.09 g., m.p. 216–217°, +0.16 g., m.p. 215–216°).

A mixture of synthetic tritisporin pentaacetate (0.357 g.; m.p. 217°) in methanol (35 ml.) containing 3% (v/v) of conc. H_2SO_4 was boiled under reflux for 1.5 hr. The solid dissolved to an orange solution in 10 min. and tritisporin began to separate as red needles after 20 min. The crystals were collected and washed with ice-cold methanol, giving tritisporin (0.188 g.), which was crystallized from dioxan (15 ml.) as brick-red prisms containing dioxan of crystallization. For analysis the crystals (0.156 g.) were dried to constant weight in high vacuum at 130°. (Found: loss on drying in high vacuum at 130°, 12.4%. $\text{C}_{16}\text{H}_{10}\text{O}_7$, $\frac{1}{2}\text{C}_{16}\text{H}_8\text{O}_8$ requires 12.7%. Found on dried material: C, 59.8; H, 3.35. Calc. for $\text{C}_{16}\text{H}_{10}\text{O}_7$: C, 59.6; H, 3.33%.) Salient details of the ultraviolet absorption spectrum of synthetic tritisporin are given in Table 1.

The general properties of synthetic tritisporin are indistinguishable from those of natural tritisporin from *H. tritici-vulgaris* when tested alongside. It melts at 278–279°, undepressed on admixture with natural tritisporin, m.p. 277–278°, but the m.p. is difficult to determine accurately. It is only slightly soluble in the usual organic solvents but may be crystallized readily from pyridine or, preferably, from dioxan. Its orange-yellow solution in glacial acetic acid has a greenish yellow fluorescence in daylight and a vivid

sulphur-yellow fluorescence in ultraviolet light. It dissolves in cold conc. H_2SO_4 to a reddish purple solution, which is much redder in tone than the bluish violet solution of catenarin in the same reagent. It gives a deep-violet ferric colour in ethanolic solution. With magnesium acetate in cold ethanol (Asahina & Shibata, 1954) it gives a deep-carmine solution with a pronounced red fluorescence in daylight. A similar but less intense colour with a pronounced red fluorescence is produced by boiling tritisporin in aqueous aluminium sulphate solution. It is insoluble in aqueous NaHCO_3 and cannot be extracted from CHCl_3 solution by this reagent. It dissolves readily in cold aqueous $\text{N-Na}_2\text{CO}_3$, which also extracts it from CHCl_3 solution. The resulting reddish purple solutions are almost identical in colour with that of its solution in conc. H_2SO_4 and are less blue than aqueous KMnO_4 . Its bluish purple solution in aqueous N-NaOH fades to colourless on standing overnight, with the formation of a slight purple precipitate.

Ultraviolet absorption spectra

The ultraviolet absorption spectra of natural and synthetic tritisporin and of 8-hydroxycatenarin were determined by means of a Hilger and Watts Uvispek spectrophotometer. The salient features of these spectra, together with those obtained by Birkinshaw (1955) on catenarin and cynodontin, are assembled in Table 1.

The following conclusions may be drawn from these spectra:

(i) The spectra of catenarin and of both natural and synthetic tritisporin (ω -hydroxycatenarin) are almost identical. Birkinshaw (1955) found a similar close agreement between the ultraviolet absorption spectra of emodin (4:5:7-trihydroxy-2-methylanthraquinone) and ω -hydroxy-emodin (4:5:7-trihydroxy-2-hydroxymethylanthraquinone). It appears therefore that the substitution of a CH_2OH group for a CH_3 group in the polyhydroxyanthraquinones has little effect on their ultraviolet absorption spectra.

(ii) The marked similarity in the 500–600 $\text{m}\mu$. region of the absorption spectra of 8-hydroxycatenarin and cynodontin confirms the chemical evidence that 8-hydroxycatenarin contains four α -hydroxyl groups.

8-Hydroxycatenarin

Finely powdered MnO_2 (1.5 g.) was added in portions over a period of 10 min., with mechanical stirring, to a solution held at 60–70° of catenarin (2 g.) in conc. H_2SO_4 (15 ml.). After stirring for a further 15 min., the mixture was cooled, poured into water (600 ml.) and then boiled for 15 min. The original brown-violet precipitate, which became dark brown on heating, was collected, washed with water and dried (2.26 g.). It was then boiled with pyridine (150 ml.) and unchanged MnO_2 was separated by filtration. The filtrate was concentrated to about 5 ml. *in vacuo* and cooled. The solid which separated was washed with a little pyridine and then with ether and finally dried (1.08 g.). It was purified by acetylation in the usual way with acetic anhydride (10 ml.) and conc. H_2SO_4 (4 drops). The resulting crude acetate (1.32 g.) was crystallized repeatedly from ethyl acetate, yielding 8-hydroxycatenarin pentaacetate as pale greenish yellow rods (0.43 g.), m.p. 235°. Raistrick *et al.* (1934) give m.p. 233–234°.

A solution of the pure acetate (0.307 g.) in methanol (30 ml.) containing 3% (v/v) of conc. H_2SO_4 was boiled

Table 1. *Ultraviolet absorption spectra in ethanol of catenarin, tritispurin, cynodontin and 8-hydroxycatenarin*

Substance	$\lambda_{\max}$ (m μ .) with (in parentheses) log ϵ							$\lambda_{\min}$ (m μ .) and (in parentheses) log ϵ
	λ 220-240	λ 240-260	λ 280-310	λ 488	λ 508-525	λ 545	λ 558-560	
Catenarin (1:4:5:7-tetrahydroxy-2-methylanthraquinone)	231 (4.51) —	255 (4.23) —	280 (4.24) 298 (4.03)	488.5 (4.16) —	508 (4.06) 515-525 (3.99)	—	—	— 345 (3.12)
Natural tritispurin (1:4:5:7-tetrahydroxy-2-hydroxymethyl-anthraquinone)	231 (4.50) —	255 (4.23) —	280 (4.26) 300.5 (4.05)	489.5 (4.18) —	510 (4.09) 525 (4.01)	—	—	— 350 (3.12)
Synthetic tritispurin (1:4:5:7-tetrahydroxy-2-hydroxymethyl-anthraquinone)	232 (4.50) —	256 (4.24) —	280 (4.27) 302 (4.05)	488 (4.20) —	508.5 (4.11) 522 (4.05)	—	—	345 (3.12) —
Cynodontin (1:4:5:8-tetrahydroxy-2-methylanthraquinone)	221 (4.22) 237.5 (4.32)	— — —	296 (3.63) —	— — —	518 (3.90) —	545 (3.88) —	558 (3.94) —	390 (2.56) —
8-Hydroxycatenarin (1:4:5:7:8-pentahydroxy-2-methyl-anthraquinone)	230 (4.33) —	244 (4.48) 258 (4.48)	308 (3.99) —	488 (4.03) —	520 (4.32) —	545 (4.30) —	560 (4.35) —	385 (3.21) —

under reflux for 1.5 hr. 8-Hydroxycatenarin began to crystallize from the eosine-coloured solution in 45 min. and was collected, after storing overnight at 0°, as a brown solid (0.177 g.) which was crystallized twice from dioxan, when it separated as brown plates with a bronze lustre containing solvent of crystallization, m.p. 318-320° with partial sublimation at about 285°. (Found: loss on drying in high vacuum at 130°, 21.7%. $C_{15}H_{10}O_7$, $C_4H_8O_2$ requires 22.6%. Found on dried material: C, 58.9; H, 3.40; $C-CH_3$, 5.6. $C_{15}H_{10}O_7$ requires C, 59.6; H, 3.33; $C-CH_3$, 5.0%.) Salient details of the ultraviolet absorption spectrum of 8-hydroxycatenarin are given in Table 1.

Solutions of 8-hydroxycatenarin and cynodontin in acetic acid are bluish red in colour, with a greenish yellow fluorescence in daylight, and both substances dissolve in cold conc. H_2SO_4 to a pure-blue solution. 8-Hydroxycatenarin gives an intense purple ferric reaction in ethanol which is very similar in shade to aqueous $KMnO_4$, a deep-violet solution with a fiery red fluorescence with magnesium acetate in ethanol and a pale-violet solution in boiling aqueous aluminium sulphate, in which it is almost insoluble. It is not soluble in aqueous $NaHCO_3$ nor is it extracted from $CHCl_3$ solution by this reagent. It is readily soluble in, and extractable from $CHCl_3$ solution by, 2% aqueous Na_2CO_3 , giving a pure-violet aqueous solution from which a deep-purple amorphous precipitate separates on standing for 2-3 hr., leaving a colourless supernatant. It forms an almost insoluble blue sodium salt in aqueous NaOH.

Dyeing tests with 8-hydroxycatenarin (VII)

White wool was mordanted with chromium, aluminium or tin, by using $K_2Cr_2O_7$, aluminium acetate or $SnCl_2$ as described by Cain & Thorpe (1913) and used by Howard & Raistrick (1955) in dyeing tests with asperthecin. A solution of 8-hydroxycatenarin (3 mg.) in dioxan (1 ml.) was added to water (5 ml.) with shaking, and the mordanted wool (0.1 g. approx. in each case) was immersed in it immediately.

The tubes were placed in a bath of cold water which was heated to boiling and held there for 1 hr. The dyed wool was then washed successively with water, soap solution and running water, and dried. Strong coloration of the wool was obtained with all three mordants: Cr, violet-blue; Al, violet; Sn, deep bluish pink. The wools dyed with 8-hydroxycatenarin are in each case much bluer in shade than those dyed with asperthecin.

SUMMARY

The mycelium of laboratory cultures of *Helminthosporium tritici-vulgaris* Nisikado contains three anthraquinone colouring matters: catenarin, tritispurin and helminthosporin. Tritispurin has been synthesized and shown to be ω -hydroxycatenarin, i.e. 1:4:5:7-tetrahydroxy-2-hydroxymethylanthraquinone. When catenarin is oxidized with manganese dioxide in concentrated sulphuric acid, it gives 8-hydroxycatenarin, i.e. 1:4:5:7:8-pentahydroxy-2-methylanthraquinone.

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The Oxidative Properties of an Oxalate-decomposing Organism, *Pseudomonas* OD1, with Particular Reference to the Synthesis of Citrate from Glycollate

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The isolation and properties of an organism belonging to the genus *Pseudomonas* which will grow on oxalate as sole source of carbon and energy have been reported (Jayasuriya, 1955). Washed suspensions of this organism, *Pseudomonas* OD1, were found to oxidize oxalate, and this reaction has been described. The oxidative metabolism of washed suspensions has now been studied further, particularly with respect to the oxidation of two carbon compounds and tricarboxylic acid intermediates, and the effect of sodium fluoroacetate. Of particular interest was the finding that citrate accumulates in the presence of glycollate plus sodium fluoroacetate, and this reaction has been investigated in detail. The present paper is a detailed report of these studies, preliminary accounts of which have already been given (Jayasuriya, 1954a, b).

MATERIALS AND METHODS

Organism. *Pseudomonas* OD1 has been described in full elsewhere (Jayasuriya, 1955). For the present studies, the organism was grown on a medium containing the following salts (mg./100 ml.): KH_2PO_4 , 136; Na_2HPO_4 , 213; $(\text{NH}_4)_2\text{SO}_4$, 50; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 20; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$, 0.25; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.25. In addition, the medium contained 1% (w/v) of one of the following carbon sources: potassium oxalate, sodium DL-lactate, sodium glycollate, sodium DL-malate, sodium succinate. Unless otherwise stated, the carbon source was

lactate. In each case, the pH was 7.0 and the medium was sterilized by autoclaving at 15 lb./in.² for 15 min. The organism was grown in penicillin flasks for 40 hr. at 25°. The conditions of growth and the preparation of washed cells have already been described fully (Jayasuriya, 1955).

With the medium containing oxalate there was no precipitation of calcium oxalate during preparation, the medium remaining clear after autoclaving, though the possibility that some calcium oxalate might be precipitated during growth cannot be excluded. The organism can, however, utilize calcium oxalate, since clear zones are produced around colonies growing on solid medium containing this compound. In addition, washed cells prepared after growth on the liquid oxalate medium described above have a low endogenous respiration, whereas they rapidly oxidize potassium oxalate (see Table 1). This would indicate that little if any calcium oxalate remains in the washed suspensions prepared from cells grown on oxalate, or that, if present, it has little effect upon the respiration.

Reagents. All reagents used, except those mentioned below, were of A.R. grade. Triethanolamine was distilled and purified by formation of the hydrochloride. Sodium glycollate was recrystallized from acetone before use. Oxaloacetic acid was kindly provided by Professor H. A. Krebs; solutions of the sodium salt were prepared immediately before use by neutralization with 0.01N-NaOH. 2-Oxoglutaric acid was kindly supplied by Dr R. L. Wickremasinghe. Sodium fluoroacetate was the gift of Dr B. C. Saunders of the Chemical Laboratory, University of Cambridge.

Analytical methods. Gas exchanges were measured with Warburg manometers at 25°. Where necessary, double-side-bulb cups were used and the reactions stopped by the addition of 0.2 ml. of 2N- H_2SO_4 from the second side arm.

Rates of substrate oxidation are expressed as $\mu\text{l. of O}_2$ consumed/mg. dry wt. of cells/hr. = Q_{O_2} . Values for CO_2

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