184. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS

68. SYNTHESIS OF CYNODONTIN (1:4:5:8-TETRA-HYDROXY-2-METHYLANTHRAQUINONE) A METABOLIC PRODUCT OF SPECIES OF *HELMINTHOSPORIUM*

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CYNODONTIN was first isolated by chloroform extraction of the dried mycelium of laboratory cultures of the fungi *Helminthosporium cynodontis* Marignoni and *H. euchlaenae* Zimmermann by Raistrick *et al.* [1933, 1] and by the same workers from *H. avenae* Eidam [1934]. It was shown to be almost certainly 1:4:5:8-tetrahydroxy-2-methylanthraquinone (III) since it was obtained *in vitro* [Raistrick *et al.* 1933, 1], by the oxidation with manganese dioxide and concentrated H_2SO_4 of helminthosporin, a metabolic product of *H. gramineum* Rabenhorst [Charles *et al.* 1933] and helminthosporin was shown by synthesis to be 4:5:8-trihydroxy-2-methylanthraquinone [Raistrick *et al.* 1933, 2].

We have now confirmed by synthesis the suggested molecular structure for cynodontin. 4-Methyl-3:6-dimethoxyphthalic anhydride (I) [Anslow & Raistrick, 1940] was condensed with 1:4-dimethoxybenzene (II) by treatment with anhydrous $AlCl_3$ in CS_2 solution. Simultaneous demethylation and ring closure of the resulting acid was effected by heating with conc. H_2SO_4 at 150° . The resulting polyhydroxyanthraquinone which must, from its method of synthesis, be 1:4:5:8-tetrahydroxy-2-methylanthraquinone (III) was identical in all its properties with cynodontin isolated from different species of *Helminthosporium*.

EXPERIMENTAL

(a) *Condensation of 4-methyl-3:6-dimethoxyphthalic anhydride with 1:4-dimethoxybenzene.* Freshly powdered anhydrous aluminium chloride (4.4 g.) was added at short intervals in five approximately equal amounts to a mixture of 4-methyl-3:6-dimethoxyphthalic anhydride (2 g.) and 1:4-dimethoxybenzene (4 g.) in dry carbon disulphide (50 ml.). The mixture, which quickly became

yellow and then orange in colour, was boiled under reflux for 8 hr. After standing overnight the supernatant CS_2 was decanted from the dark brown semi-solid mass which was decomposed with 2 *N* HCl (75 ml.), the mixture being cooled during the process. The residue remaining after evaporation of the CS_2 solution was added and the excess of 1:4-dimethoxybenzene was removed by distillation in steam. The oily residue quickly solidified on cooling and was extracted with ether (1 l.). The ether solution was dried over anhydrous MgSO_4 and on removal of the solvent the crude benzoyl acid was obtained as a brownish yellow oil which later solidified (2.44 g.).

(b) *Demethylation and ring closure of the substituted benzoyl acid.* The crude acid (2.44 g.), without further purification, was heated with conc. H_2SO_4 (20 ml.) in an oil bath at 150° for 35 min. The H_2SO_4 solution, which was initially greenish-blue in colour with a fine green fluorescence, became deep blue in colour with an intense red fluorescence after 15 min. heating. The anthraquinone, which was separated by pouring the cooled reaction mixture into iced water (600 ml.), was coagulated by heating on a boiling water bath for 30 min. and was separated by filtration as a chocolate brown amorphous powder (1.40 g.). This crude material was extracted with chloroform (Soxhlet) and there separated from the eosin-coloured extract brown leaflets with a bronze lustre (0.76 g.; M.P. $255\text{--}260^\circ$). Evaporation of the chloroform mother liquors and sublimation of the residue in a high vacuum gave a further 0.36 g. of crude anthraquinone.

(c) *1:4:5:8-Tetraacetoxy-2-methylantraquinone (cynodontin tetraacetate).* The anthraquinone (0.76 g.; M.P. $255\text{--}260^\circ$) separating from chloroform was purified by conversion into the acetate. It was dissolved in a mixture of pyridine (25 ml.) and acetic anhydride (5 ml.) and held at 37° for 2 days. The reaction mixture was filtered and the filtrate was poured into ice-water (400 ml.). The acetate (0.87 g.) which separated was crystallized twice from glacial acetic acid (norite). Pale yellow prisms (0.44 g.), M.P. $224\text{--}226^\circ$ alone or in admixture with the tetraacetate prepared from natural cynodontin. (Found: C, 60.57, 60.42; H, 3.99, 4.12 %. $\text{C}_{23}\text{H}_{18}\text{O}_{10}$ requires C, 60.77; H, 4.00 %.) A further 0.11 g. of pure acetate was obtained by acetylating in the same way the 0.36 g. of crude anthraquinone obtained from the chloroform mother liquors.

(d) *1:4:5:8-Tetrahydroxy-2-methylantraquinone (cynodontin).* Pure 1:4:5:8-tetraacetoxy-2-methylantraquinone (0.4 g.) was hydrolysed by heating under reflux on a boiling water bath with 2 *N* aqueous NaOH (30 ml.) in an atmosphere of nitrogen for 2 hr. The tetraacetate quickly dissolved to give an intensely blue-violet solution. The anthraquinone was precipitated with 2 *N* HCl (40 ml.), coagulated by heating on a boiling water bath for 30 min., filtered, washed and dried. This material (0.25 g.) was crystallized from pyridine (30 ml. with norite) and 1:4:5:8-tetrahydroxy-2-methylantraquinone was thus obtained in brown leaflets with a fine bronze lustre (0.19 g.; M.P. $260\text{--}261^\circ$), alone or in admixture with cynodontin from *Helminthosporium cynodontis*. (Found: C, 62.94, 62.88; H, 3.67, 3.65 %. $\text{C}_{15}\text{H}_{10}\text{O}_6$ requires C, 62.91; H, 3.52 %.) The synthetic and natural specimens are both insoluble in cold dilute aqueous Na_2CO_3 . Both dissolve in cold aqueous 2 *N* KOH to give deep blue-violet solutions and solutions of both in cold conc. H_2SO_4 are intense pure blue in colour with a fine red fluorescence.

SUMMARY

Cynodontin (1:4:5:8-tetrahydroxy-2-methylantraquinone), a metabolic product of different species of *Helminthosporium*, has been synthesized. 4-Methyl-3:6-dimethoxyphthalic anhydride was condensed with 1:4-dimethoxybenzene

and the resulting acid was simultaneously demethylated and converted into 1:4:5:8-tetrahydroxy-2-methylanthraquinone by heating with conc. H_2SO_4 . The synthetic and natural products were shown to be identical.

REFERENCES

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