115. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS

69. SYNTHESIS OF CATENARIN (1:4:5:7-TETRAHYDROXY- 2-METHYLANTHRAQUINONE), A METABOLIC PRODUCT OF SPECIES OF *HELMINTHOSPORIUM*

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CHARLES *et al.* [1933] isolated, by chloroform extraction of the dried mycelium of laboratory cultures of the plant pathogen *Helminthosporium gramineum* Rabenhorst, two hitherto undescribed polyhydroxyanthraquinones which they named helminthosporin and hydroxyisohelminthosporin. Later, Raistrick *et al.* [1934] isolated hydroxyisohelminthosporin from *H. velutinum* Link, *H. tritici-vulgaris* Nisikado and *H. catenarium* Drechsler, and since this polyhydroxyanthraquinone occurs in only relatively small amounts in the mycelium of *H. gramineum* but is the main coloured product of *H. catenarium* it was re-named catenarin. More recently, Anslow & Raistrick [1940, 1] showed by analytical methods that catenarin is 1:4:5:7-tetrahydroxy-2-methylantraquinone (V) and we have now confirmed this conclusion by synthesis.

Considerable difficulty was experienced before a satisfactory synthesis of catenarin was effected. Thus, although condensation of 4-methyl-3:6-dimethoxyphthalic anhydride with 1:4-dimethoxybenzene and subsequent ring closure of the resulting substituted benzoylbenzoic acid led to a straightforward synthesis of cynodontin (1:4:5:8-tetrahydroxy-2-methylantraquinone [Anslow & Raistrick, 1940, 2], this method failed when the same anhydride was condensed with 1:3-dimethoxybenzene. A substituted benzoylbenzoic acid was readily obtained but all attempts to effect ring closure proved fruitless. This recalls a similar experience of Graves & Adams [1923] in attempting to synthesize emodin (4:5:7-trihydroxy-2-methylantraquinone) from 3:5-dimethoxyphthalic anhydride and *m*-cresol and supports the conclusion of Jacobson & Adams [1924] that 'where, in the benzoylbenzoic acids, there is an *ortho* or *para* directing group *meta* to the position in which condensation to the anthraquinone must take place, the difficulty of forming the anthraquinone is especially pronounced'.

A successful synthesis of catenarin was finally achieved by a modification of the method used by Jacobson & Adams [1924] for the synthesis of emodin. 3:5-Dimethoxyphthalic anhydride (I) was condensed with *m*-cresol (II) and the resulting 3:5-dimethoxy-2-(2'-hydroxy-4'-methylbenzoyl)benzoic acid (III) was brominated to give 3:5-dimethoxy-2-(2'-hydroxy-4'-methyl-5'-bromobenzoyl)benzoic acid (IV). Ring closure of this substance was effected by heating at 100° with conc. H₂SO₄ and boric acid and the resulting condensation product, without isolation, was simultaneously demethylated and its bromine atom replaced by hydroxyl, by raising the temperature to 150–160°. Similar direct replacement of a halogen atom by hydroxyl has been previously described in the

conversion of 1-chloro-4-hydroxy-2-methylantraquinone into 1:4-dihydroxy-2-methylantraquinone, i.e. 2-methylquinizarin (Bayer and Co., D.R.P. 203,083; Ullmann & Schmidt, 1919).

The synthetic 1:4:5:7-tetrahydroxy-2-methylantraquinone agreed in all its properties with natural catenarin from *H. catenarium*.

EXPERIMENTAL

The starting point of the synthesis was orcin (3:5-dihydroxytoluene). This was converted into 3:5-dimethoxyphthalic anhydride by the following stages. The yields obtained are given in brackets. (a) Orcin $\rightarrow$ 3:5-dimethoxytoluene (76%); (b) 3:5-dimethoxybenzoic acid (26%); (c) methyl 3:5-dimethoxybenzoate, esterification effected with diazomethane in ether (95%); (d) 3:5-dimethoxytrichloromethylphthalide (94%); (e) 3:5-dimethoxyphthalide carboxylic acid (93.5%); (f) 3:5-dimethoxyphthalide (93%); (g) 3:5-dimethoxyphthalic acid (92%); (h) 3:5-dimethoxyphthalic anhydride (87%). The methods used were, in general, those described by Fritsch [1897] and Graves & Adams [1923] but the following modifications were introduced with advantage.

Oxidation of 3:5-dimethoxytoluene to 3:5-dimethoxybenzoic acid. 3:5-Dimethoxytoluene (20 g.) was stirred vigorously with a hot solution of KMnO_4 (40 g.) in water (1 litre), and heated to boiling under reflux. After boiling for 10 min. the permanganate was completely decolorized. Unchanged dimethoxytoluene was removed by steam distillation, the hot solution was filtered and the MnO_2 was well washed with hot water. The filtrate was cooled, acidified with conc. HCl and kept at 0° overnight, when 3:5-dimethoxybenzoic acid (3.1 g., m.p. 181°) separated. Only traces of the acid remained in the filtrate. The recovered dimethoxytoluene was re-treated with KMnO_4 . In this way 82 g. 3:5-dimethoxytoluene gave 25.9 g. 3:5-dimethoxybenzoic acid (26% of theory).

Oxidation of 3:5-dimethoxyphthalide to 3:5-dimethoxyphthalic acid. 3:5-Dimethoxyphthalide (22.45 g.) was dissolved by heating in a mixture of ethanol (250 ml.) and water (115 ml.) containing NaOH (11.5 g.). The ethanol was removed by steam distillation and to the cooled aqueous solution thus obtained there was added 3% KMnO_4 (850 ml.). The mixture was kept at room temperature overnight. The excess KMnO_4 was destroyed by adding an excess of ethanol and heating to 50° . The MnO_2 was separated by filtration, washed and

the filtrate evaporated *in vacuo* to 150 ml. After acidification with HCl the mixture was kept at 0° overnight. 3:5-Dimethoxyphthalic acid separated (24.0 g.; m.p. 155–156°; 92% of theory).

Condensation of 3:5-dimethoxyphthalic anhydride with m-cresol. 3:5-Dimethoxy-2-(2'-hydroxy-4'-methylbenzoyl)benzoic acid (III)

3:5-Dimethoxyphthalic anhydride (5 g.) was dissolved in warm freshly distilled *m*-cresol (30 ml.). The solution was cooled and to it was added freshly powdered anhydrous AlCl₃ (10 g.) in small amounts to control frothing. The deep red mixture was kept overnight and was then heated in an oil bath at 70° for 6 hr. with mechanical stirring. The reaction mixture became darker red in colour and, after 5½ hr. heating, was very viscous and difficult to stir. After standing overnight at room temperature, ice was added together with 2*N* HCl (50 ml.) and the excess *m*-cresol was removed by steam distillation. While still hot the supernatant aqueous layer (from which 0.65 g. of 3:5-dimethoxyphthalic acid separated on cooling) was decanted. The residual oil quickly solidified and was dissolved in *N* NaOH (180 ml.) and the solution saturated with CO₂. The dull yellow phthalein by-product which separated was filtered, washed and dried. Wt. 3.1 g. Acidification of the filtrate led to the separation of the crude benzoyl acid as a brick red solid, wt. 3.3 g., m.p. 198–203°. Pure 3:5-dimethoxy-2-(2'-hydroxy-4'-methylbenzoyl)benzoic acid was obtained from the crude product by trituration with ether (50 ml.) followed by crystallization from methanol (25 ml. + norite). The pure acid (1.45 g.; net yield 22%) was obtained as colourless rosettes of needles m.p. 233° which gave the following colour reactions: (a) with cold conc. H₂SO₄, orange red solution; (b) with FeCl₃ in alcohol, an intense brownish purple colour; (c) with *N* NaOH a bright yellow solution. [Found: C, 64.72; H, 5.12; OCH₃, 18.9%. C₁₇H₁₆O₆ requires C, 64.54; H, 5.10; 2CH₃O, 19.6%.] Graves & Adams [1923] first described 3:5-dimethoxy-2-(2'-hydroxy-4'-methylbenzoyl) benzoic acid (m.p. 233°) and claim a yield of 45% but Jacobson & Adams [1924] state 'the yields varied considerably and frequently did not exceed 20% of that calculated'.

Bromination of 3:5-dimethoxy-2-(2'-hydroxy-4'-methylbenzoyl)benzoic acid

Pure 3:5-dimethoxy-2-(2'-hydroxy-4'-methylbenzoyl)benzoic acid (2.43 g.) was dissolved in hot glacial acetic acid (86 ml.). The solution was immersed in a bath at 50° and to it was added, with mechanical stirring, 10% w/v solution of bromine in glacial acetic acid (13.85 ml.; calculated amount 12.28 ml.). The reaction mixture was maintained, with constant stirring, at 50° for 2 hr. The acetic acid was removed by vacuum distillation and the dry residue was refluxed with methanol (20 ml.) for 5 min. and filtered hot. Treatment of the filtrate is described below. The undissolved portion was dried, wt. 1.45 g. m.p. 256–258° and recrystallized from ethanol. 3:5-Dimethoxy-2-(2'-hydroxy-4'-methyl-5'-bromobenzoyl)benzoic acid (IV) was thus obtained as thick colourless rods; m.p. 260°, Jacobson & Adams [1924] give m.p. 258–259°. [Found: C, 51.8; H, 3.79; Br, 20.2; OCH₃, 15.6%. C₁₇H₁₅O₆Br requires C, 51.64; H, 3.83; Br, 20.2; 2CH₃O, 15.7%.] This compound gives the following reactions: (a) with cold conc. H₂SO₄ a dark red colour with an orange tone in thin layers; (b) in cold 2*N* NaOH it readily dissolved and on standing over the week-end deposited diamond shaped plates; (c) with FeCl₃ in alcohol an iodine brown colour.

Jacobson & Adams [1924] state that retreatment of the evaporated methyl alcohol filtrate, referred to above, with the calculated amount of bromine leads

to quantitative yields of 3:5-dimethoxy-2-(2'-hydroxy-4'-methyl-5'-bromobenzoyl)benzoic acid. We have been unable to confirm this statement. In our hands such treatment led to the formation of a dibromobenzoyl acid. This compound is more conveniently prepared in one stage as follows. 3:5-Dimethoxy-2-(2'-hydroxy-4'-methylbenzoyl)benzoic acid (0.5 g.) was dissolved in hot glacial acetic acid (20 ml.) and immersed in a bath at 50°. There was added, with mechanical stirring, a 10% w/v solution of bromine in glacial acetic acid (5.06 ml. i.e. twice the amount used in the preparation of the monobromo-acid). The reaction mixture was maintained with constant stirring at 50° for 2 hr., during which time pale yellow rods separated, wt. 0.64 g., m.p. 249° (efferv.). Recrystallization from ethanol gave pale yellow rectangular tablets m.p. 251°. [Found: C, 43.18; H, 3.04; OCH₃, 12.1; Br, 33.7%. Calc. for C₁₇H₁₄O₆Br₂; C, 43.04; H, 2.98; 2CH₃O, 13.1; Br, 33.7%.] This compound, which is probably 3:5-dimethoxy-2-(2'-hydroxy-4'-methyl-3':5'-dibromobenzoyl)benzoic acid gave the following reactions: (a) with cold conc. H₂SO₄ a dark red colour very similar to that given by the monobromobenzoyl acid but appearing blue in thin layers; (b) in cold 2N NaOH it was almost insoluble; after standing over the week-end the liquid was full of fluffy needles; (c) FeCl₃ in alcohol, iodine-brown colour.

Preparation of 1:4:5:7-tetrahydroxy-2-methylantraquinone (catenarin)

3:5-Dimethoxy-2-(2'-hydroxy-4'-methyl-5'-bromobenzoyl)benzoic acid (IV; 1 g.) was dissolved in conc. H₂SO₄ (25 ml.) containing boric acid (2.5 g.). The orange red solution was heated in an oil bath at 100° for 2½ hr. It rapidly became iodine-brown in colour and developed a purple tint after 20 min. The temperature was raised during 30 min. to 150–160° and was maintained at this temperature for 1 hr. The solution quickly became permanganate-purple in colour, changing after 30 min. to violet with a red fluorescence.

The reaction mixture was cooled, poured on ice, diluted with water (750 ml.) and boiled for 10 min. The flocculated precipitate, after cooling, was collected by centrifuging. It was washed with water, extracted with N/2 Na₂CO₃ (60 ml.) and separated from a trace of insoluble matter by centrifuging. The clear violet-red solution was saturated with CO₂ and the crude 1:4:5:7-tetrahydroxy-2-methylantraquinone which separated was collected by centrifuging. It was washed, suspended in water (50 ml.) and extracted with ether (2½ l.). Removal of the solvent from the dried ether solution yielded crude 1:4:5:7-tetrahydroxy-2-methylantraquinone (0.19 g.; m.p. 236–242°) as a dark red solid. This was purified by sublimation in a high vacuum at 170–180°, followed by recrystallization from ethanol. 1:4:5:7-Tetrahydroxy-2-methylantraquinone was thus obtained as glistening, deep red, rhombic plates, m.p. 246°, alone or admixed with catenarin from *Helminthosporium catenarium* Drechsler (m.p. 246°). [Found, on material dried to constant weight in a high vacuum at 100°; C, 62.65, 62.70; H, 3.70, 3.64%. Calc. for C₁₅H₁₀O₆; C, 62.91; H, 3.52%.] The colour reactions of the synthetic and natural materials were identical: (a) with cold conc. H₂SO₄; red-violet by transmitted light and blue-violet by reflected light, becoming bluer by transmitted light on the addition of boric acid and exhibiting a slight fluorescence; (b) with N NaOH; a violet-red solution bleaching to pale yellow on standing overnight; (c) with N Na₂CO₃ and N NH₄OH readily soluble in the cold, violet-red solution; (d) FeCl₃ in alcohol, purple-brown colour; (e) glacial acetic acid, orange yellow solution with a reddish tint in thin layers and a marked fluorescence.

1:4:5:7-Tetraacetoxy-2-methylantraquinone (catenarin tetraacetate). Synthetic 1:4:5:7-tetrahydroxy-2-methylantraquinone (0.38 g.) was suspended in 4 ml. of

a mixture of acetic anhydride (50 ml.) and conc. H_2SO_4 (1 ml.) and heated at 60–70° for 30 min. On cooling, 1:4:5:7-tetraacetoxy-2-methylantraquinone separated. Wt. 0.35 g. m.p. 221–226°. Two crystallizations from acetic anhydride raised the m.p. to 235°, not depressed on admixture with catenarin tetraacetate from *H. catenarium* (m.p. 235°). [Found: C, 60.94, 60.88; H, 3.80, 3.90; CH_3CO , 37.8, 38.05%. Calc. for $\text{C}_{23}\text{H}_{18}\text{O}_{10}$; C, 60.77; H, 3.99; $4\text{CH}_3\text{CO}$, 37.9%.]

SUMMARY

1:4:5:7-Tetrahydroxy-2-methylantraquinone has been synthesized. This compound was shown to be identical with natural catenarin, a metabolic product of different species of *Helminthosporium*.

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