CVIII. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS

LVIII. SYNTHESIS OF SPINULOSIN (3:6-DIHYDROXY-4-METHOXY-2:5-TOLUQUINONE) A METABOLIC PRODUCT OF *PENICILLIUM SPINULOSUM* THOM

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It was shown recently by Anslow & Raistrick [1938] that fumigatin (3-hydroxy-4-methoxy-2:5-toluquinone) could be converted by the Thiele-Winter [1900] reaction into a tetraacetate, the tetrahydric phenol from which yielded on oxidation a substance identical with the dihydroxymonomethoxytoluquinone isolated by Birkinshaw & Raistrick [1931] from cultures of *Penicillium spinulosum* Thom. The resulting conclusion that this substance, named by Anslow & Raistrick spinulosin, must be 3:6-dihydroxy-4-methoxy-2:5-toluquinone (V) has now been confirmed by synthesis.

The starting-point of the synthesis was toluquinone, which was converted into 4-methoxytoluquinone (I) [Luff *et al.* 1910; Ashley, 1937], from which, by the Thiele-Winter reaction, 2:3:6-triacetoxy-4-methoxytoluene was prepared. This substance, on hydrolysis, gave 2:3:6-trihydroxy-4-methoxytoluene (II) which, on oxidation with ferric chloride, gave 6-hydroxy-4-methoxy-2:5-toluquinone (III) [Anslow *et al.* 1938; Aulin & Erdtman, 1938]. The orientation of the latter follows from the following facts: (a) Konya [1900] obtained it by oxidizing 3-amino-2:6-dihydroxy-4-methoxytoluene with FeCl_3 ; (b) Pollak & Solomonica [1901] synthesized it by oxidizing 3-amino-2-hydroxy-4:6-dimethoxytoluene with FeCl_3 ; (c) on methylation it gives the same dimethoxytoluquinone, i.e. 4:6-dimethoxy-2:5-toluquinone, as does 4-hydroxy-6-methoxy-2:5-toluquinone on methylation [Anslow *et al.* 1938]. On treating 6-hydroxy-4-methoxy-2:5-toluquinone (III) with a large excess of methylamine in alcoholic solution, 4-methoxy-3:6-di(methylamino)-2:5-toluquinone (IV) was rather unexpectedly obtained. The formation of di(methylamino) derivatives of *p*-quinones by treatment with methylamine in alcoholic solution has been known for some time [Zincke, 1885; Kehrmann, 1891; Fichter, 1904; 1908; Hasan & Stedman, 1931; Kögl, 1926; Kögl & Becker, 1928]. So far as we are aware, however, in all recorded cases the methylamino group or groups enter the quinone molecule by substitution of a hydrogen atom attached directly to the nucleus. In the case of 6-hydroxy-4-methoxytoluquinone (III), however, not only is a nuclear hydrogen atom replaced by the CH_3NH grouping, but the OH group is also so replaced. 4-Methoxytoluquinone (I) reacts with methylamine under the above conditions but does not give rise to (IV). On hydrolysis of (IV) with 10*N* H_2SO_4 , spinulosin (V) was obtained identical in all respects with that isolated from cultures of *P. spinulosum*. The yields of intermediates and final product were good, 8.2 g. of spinulosin being obtained from 27.3 g. of 4-methoxytoluquinone.

On reduction with $\text{Na}_2\text{S}_2\text{O}_4$ spinulosin (V) gives the hitherto undescribed 3:6-dihydroxy-4-methoxytoluquinol (VI).

EXPERIMENTAL

2:3:6-Triacetoxy-4-methoxytoluene: 4-methoxytoluene (I) (27.3 g.), m.p. 172°, prepared from toluquinone by heating with ZnCl_2 in methyl alcohol [Ashley, 1937] was treated with 290 ml. of a cold mixture of acetic anhydride and conc. H_2SO_4 in the proportion of 100 ml. of the former to 5 ml. of the latter. The quinone slowly dissolved and the mixture stood at room temperature for 3 days with occasional shaking. It was then poured into iced water when the crude 2:3:6-triacetoxy-4-methoxytoluene quickly crystallized. It was filtered, washed well with water and dried *in vacuo* (yield 45.9 g.; 86.3%). m.p. 89–90° [Anslow *et al.* 1938, give 89–90°; Erdtman, 1933, gives 91–92°].

2:3:6-Trihydroxy-4-methoxytoluene (II) [Konya, 1900; Anslow *et al.* 1938]. 45.9 g. of the above crude acetyl compound were hydrolysed by heating under reflux for $\frac{3}{4}$ hr. in an atmosphere of N_2 with 230 ml. of a mixture of methyl alcohol (300 ml.) and conc. H_2SO_4 (10 ml.). Water was now added and residual methyl alcohol and methyl acetate formed during hydrolysis removed by distillation *in vacuo*.

6-Hydroxy-4-methoxytoluquinone (III). It was found to be unnecessary to isolate the phenol (II) before oxidation to the quinone. The hydrolysis solution was diluted with water to about 500 ml. and 115 ml. of a solution of FeCl_3 (50 g. anhydrous in 110 ml. H_2O) were quickly added with vigorous shaking. The quinone separated at once in golden brown microcrystals which were immediately filtered, washed with saturated aqueous NaCl , then with cold water and dried *in vacuo* (yield 24.5 g., 94.1%). m.p. 203° [Anslow *et al.* 1938, give 203–204°; Aulin & Erdtman, 1938, give 200–204°].

4-Methoxy-3:6-di(methylamino)-2:5-toluquinone (IV). It was found in small scale experiments that, if the quinone (III) is treated with the theoretical amount or a slight excess of methylamine in alcohol, the methylamine salt of (III) separates, but there is no formation of the di(methylamino) compound (IV). The

optimum conditions for the formation of (IV) were the following. The crude 6-hydroxy-4-methoxytoluquinone (III) (24.5 g.) was dissolved in boiling ethyl alcohol (50 ml. per g.), quickly cooled to 35° without shaking (so as to avoid crystallization) and 120 ml. of a solution of methylamine in ethyl alcohol (33 % w/w) were added (6–7 times the theoretical amount). The mixture which immediately became intensely purple in colour was held at room temperature for 3 days. 4-Methoxy-3:6-di(methylamino)-2:5-toluquinone (IV) slowly separated in large shining purple-black plates which were collected at the end of each day. The crystals, which were analytically pure, were filtered, washed with alcohol and dried, m.p. 215° (yield 11.6 g.; 75.6 %). (Found (Weiler): C, 57.02, 57.34; H, 6.63, 6.61; N, 13.0; OCH₃, 14.8 %. C₁₆H₁₄O₃N₂ requires C, 57.11; H, 6.71; N, 13.3; 1OCH₃, 14.8 %.)

3:6-Dihydroxy-4-methoxy-2:5-toluquinone, spinulosin (V). The above di(methylamino) compound (IV) (11.6 g.) was hydrolysed in 2 g. amounts by boiling with 10*N* H₂SO₄ (20 ml. per 1 g.). The di(methylamino) compound dissolved in the acid to give an intense purple-blue solution and after 3 min. boiling spinulosin crystallized out. The mixture was then cooled, spinulosin separated by filtration, washed well with cold water and dried; wt. 7.8 g., m.p. 200°. The filtrate and washings on extraction with ether gave a further 0.4 g. Total wt., 8.2 g. = 80.7 % of theory. The whole was recrystallized from boiling toluene in purple-bronze plates appearing brown under the microscope. A portion was readily sublimed at 110–120° in a high vacuum in purple-black micro-crystals. (Found (Weiler): C, 52.42, 52.34; H, 4.19, 4.35; OCH₃, 17.0, 16.7 %. C₈H₈O₅ requires C, 52.17; H, 4.38; 1OCH₃, 16.9 %.) m.p. 201° alone or in admixture with spinulosin isolated from cultures of *P. spinulosum* [Birkinshaw & Raistrick, 1931]. The synthetic and natural specimens also gave the same colour reactions with conc. H₂SO₄ (a deep pure blue), with 2*N* NaOH (an intense bluish purple) and with FeCl₃ in alcohol (a very dark rich brown).

3:6-Dihydroxy-4-methoxytoluquinol (dihydrospinulosin) (VI). Spinulosin (0.2 g.) was shaken for a few moments with Na₂S₂O₄ (4 g.) in water (20 ml.) when a colourless solution was obtained. This was extracted five times with an equal volume of ether and the ether removed, leaving a colourless crystalline residue (0.18 g.) which was sublimed in a high vacuum at 130–140°. The quinol sublimed in colourless prisms, m.p. 167°. (Found (Weiler): C, 51.68, 51.76; H, 5.28, 5.41; OCH₃, 16.6, 16.9 %. C₈H₁₀O₅ requires C, 51.59; H, 5.42; 1OCH₃, 16.7 %.) Dihydrospinulosin gives the following colour reactions: (a) with conc. H₂SO₄, bright yellow, becoming emerald green after 5 min. and plum colour overnight; (b) with 2*N* NaOH, yellow, becoming, on shaking, brown and finally an intense bluish purple indistinguishable from that given by spinulosin; (c) with FeCl₃ an alcoholic solution gives an intense greenish black.

SUMMARY

Spinulosin (3:6-dihydroxy-4-methoxy-2:5-toluquinone), a metabolic product of *Penicillium spinulosum* Thom, has been synthesized by the following series of reactions. Toluquinone → 4-methoxytoluquinone → 2:3:6-triacetoxy-4-methoxytoluene → 2:3:6-trihydroxy-4-methoxytoluene → 6-hydroxy-4-methoxytoluquinone → 4-methoxy-3:6-di(methylamino)-2:5-toluquinone → spinulosin. On reduction spinulosin gives 3:6-dihydroxy-4-methoxytoluquinol.

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