2, 4, 2′, 4′-Tetrahydroxyazobenzene, Synthetic Estrogen of a New Type

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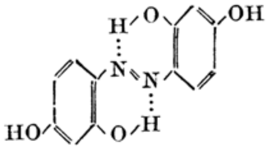
The authors synthesized 2, 4, 2′, 4′-tetrahydroxyazobenzene (4, 4′-azoresorcinol), 2, 4-$(HO)_2C_6H_3N{=}NC_6H_3(OH)_2$-2′, 4′, expecting that it might be estrogenic, because it might have a chrysene-shaped, rigid ring system by virtue of double chelation and thus its molecular shape might resemble those of potent natural and synthetic estrogens, except that it would be quite flat:



The diazo-oxide from 4-aminoresorcinol 1-benzoate, $C_6H_3(OCOC_6H_5)(OH)(NH_2)$-1, 3, 4, was coupled with resorcinol. 2, 4, 2′, 4′-Tetrahydroxyazobenzene 4-benzoate was obtained in yellow fine crystals melting at 235°. Saponification gave the desired tetrahydroxyazobenzene in orange-colored crystals. It did not melt at 300° but gradually turned black.

Estrogenic activity of the tetrahydroxyazobenzene was examined by the vagina smear test with ovariectomized mice, the substance in oil being injected subcutaneously in two portions. A weighed amount dissolved in acetone was mixed with camellia oil and acetone was evaporated up. The substance partly separated out in suspension. 20 γ produced full estrus in 100% animals, and 10 γ in 60%. By the same test method 1 mg. of 4, 4′-dihydroxyazobenzene⁽¹⁾ showed full action in 80% animals, and 500 γ in 20%.

The presumed position of the coupling was *a priori* very probable, because W. Will and W. Pukall⁽²⁾ obtained 2, 4-dihydroxyazobenzene as the main product by coupling benzenediazonium chloride with resorcinol. H. Kauffmann and W. Kugel⁽³⁾ obtained the same 2, 4-dihydroxyazobenzene by coupling benzenediazonium chloride with resorcinol monobenzoate and saponifying the formed 2-benzoate. The present authors coupled the diazo-oxide from 4-aminoresorcinol 1-benzoate with resorcinol monobenzoate. The product should be 4, 2′-dibenzoate of 2, 4, 2′, 4′-tetrahydroxyazobenzne by analogy. The substance obtained by saponification was the same in every respect as the tetrahydroxyazobenzene synthesized by using free resorcinol, but owing to the infusibilty of the substance it was impossible to identify both specimens by a mixed melting point.

The considerably strong action of the tetrahydroxyazobenzene provides a strong evidence for the structure consisting of the four hydroxy groups at 2, 4, 2′, 4′-positions and the formation of the chelate rings. Extra hydroxy groups introduced into natural or synthetic estrogens have hitherto always diminished activity to a great extent. The fact that the two more hydroxy groups at 2, 2′-positions give the tetrahydroxyazobenzene an activity very much stronger than that of 4, 4′-dihydroxyazobenzene proves that they form chelate rings with the azo nitrogen atoms and thus constitute a molecular structure, rigid and similar to those of strong natural and synthetic estrogens.

The 2, 4, 2′, 4′-tetrahydroxyazobenzene is an estrogen of a new type in that the molecular structure favorable for estrogenic activity is formed by chelation. But, as it is quite flat, such a potency as is comparable with that of diethylstilbestrol or hexestrol cannot be expected.⁽⁴⁾

Experimental Part

A 10% aqueous solution of sodium nitrite was added gradually to a solution of 4-aminoresorcinol 1-benzoate⁽⁵⁾ (2 g.) in 5% hydrochloric acid (30 g.), with cooling at 0~2° and occasional test with potassium iodide-starch paper. The precipitated diazo-oxide was collected, and washed with water. Pale yellow crystals melting at 117~119° with decomposition (yield nearly theoretical). Found: N, 11.75. Calculated for C_{13}-

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(1) R. Willstätter and M. Benz, *Ber.*, **39**, 3495 (1906).

(2) *Ber.*, **20**, 1121, 1145 (1887).

(3) *Ber.*, **44**, 2387 (1911).

(4) For molecular structure and estrogenic activity see: M. Ōki and Y. Urushibara, This Bulletin, **25**, 109 (1952).

(5) D. H. R. Barton, W. H. Linnell and N. Senior, *J. Chem. Soc.*, **1945**, 436.

$H_8O_3N_2$: N, 11.66%.

The diazo-oxide (1.8 g.) was added gradually with stirring to a solution prepared from potassium carbonate (5 g.), resorcinol (2 g.), and water (100 cc.). After standing overnight the reaction mixture was acidified with dilute hydrochloric acid, and the precipitate was collected and recrystallized from methanol (yield 2 g.). 2, 4, 2′, 4′-Tetrahydroxyazobenzene 4-benzoate thus obtained forms yellow crystals melting at 235°. Found: N, 7.95. Calculated for $C_{19}H_{14}O_5N_2$: N, 8.00%.

The above benzoate (1.5 g.) was dissolved in 15 % aqueous caustic potash, and the solution was heated for a few minutes on a waterbath, left to stand overnight, and acidified with hydrochloric acid. Repeated recrystallization of the precipitate from methanol with use of active charcoal gave 2, 4, 2′, 4′-tetrahydroxyazobenzene in fine orange-colored crystals (yield 1 g.). It did not melt but turned black gradually at 265~300°. Found: N, 11.10. Calculated for $C_{12}H_{10}O_4N_2$: N, 11.38%.

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