Estrogenic Action of Methylenimine Derivatives

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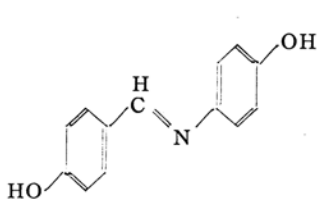
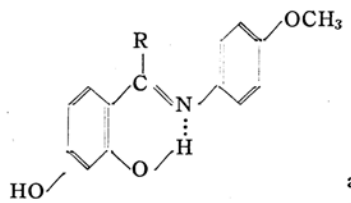
F. W. Schueler suggested that a compound, constituted of a rather large, rigid, relatively inert, and lipoid soluble molecular structure with two active hydrogen-bond forming groups located at an optimum distance of 14.5 Å units from each other may show estrogenic action.[1] He prepared sixteen azomethines and found an example to meet his hypothesis in 4, 4′-dihydroxydiphenylmethylenimine (I), which, administered subcutaneously, produced a true estrogenic response in rats in a total dose of 12.5 mg.[2] However, it must be noted that the activity is rather small for the fulfilment of the requirements.

M. Ōki and Y. Urushibara deduced the non-planar structure of stilbestrol and its homologs from their ultraviolet absorption data and presented a hypothesis that in addition to other requirements an optimum thickness of the molecule is an important factor for a strong estrogenic action.[3] The 4, 4′-dihydroxydiphenylmethylenimine prepared by Schueler has a thin structure, and its low activity might be attributed to the lack of the moderate thickness favorable for the development of a high activity.



I



II

R

a) CH_3

b) C_2H_5

c) $(CH_3)_2CH$

The author prepared alkyl 2,4-dihydroxyphenyl-4′-methoxyphenylmethylenimines (II), which probably have rigid structures by virtue of the chelate ring formation between the nitrogen atom and the ortho-standing phenolic hydrogen atom[4], and at the same time a thickness given by the steric interference of the alkyl substituent with the 4′-methoxyphenylimino group.[5]

The substituted methylenimines were prepared by condensing alkyl 2, 4-dihydroxyphenyl ketone with *p*-anisidine in the presence of anhydrous zinc chloride:

$$1\text{-}RCOC_6H_3(OH)_2\text{-}2,4+p\text{-}NH_2C_6H_4OCH_3 \rightarrow II$$

Estrogenic activities were evaluated by the vaginal smear test with ovariectomized mice by subcutaneous injection of oil solutions in two portions. Substance IIa (R: CH_3) was inactive in 1 mg. dosage. The substance IIb (R: C_2H_5) was fully active in 1 mg. dosage, and the substance IIc (R: $(CH_3)_2CH$) produced estrus in 40 % of the animals in the same dosage. The ethyl compound was the most active of the present series as in the stilbestrol homologs. As Schueler pointed out[2], a stronger estrogenic response may be expected when applied by intravaginal injection, because the methylenimine linkage is probably in part cleavaged in vivo before the compound reaches its site of action.

Experimental Part*

Alkyl 2, 4-dihydroxyphenyl Ketone. 1-$RCOC_6H_3(OH)_2$-2, 4.—Prepared according to Org. Syn. Vol. XXI, p. 103 (1941). 55 g. of resorcinol yielded 49 g. of methyl 2, 4-dihydroxyphenyl ketone melting at 142.3–144.3°C, and 48.5 g. of ethyl 2, 4-dihydroxyphenyl ketone melting at 98.2–99.2°C., respectively. 16 g. of resorcinol yielded 8.5 g. of isopropyl 2, 4-dihydroxyphenyl ketone bioling at 164°C./5 mm.

Methyl-2,4-dihydroxyphenyl-4′-methoxyphenyl-methylenimine (II a) 5 g. of methyl 2, 4-dihydroxyphenyl ketone and 6 g. of *p*-anisidine were mixed in a 20 cc. flask and 0.1 g. of anhydrous zinc chloride was added. The flask was immersed in an oil bath and heated at 140°C. for five minutes in a stream of carbon dioxide. The reaction mixture was cooled to the room tempera-

1) F. W. Schueler, *Science*, **107**, 94 (1948).
2) F. W. Schueler, *J. Amer. Pharm. Assoc.*, **39**, 87 (1950).
3) M. Oki and Y. Urushibara, This Bulletin, **25**, 109 (1952).
4) Cf. Y. Urushibara and T. Takahashi, This Bulletin, **26**, 162 (1953).
5) Cf. Reference 3.

* Melting points of methylenimines were determined in sealed capillaries, and corrected.

ture and digested with methanol. Recrystallization from acetone gave yellow plates melting at 262°C. with decomposition (Yield 8.2 g.). Found: C, 70.26; H, 5.80; N, 5.43. Calculated for $C_{15}H_{15}O_3N$ (II a): C, 70.02; H, 5.88; N, 5.44 %.

Ethyl- and isopropyl-2, 4-dihydroxyphenyl-4'-methoxyphenyl-methylenimine (IIb and IIc) Synthesized in the same way as the methyl compound. 5 g. of ethyl 2,4-dihydroxyphenyl ketone and 6 g. of *p*-anisidine yielded 6 g. of ethyl-2, 4-dihydroxyphenyl-4'-methoxyphenyl-methylenimine. Recrystallization from acetone gave yellow leaflets melting at 213°C. with decomposition. Found: C, 71.07; H, 6.07; N, 5.21. Calculated for $C_{15}H_{17}O_3N$ (II b): C, 70.83; H, 6.32; N, 5.16 %.

7.26 g. of isopropyl 2, 4-dihydroxyphenyl ketone and 7.06 g. of *p*-anisidine yielded 4 g. of isopropyl-2, 4-dihydroxyphenyl-4'-methoxyphenyl-methylenimine. Recrystallization from methanol gave pale yellow crystals melting at 197°C. with decomposition. Found: C, 71.42; H, 6.88; N, 4.82. Calculated for $C_{17}H_{19}O_3N$ (II c): C, 71.56; H, 6.71; N, 4.91 %.

These methylenimine derivatives were readily hydrolyzed by diluted hydrochloric acid to *p*-anisidine and corresponding alkyl 2, 4-dihydroxyphenyl ketone.

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