CXC. THE CONSTITUTION OF EQUOL.

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MARRIAN AND HASLEWOOD [1932], while endeavouring to isolate oestrone from a mare's urine extract supplied by Schering-Kahlbaum, A. G., isolated a new non-oestrogenic, optically active compound of the formula $C_{15}H_{14}O_3$. It was found that two of the oxygen atoms were present as hydroxyl groups and since the third oxygen atom failed to show reactivity, even as a carbonyl group, it was suggested that it was present in a cyclic ether linkage. Subsequent efforts to obtain further quantities of material for a more detailed chemical examination from extracts from other sources were unsuccessful. The present authors were therefore confronted with the problem of obtaining large amounts of equol and of discovering an explanation for its irregular appearance in mare's urine extracts.

During the summer of 1934 application of the somewhat lengthy isolation process used by Marrian and Haslewood gave regular yields of equol from the toluene extracts of pregnant mare's urine. Subsequently the isolation was considerably simplified by the discovery that equol could be directly crystallised by chloroform from the unpurified ether-soluble phenolic fraction of the toluene extract. Using this simplified process equol was also isolated in appreciable amounts from the urine of stallions and non-pregnant mares. Therefore its excretion is quite unspecific for pregnancy, and since large amounts were found in the urine of non-pregnant mares, there seems to be no reason to associate its presence in urine with the presence of large amounts of the oestrogenic hormones. During the autumn the amounts of equol isolated from urine extracts steadily decreased and in the winter months it was not possible to isolate it at all. So far as can be determined, no dietary factor was the cause of this variation and at present it is impossible to say whether this apparent seasonal fluctuation is fortuitous or not.

Preliminary experiments (unpublished) by Marrian and Haslewood showed that equol dimethyl ether was remarkably resistant to oxidation by alkaline permanganate whereas equol was readily attacked by potash fusion yielding resorcinol. The latter method of decomposition was therefore resorted to by the present authors in obtaining evidence on the structure of the compound. In the first instance a mild potash fusion of equol yielded β -resorcylic acid (2:4-dihydroxybenzoic acid), resorcinol and a phenol $C_{15}H_{14-16}O_3$ (isolated as its benzoate and subsequently hydrolysed). Since β -resorcylic acid is readily decarboxylated by potash fusion it is probable that resorcinol was formed indirectly from the former. The benzoate of the C_{15} phenol was found, on analysis, to contain 3 benzoyl groups, showing that the fusion had introduced a third hydroxyl group into the molecule. Methylation of the phenol with diazomethane yielded a dimethyl ether which gave a strongly positive Millon reaction indicating a free phenolic group.

Since it is well known that a phenolic hydroxyl group in the *o*-position to a side chain will not methylate with diazomethane, it seems probable that one

hydroxyl must occupy such a position. Further, the fact that equol on treatment with diazomethane yields the same dimethyl ether as with dimethyl sulphate indicates that it is the hydroxyl group introduced by the potash fusion which occupies the *o*-position to a side-chain.

Potash fusion of compounds in which an oxygen atom is linked directly to two aromatic rings yields products containing two new phenolic hydroxyl groups. Thus xanthone yields 2:2-dihydroxybenzophenone [Richter, 1883]; diphenylene oxide yields 2:2'-dihydroxydiphenyl [Kraemer and Weissgerber, 1901]; thyronine yields quinol and *p*-hydroxybenzoic acid [Harington, 1926]. On the other hand potash fusion of 1:2:3:4-tetrahydrodiphenylene oxide yields 2-hydroxydiphenyl [Hönigschmid, 1901], whilst decomposition of 3-phenylcoumarane with alcoholic potash at 200° yields α -(2-hydroxyphenyl) phenylethylene [Stoermer and Kippe, 1903]. It therefore seemed probable that the new hydroxyl group introduced was formed by the opening of an oxygen linkage between an aromatic ring and an aliphatic carbon atom.

On the basis of this the partial formula (I) could be constructed for equol, and since the residue ($C_7H_{10}O$) must contain an aromatic ring carrying the second phenolic hydroxyl group, the formula could be extended to (II).

A second potash fusion under more vigorous conditions was then carried out in order to ascertain if possible the nature of the second aromatic ring. The following products were isolated: resorcinol, *p*-hydroxybenzoic acid and *p*-ethylphenol. The two latter compounds were clearly formed from the second aromatic ring of equol. Since the latter contains only fifteen carbon atoms and since the partial formula (II) accounts for eight of them, it follows that the ethyl group of the *p*-ethylphenol must represent two carbon atoms of the aliphatic part of the molecule joining the two aromatic rings. Either carbon atom (*a*) or (*b*) of formula (II) must therefore be the β -carbon atom of the *p*-ethylphenol. In the

first case, formula (III) or (IV), in the second case formula (V) or (VI) must represent equol. Of these possibilities (VI) can almost certainly be excluded since it does not account for the formation of β -resorcylic acid and *p*-ethylphenol by potash fusion. Equol must therefore be 7-hydroxy-2-(4'-hydroxyphenyl)-chromane (III), 7-hydroxy-3-(4'-hydroxyphenyl)chromane (V), or 6-hydroxy-2-(4'-hydroxybenzyl)coumarane (IV).

EXPERIMENTAL.

Mild potash fusion of equol.

2 g. of equol were intimately mixed with 20 g. of finely powdered KOH and heated in a silver crucible to 210–240° for 30 minutes. After cooling, the melt was dissolved in water, the resulting solution saturated with carbon dioxide and extracted with ether. The ethereal solution after drying over calcium chloride was evaporated to dryness and the residue was distilled at 100°/0.02 mm. for several days. The sublimate consisted of 0.16 g. of a white crystalline substance (A) and 0.49 g. of a colourless "glass" (B).

(A) gave a positive fluorescein test and a slate-purple colour with aqueous ferric chloride. After one crystallisation from benzene it melted at 108–109°. Mixed with an authentic specimen of resorcinol (m.p. 110–111°), the m.p. was 109–111°. On nitration in the cold with a mixture of sulphuric and nitric acids, and recrystallisation of the product from dilute alcohol nitroresorcinol, m.p. 176–177° (not depressed by admixture with an authentic sample) was obtained.

Since (B) could not be crystallised it was benzoylated by the Schotten-Baumann method. The product after repeated crystallisations from ethyl alcohol melted at 133–134°.

(Found: C, 77.49, 77.21, 77.36; H, 5.60, 5.79, 5.78 %. $C_{15}H_{11}(O.CO.C_6H_5)_3$ requires C, 77.95; H, 4.73 %. $C_{15}H_{13}(O.CO.C_6H_5)_3$ requires C, 77.67; H, 5.07 %.)

0.1225 g. of the benzoate was heated to boiling with 10 ml. of 10 % alcoholic KOH. The mixture was then evaporated to dryness under a stream of carbon dioxide, and the dry residue extracted with ether. The ethereal solution was evaporated and the residue distilled at 140–150°/0.02 mm. for 5 hours. The sublimate was a clear, hard, slightly yellow glass and weighed 40.9 mg. After long standing the regenerated phenol (B) crystallised and melted at 136–137° with preliminary sintering at 130°.

(Found: C, 74.26, 74.14; H, 6.26, 6.61 %. $C_{15}H_{14}O_3$ requires C, 74.35; H, 5.83 %.)

33.3 mg. of the phenol (B) were methylated with diazomethane in dry ether. The product was purified by sublimation at 100–110°/0.03 mm. After long standing the sublimate crystallised and melted at 66.5–69° with preliminary sintering at 60°. The methylated product gave a strongly positive Millon reaction.

(Found: C, 75.50, 75.37; H, 7.25, 7.23 %; CH_3O , 22.74, 22.50 %. Mol. wt. 249, 250, 273. $C_{15}H_{12}O(OCH_3)_2$ requires C, 75.52; H, 6.72; CH_3O , 22.97 %. Mol. wt. 270.)

The aqueous phase from the solution saturated with carbon dioxide, from which the phenols had been extracted, was acidified with hydrochloric acid and extracted with ether. The ethereal solution after drying over calcium chloride was evaporated to dryness and the residue was distilled at 110°/0.02 mm. for 20 hours. 0.048 g. of a white crystalline sublimate was obtained. This material was purified by washing with cold benzene, recrystallisation from water and finally resubliming at 110°/0.02 mm. It melted with decomposition and evolution

of gas at 198°, and gave an intense purple colour with ferric chloride in aqueous solution. Mixed with an authentic specimen of β -resorecylic acid (m.p. 196°, evolution of gas) which had been purified by crystallisation from water and sublimation *in vacuo*, it melted at 196° with evolution of gas. The identification of the product as β -resorecylic acid was therefore considered to be complete.

Drastic potash fusion of equol.

1.76 g. of equol were heated at 300–320° for 80 minutes with 15 g. of KOH. The reaction product was worked up in the same manner as in the first fusion.

The phenolic fraction on distillation at 90–100°/0.04 mm. yielded 0.447 g. of resorcinol and a nearly colourless oil which on standing crystallised. The latter substance (C) melted at about 40° and boiled at 207°. With ferric chloride in aqueous solution it gave a blue colour. It had a faint cresol-like smell, and on benzylation yielded a benzoate which after two crystallisations from aqueous alcohol melted at 58–61° (unchanged when mixed with an authentic specimen of *p*-ethylphenyl benzoate). (C) was therefore *p*-ethylphenol.

The acidic fraction on sublimation at 140–150°/0.04 mm. yielded a white crystalline substance (D) which after two crystallisations from hot water melted at 208–211°. Mixed with authentic *p*-hydroxybenzoic acid (m.p. 211–213°), the m.p. was 209–213°.

On acetylation with acetic anhydride (D) yielded an acetate which after crystallisation from aqueous alcohol melted at 186–190°. Mixed with an authentic specimen of *p*-acetoxybenzoic acid (m.p. 187–189°), the m.p. was 184–189°. The identification of (D) as *p*-hydroxybenzoic acid was therefore considered to be complete.

SUMMARY.

1. Equol may be isolated directly from the ether-soluble phenolic fraction of toluene extracts of the urine of pregnant and non-pregnant mares and of stallions, by crystallisation from chloroform. Its presence in such extracts is irregular. No explanation for this irregularity can be advanced.

2. From the products of the fusion of equol with potassium hydroxide, the following substances have been isolated: a trihydroxyphenol, $C_{15}H_{14}O_3$ or $C_{15}H_{16}O_3$; resorcinol, β -resorecylic acid, *p*-ethylphenol, and *p*-hydroxybenzoic acid.

3. On the basis of these decomposition products equol is considered to be 7-hydroxy-2-(4'-hydroxyphenyl)chromane, 7-hydroxy-3-(4'-hydroxyphenyl)chromane, or 6-hydroxy-2-(4'-hydroxybenzyl)coumarane.

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