LXXXIV. PHENOL METHYL ETHERS AS OESTROGENIC AGENTS

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THE experimental material presented in this paper derives from two sources: (a) the observation that oestrone methyl ether is a powerful oestrogenic agent, not much less active than oestrone itself; (b) the occasional finding that fennel oil (*oleum foeniculi*) has a remarkable oestrogenic potency (500 rat units per ml. tested on infantile rats).

(a) Conventionally, the oestrogenic activity of oestradiol benzoate is ascribed to its hydrolytic fission in the body, the real agent being assumed to be the free phenolic compound [Miescher & Scholz, 1937]. This assumption is somewhat invalidated by the fact that oestrone methyl ether and oestradiol methyl ether produce the full vaginal cycle even in doses of 1–5 γ (under conditions where 0.5 γ of oestrone is active) although no demethylation would take place in the cell. Apparently, the free hydroxyl group is not at all essential for the activity of the molecule; its particular "haptophoric" properties are not required.

(b) Fennel oil is not the first product of the vegetable kingdom to exhibit oestrogenic activity. Oestrogenic activity has been found in flowers, potatoes, beetroot, yeast, by various authors as early as 1927 and 1929 [Loewe *et al.* 1927; Faure *et al.* 1927; Dingemanse & Laqueur, 1929]. Furthermore, it has been stated [Butenandt & Jacobi, 1933] that palm kernel oil produces the vaginal cycle in castrated mice, and the same holds true according to Aschheim & Hohlweg [1933] for petroleum and peat. But their activities are of quite a different magnitude from that of fennel oil (petroleum, peat and coal contain about 4000–2000 M.U./kg., the plant products mentioned even less, fennel oil 500,000 infantile R.U./kg.). A second main difference is that the activity of the above-named vegetable products is due to the presence of oestrone which has actually been isolated in crystalline state from palm kernel oil, while this possibility is excluded for fennel oil, which is prepared by steam-distillation. The action of the latter has undoubtedly to be ascribed to other "unspecific" oestrogenic agents.

In extending these experiments, a series of other natural essential oils has been tested. The results are reported in Table I. It is noteworthy that both the oils (fennel and anise oils) which showed marked oestrogenic activities derive from closely related plant species (Umbelliferae). On the other hand, the inactive dill oil derives also from an Umbellifera.

We were therefore led to the assumption that the activity is due to certain specific constituents, and started an investigation as to the nature of these constituents. Amongst them, anethole, which is present in both fennel and anise oils,

proved to be fairly active, and as it is related to oestrone methyl ether and oestradiol methyl ether, experiments were conducted to include various phenolic

Table I. *Oestrogenic activity of essential oils*

Name of the oil	Activity per ml.	Main constituents of the oil [Wehmer, 1929, 1931]
Fennel oil (<i>oleum foeniculi</i>)	500 R.U. (<50 M.U.)	Anethole, <i>p</i> -methoxyphenylacetone, <i>d</i> -fenchone, etc.
Anise oil (<i>oleum anisi</i>)	100 R.U. (<10 M.U.)	Anethole, estragol
Muscat oil (<i>oleum macidis</i>)	—	(+)-Pinene, (+) camphene, eugenole, <i>iso</i> eugenol, safrole
Eucalyptus oil	10 R.U. (0 M.U.)	Cineol, (+)-pinene, fenchene, camphene
Dill oil (<i>Anethum graveolens</i>)	—	Carvone, phellandrene, apiole
Bay oil (<i>Pimenta officinalis</i>)	—	Eugenol
Pine oil (<i>Pinus silvestris</i>)	—	Pinene, camphene, fenchone, etc.
Oil from Jaffa orange peel	—	Limonene, <i>d</i> -linalool, methyl anthranilate
Thymus oil (<i>Thymus vulgaris</i>)	—	Thymol, <i>p</i> -cymene, borneol
Oil of cloves (<i>oleum caryophyllorum</i>)	— (toxic)	Eugenol, acetyleneugenol, caryophyllene, etc.
Oil of cinnamon (<i>Cinnamomum ceylonium</i>)	— (toxic)	Cinnamic aldehyde, eugenol, phellandrene

methyl ethers and similar derivatives.¹ We detected a certain activity in some of the tested materials, as may be seen from Table II. Even the most potent agents amongst them are much less active than oestrone itself. Unfortunately, no rule governing the correlation between chemical structure and oestrogenic activity in these series can be derived from Table II. In the *p*-methoxy-series, e.g. the propenyl compound is active, the allyl derivative not, while for the 3:4-dimethoxy-series the reverse holds true.

Table II. *Oestrogenic activity of phenolic methyl ethers*

Name	Activity
Anethole	50 M.U., 100 R.U./ml.
Estragol	—
Dihydroanethole	<10 M.U., 10 R.U./ml.
Anisole	10 M.U./ml. (toxic)
Eugenol methyl ether	— (toxic in 0.1 g. doses)
<i>iso</i> Eugenol methyl ether	— (toxic in 0.1 g. doses)
Dihydroeugenol methyl ether	—
Veratrole	—
Anisaldehyde	—
Veratric aldehyde	10 R.U./ml., negative with mice
Vanillin	—
Piperonal	—
<i>p</i> -Methoxycinnamic aldehyde*	— (toxic in 0.1 g. doses)
Veratryl alcohol	— (toxic)
<i>p</i> -Methoxyphenylacetone†	—

* Cinnamic aldehyde and cinnamyl alcohol were also negative.

† Constituent of fennel oil.

It may be noted that hydrogenation of the carbonyl group in veratric aldehyde destroys the activity, while the same operation in the oestrone and androsterone series increases the activity [David *et al.* 1933; 1935; Schoeller *et al.* 1935; Doisy *et al.* 1933]. One obtains the impression from the above data and

¹ As fennel oil is more active than anethole, its oestrogenic activity cannot be ascribed exclusively to that substance, but rather to another still unknown constituent of the natural oil.

from those presented by previous authors [Dodds, 1936, 1, 2], that the activity of a given organic molecule is not determined directly by constitution but by certain other factors, which may or may not be connected with structural features.

It has been pointed out already that the magnitude of activity is much smaller for the substances studied than for oestrone. It was unexpected, therefore, when Dodds & Lawson [1937, 1] reported anol (*p*-propenylphenol), the mother-substance of anethole, to have the same degree of oestrogenic activity as oestrone. We too were unable to confirm these results, which have been recently withdrawn by the British authors [Dodds & Lawson, 1937, 2]. Pure anol is only slightly active (100 M.U., 1000 R.U./g.), but when its preparation from anethole has not been carried out with special care, it is contaminated by a non-crystallizable substance which increases the total activity (1000 M.U., 5000–10,000 R.U./g.), but still many times smaller than that of follicular hormone. Dodds & Lawson tentatively ascribe the formation of this active by-product to a polymerization reaction.²

Finally it has to be reported that our experiments showed a different behaviour towards the tested preparations of infantile female rats and castrated mice, respectively. The relevant results are included in Table II. (Similar observations have been reported by Behrens *et al.* [1937] and by Rowe & Simonds [1937].

EXPERIMENTAL

Anethole, anisole, eugenol methyl ether, veratrole, anisaldehyde, vanillin and piperonal were commercial products which we purified by careful distillation or recrystallization; *p*-methoxycinnamic aldehyde was prepared according to Scholtz & Wiedemann [1903]. Estragol was prepared according to Tiffeneau [1904].

Dihydroanethole. Anethole (14.8 g.) in alcoholic solution (300 ml.) absorbed in presence of palladized BaSO₄ (1 g., 5%) the theoretical amount of hydrogen (2519 ml., 759 mm.; 34°) within 55 min. at room temperature. The product had B.P. 119°/42 mm. and was obtained in quantitative yield (cp. Albright [1914]).

isoEugenol methyl ether. Eugenol methyl ether (10 g.) was boiled with 7.5% methyl-alcoholic KOH (40 ml.) for 2 hr. The methyl alcohol was evaporated from a water bath as far as possible and the residue taken up with water and ether. B.P. 152°/45 mm.; yield, 7 g. [Eijkman, 1890].

Dihydroeugenol methyl ether. Eugenol methyl ether (8.9 g.) was hydrogenated as above in alcoholic solution (25 ml.). The required amount of hydrogen (1250 ml.; 29°, 756 mm.) was absorbed quickly; the hydrogenated product had B.P. 151–153°/46 mm.¹

Veratric aldehyde was prepared according to "Organic Syntheses" (vol. 13, p. 102 (1933); 16, 91 (1936)) and was converted into *veratryl alcohol* according to Decker & Pschorr [1904]. The aldehyde (46 g.) was boiled for 1 hr. with 15% methyl-alcoholic KOH (140 ml.). The mass was poured out into water (1 l.) and the veratryl alcohol extracted from the alkaline mixture by means of ether. B.P. 125–130°/0.2 mm.; yield, 13 g.

p-Methoxyphenylacetone. Estragol (10 g.) in alcohol (150 ml.) was treated with mercuric oxide (45 g.) and solid iodine (51 g.) added in small portions, so as

¹ Catalytic hydrogenation has not been applied before to eugenol methyl ether. Cp. Beilstein, vol. 6, p. 920 (Berlin 1923).

² Recently synthetic dimeric anol has been shown to be active [Campbell *et al.* 1938; cf. Serini & Steinwerk, 1937].

to avoid too great a rise in temperature. After 48 hr. the mass was poured into a large volume of water and the estragol methoxyiodide,

formed [Daufresne, 1907; 1908, 1, 2; Bougault, 1900] extracted with ether. Even in a high vacuum (B.P. 165–170°/1.5 mm.) the product could not be obtained completely iodine-free and colourless; it was converted directly into *p*-methoxyphenylacetone according to Daufresne.¹ The preparation obtained (37 g.) was kept with silver nitrate (24 g. finely ground) in alcohol (250 ml.) at room temperature for 2 hr. and then boiled for 7 hr. The filtered solution was concentrated, the residue taken up with water and ether and the ethereal layer distilled. B.P. 118–120°/0.2 mm.; yield, 17 g.

Anol. Anethole was hydrolysed according to Stoermer & Kahlert [1901] with alcoholic KOH in an autoclave filled with nitrogen (10 atm. pressure at the beginning). The reaction mixture was taken up with water and ether, the alkaline solution acidified and the anol so obtained extracted with ether. It boils at 95°/0.3 mm., and the colourless distillate crystallizes spontaneously. The crystals, after drying on a porous plate, have M.P. 90°.

Oestrone methyl ether was prepared from oestrone (1 g.) with methyl *p*-toluene-sulfonate (1.3 g.) and 10% KOH (9 ml.) [Cohen *et al.* 1935; cp. Butenandt & Hildebrandt, 1931; Marrian & Haslewood, 1932]. The precipitate was recrystallized from methanol, M.P. 170°. In the same way oestradiol gave pure *oestradiol methyl ether*: it was obtained first as an oil, which crystallized on trituration with methyl alcohol and could be recrystallized from the same solvent. M.P. 97° [cp. Danielli *et al.* 1933].

Test methods

For the animal tests, the substances were injected in three portions, within 24 hr.; liquids were used in the homogeneous state, solids in oil solution. Twice daily vaginal smears have been taken; only full oestrus has been taken as positive.

The tests have been carried out with (a) adult castrated mice, average weight 20 g., and (b) infantile non-castrated rats, average weight 25–30 g., occasionally with (c) adult castrated rats, average weight 150–200 g. R.U. (rat units) and therefore do not refer to the conventional test carried out on castrated sexually mature rats, but to infantile rats. As pointed out in the theoretical part of this paper, an unexpected observation has been made in these comparative experiments. Although the infantile rat has about the same weight as the adult mouse, some of the tested materials showed higher activities with rats than with mice (more than tenfold). These observations recall the well-known fact, that for oestrone the requirements of an adult castrated rat (100 g.) are 5 times as great as those of an adult castrated mouse (20 g.), while for oestradiol benzoate the requirements of both the test animals are equal, in spite of the difference in weight. Both the methyl ethers of oestrone and oestradiol proved to behave like oestrone, while fennel oil and anise oil are similar to oestradiol benzoate, but the difference is still more conspicuous (10 times more active for the infantile rat than for the mouse). No difference has been observed between castrated and non-castrated rats, as occasionally asserted for oestriol.

¹ In disagreement with this author's description the ketone was obtained directly, instead of the unstable methoxynitrate.

SUMMARY

1. The methyl ethers of oestrone and oestradiol show not much lower oestrogenic activity than the *O*-methyl-free compounds, their free hydroxyl groups apparently being not essential for their action.

2. In contradistinction to a number of other essential oils, fennel and anise oils and to a very slight extent eucalyptus oil too have definite oestrogenic activities.

3. These activities are to be ascribed to anethole present in these oils. A number of other phenol methyl ethers have been tested for oestrogenic activity among which dihydroanethole and veratric aldehyde gave slight positive responses.

4. Pure anol is also slightly active.

5. A surprising difference in behaviour of non-castrated infantile rats and castrated adult mice towards the substances tested has been observed.

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