Estrogenic Strength of 5-(*p*-Hydroxyphenyl)-cyclohexanedione-1,3

By PHILIPPOS E. PAPADAKIS† and THEODORE J. URBAN

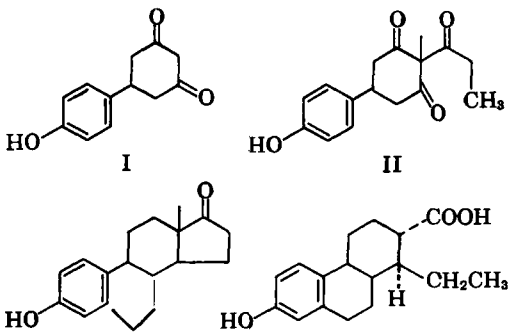
A technique is described for detecting the estrogenic activity of a nonsteroidal compound, 5-(*p*-hydroxyphenyl)-cyclohexanedione-1,3. Under the conditions of this experiment, the uterine weight assay indicated that 0.25 mg./ml. of this compound has estrogenic activity of 5.0 I.U. and 0.75 mg./ml. of the same compound has estrogenic activity of 15 I.U. Vaginal smears indicated that this compound gave a positive response.

BESIDES the natural estrogenic substances, there are synthetic compounds which, although they do not have the steroid ring system, exhibit estrogenic activity. Examples of such synthetic compounds which differ considerably in structure from the natural estrogens are stilbestrol, hexestrol (1), doisynolic acid and its stereoisomers (2–4).

This paper presents the bioassay of an estrogenic compound, 5-(*p*-hydroxyphenyl)-cyclohexanedione-1,3 (I) (5), surnamed by students at Creighton "papadakisone."

Derivatives of I having similarities to compounds of physiological importance have been synthesized. Some of these have been reported (6–8). A C-acyl derivative (7), represented by II, may be considered as an incomplete ring system of estrone (III). Doisynolic acid (IV) is also an incomplete ring system of estrone (III). Since papadakisone (I) is an incomplete ring system of estrone, it seemed reasonable to investigate first its estrogenic activity and then in later experiments proceed to examine the activity of other derivatives which have closer structural resemblance to estrone ex. (II).

The assay of papadakisone is based on an increase of the uterine weight of rats. A typical vaginal smear technique was also used, but since Stob, *et al.* (9), and Rubin, *et al.* (10), indicate that the uterine weight assay is more sensitive than the vaginal smear method, the estimation of estrogenic strength in this experiment is based on the uterine weight assay.



MATERIALS AND METHODS

The test animals used for assay purposes were ovariectomized Holtzman white rats. After the gonads were removed, 14 to 21 days elapsed to allow for uterine atrophy. The rats were killed 24 hours after the last subcutaneous injection of the test material. The rats received 0.1-ml. injections of the aqueous solution of papadakisone every day for 6 days. The solution was made by adding 1 ml. of 6 *N* sodium hydroxide which converts the compound to its soluble salt. The strength of the solution of the dosages used corresponded to 0.25 mg./ml. and 0.75 mg./ml., respectively. The commercial estrogen[1] was used daily in dosages of 10, 25, and 50 I.U. per injection for 6 days (see Table I). The volume of the oil solution of estrogen used for each injection was 0.1 ml. The uteri were weighed using a Roller-Smith precision balance. The uterine weights of the test animals treated with various dosages of commercial estrogen and the increase in uterine weight was used to indicate estrogenic activity.

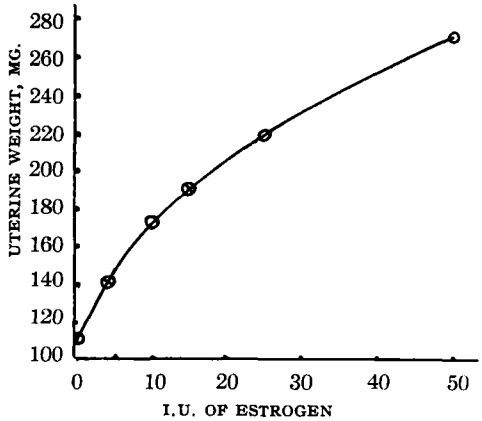


Fig. 1.—Uterine weight response to various dosages of estrogenic substances. ○ = Urestrin; ⊕ = papadakisone.

RESULTS

Data on the changes in uterine weight due to different dosages of estrogen and papadakisone are presented in Tables I and II. Data showing vaginal smear responses are presented in Table III.

In order to establish a means of reference for papadakisone-induced increases in uterine weight, a standard response curve (Table I) was determined using subcutaneous injections of estrogen. The rats receiving papadakisone all exhibited uterine weights that were heavier than the weights of the controls, and these differences also applied when the uterine weights were expressed as a percentage of body weight.

The work with vaginal smears indicates that papadakisone gives a positive response (Table III).

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[1] Marketed as Urestin by The Upjohn Co.

TABLE I.—STANDARD DOSE RESPONSE TO SUBCUTANEOUS INJECTIONS OF ESTROGEN[a] BASED ON UTERINE WEIGHT RESPONSE

Estrogenic Hormone (I.U.)	No. of Animals	Mean Body Wt. (Gm.)	Range of Rats' Wt. (Gm.)	Body Wt., %	Mean Uterine Wt. (mg.)
None	15	228.40	233.4–261.0	0.0503	115.10
10 I.U.	9	247.40	233.2–266.8	0.0702	173.62
25 I.U.	8	248.76	225.4–272.9	0.0893	220.52
50 I.U.	10	245.06	223.4–263.9	0.1101	269.88

[a] Urestin.

TABLE II.—RESPONSE TO SUBCUTANEOUS INJECTIONS OF PAPADAKISONE BASED ON UTERINE WEIGHT RESPONSE

Hormone, mg./ml.	No. of Animals	Mean Body Wt. (Gm.)	Range of Rats' Wt. (Gm.)	Body Wt., %	Mean Uterine Wt. (mg.)
None	10	218.87	165.1–277.9	0.0431	94.46
0.25	10	239.00	217.8–255.3	0.0589	140.96
0.75	10	244.40	230.4–260.4	0.0781	190.88

TABLE III.—RESPONSE TO SUBCUTANEOUS INJECTIONS OF ESTROGENIC COMPOUNDS BASED ON THE VAGINAL SMEAR RESPONSE

Treatment	No. of Animals	No. of Positive Smears	Positive, %
None	10	0	0
0.25 mg./ml.[a]	10	10	100.0
0.75 mg./ml.[a]	10	8	80.0
50 I.U.[b]	12	11	91.88

[a] Papadakisone. [b] Urestrin.

DISCUSSION

It seems quite safe to say that the nonsteroidal compound (called papadakisone here) has estrogenic activity. This is evidenced by positive vaginal smears and an increase in uterine weights. Comparing the responses of the experimental substance to the responses of I.U. of estrogen, the results indicate that 0.25 mg./ml. of papadakisone has an estrogenic activity in the neighborhood of 5.0 I.U. (Fig. 1). The dose of 0.75 mg./ml. of papadakisone causes a uterine weight response about that of a 15 I.U. dosage of estrogen. In searching the literature it was found that Lespagnol and Schmitt (11) compared the estrogenic activity of 5-(*p*-methoxyphenyl)-cyclohexanedione-1,3 to that of estrone and found the ratio of 1/500.

When treated with sodium hydroxide solution structure I is converted to its sodium salt, so that the estrogenic activity measured should be considered as due to the sodium salt of I.

REFERENCES

(1) Fieser, L. F., and Fieser, M., "Organic Chemistry," 3rd ed., Reinhold, N. Y., 1956, p. 972.
(2) MacCorquodale, D. W., Thayer, S. A., and Doisy, E. A., *J. Biol. Chem.*, **99**, 327(1933).
(3) MacCorquodale, D. W., Thayer, S. A., Doisy, E. A., and Levin, L., *ibid.*, **101**, 753(1933).
(4) See "The Merck Index," 7th ed., Merck and Co., Rahway, N. J., 1960, p. 390, for other references for stereoisomeric doisynolic acids.
(5) Papadakis, P. E., *J. Am. Chem. Soc.*, **67**, 1799(1945).
(6) Papadakis, P. E., Scigliano, J., Chin, C., and Adrienne, A., *ibid.*, **72**, 4256(1950).
(7) Papadakis, P. E., Scigliano, J., and Pirrucello, S., *ibid.*, **75**, 5436(1953).
(8) Papadakis, P. E., and Scigliano, J., *ibid.*, **75**, 5437 (1953).
(9) Stob, M., Andrews, F. N., and Zarrow, M. X., *Am. J. Vet. Res.*, **15**, 319(1954).
(10) Rubin, B., Dorfman, A. S., Black, L., and Dorfman, R., *Endocrinology*, **49**, 429(1951).
(11) Lespagnol, A., and Schmitt, J., *Bull. Soc. Chim. France*, 458(1950); through *Chem. Abstr.*, **45**, 563(1951).

Isolation of Lupeol from *Sweetia panamensis*

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SWEETIA PANAMENSIS Benth. is one of several medicinal Central American plants currently being investigated in this laboratory. For the purpose of facilitating subsequent extractions, the bark of this plant was defatted with skellysolve B, and from this extract the triterpene, lupeol, was isolated. Thus *S. panamensis* may be added to the already extensive list of plants in which this compound is found.

The isolation of lupeol from this particular source is of some historical interest. Thompson (1) reported the isolation from *S. panamensis* of a compound which according to his description is quite similar to lupeol. Indeed, when we repeated his work, lupeol was isolated from an ethanolic extract of the bark. Inasmuch as Thompson's investigation was reported in 1884 this would seem to constitute the first known isolation of lupeol rather than that of Schulze and Steiger in 1889 (2).

Also, this investigation has shown that the lupeol occurs in the bark of this plant as the free alcohol. The triterpene was characterized through formation of the acetate, benzoate, and by use of infrared spectra.

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EXPERIMENTAL

Isolation of Lupeol.—The bark[1] was powdered

[1] Obtained from S. B. Penick and Co.