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.

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Isolation of Lupeol from *Sweetia panamensis*

By THOMAS J. FITZGERALD, JACK L. BEAL, and JULES B. LAPIDUS

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.

in a Wiley mill, and 1000 Gm. was extracted to exhaustion in a Soxhlet apparatus with 2.5 L. of skellysolve B. The light green solution was reduced to dryness *in vacuo* and yielded a light greenish-yellow residue weighing 20.1 Gm. The residue, when subjected to the Liebermann-Burchard test, gave the characteristic red color of lupeol.

Ten grams of this residue was refluxed for 3 hours with 250 ml. of alcohol containing 12 Gm. of sodium hydroxide. After cooling, the brown solution was poured into 300 ml. of water, and a flocculent yellow precipitate formed. The alcohol was evaporated on a steam bath, and after filtering the suspension, the precipitate was washed with 500 ml. of water. After drying at 105° for 12 hours the crude unsaponifiable material weighed 5.7 Gm. Recrystallization from ethanol gave needles, m.p. 212–213°.[2] When this material was mixed with an authentic sample of lupeol[3] (m.p. 214.5°) and melted, no depression occurred. Further purification was achieved by preparing the acetate and saponifying. This procedure gave a product, after recrystallization from ethanol, m.p. 214 to 214.5°; lit. value, 215° (4). The infrared spectrum of this material was identical with that of an authentic sample.

[2] Melting points are uncorrected.

[3] The authentic samples of lupeol and its derivatives were provided by Dr. Jack L. Beal from a previous investigation (3).

Lupeol Acetate.—The acetate was prepared according to Wagner (5). Recrystallization from ethanol gave the compound as needles with a melting point range of 217 to 217.5°; lit. value, 218° (4).

Lupeol Benzoate.—The benzoate was also prepared according Wagner (5). This material was obtained as small platelets when recrystallized from benzene-ethanol (1:5) and gave a melting point range of 264–266°; lit. value, 273–274° (4). The infrared spectrum of this compound was identical with that of an authentic specimen of lupeol benzoate.

Presence of Free Lupeol.—A portion of the skellysolve residue was washed with methanol and then dissolved in chloroform. The needle-shaped crystals which formed on evaporation of the solvent at room temperature, m.p. 211–213°, gave an infrared spectrum which was identical with that of the material obtained by saponification.

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Communications

Absence of 6-Methoxybenzoxazolinone in Uninjured Maize Tissue

Sir:

The peculiar phenomenon has been demonstrated (1) that many living plants contain stable precursors which after the crushing of plants are enzymically decomposed to substances which (or after further chemical or enzymic decomposition) have different biological activities. One of the typical cases is the formation of benzoxazolinone (BOA) and its 6-methoxy derivative (MBOA) in crushed rye and maize plants, respectively. When these compounds were isolated and characterized chemically in this laboratory, it was at first believed that they were present in the intact plants (2). Later on we could relate their formation to the precursors present in these plants. The reaction series is:

precursor, glucoside of 2,4-dihydroxy-7-methoxy- —enzymic→ aglucone —chemical→ 1, 4-benzoxazin-3-one —chemical→ 6-methoxybenzoxazolinone (3, 4).

The last step in the reaction is of an unusual type—a six-membered ring is transformed into a five-membered one by the splitting of formic acid. When hydrolysis of the glucoside is completely prevented in the intact plant, we have never detected any formation of BOA or MBOA.

Smissman, *et al.* (5), recently reported that they found MBOA as an original substance in maize tissue. Quantitative data were not given. Their procedure was complicated, however, and involved some steps during which the formation of MBOA from the aglucone can take place. If the aglucone was already present in the ground plants when they were placed in 95% ethanol, the formation of some MBOA would be expected. Kinetic studies in this laboratory have shown that aglucone of the rye glucoside is converted into BOA at a much higher rate in 80% ethanol than in aqueous solution (6). The rapid conversion of the aglucone of the maize glucoside into MBOA in 95% ethanol solution at 40° is shown in Fig. 1.

The other step at which MBOA could surely be formed from the maize aglucone is the fractiona-