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Constituents of *Cannabis sativa* L. XXI: Estrogenic activity of a non-cannabinoid constituent[1]

Ph.W. Wirth, J.C. Murphy[2], F.S. El-Feraly[3] and C.E. Turner

Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University (Miss. 38677, USA), 13 October 1980

Summary. A non-cannabinoid phenol (4,4,dihydroxy-5-methoxybibenzyl) increased uterine weight in prepubescent female rats, suggesting non-cannabinoids contribute to the estrogenic effects of *Cannabis*.

Cannabis preparations have been reported to produce female characteristics in male rodents[4], depress plasma testosterone levels[5] and to produce excessive enlargement of male mammary glands[6]. Whether or not Δ^9-tetrahydrocannabinol produces these effects remains controversial[4,5]. Most research is involved only with Δ^9-tetrahydrocannabinol[7]. However, over 400 other compounds are known to occur in *Cannabis* and some of these resemble known estrogenic agents more closely than does Δ^9-tetrahydrocannabinol. Examples of such compounds are the non-cannabinoid phenolic spiro-indans and dihydrostilbenes recently isolated in our laboratories.

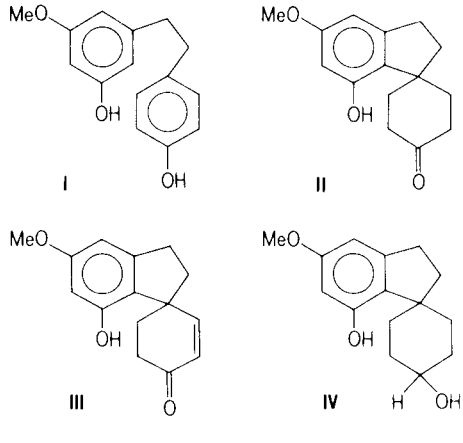
The first of the non-cannabinoid phenols isolated was cannabispiran[8], which previously may have been misidentified as (−)-Δ^8-tetrahydrocannabinol since both compounds have the same relative retention time in GC analysis. Several spiro-indans other than cannabispiran have since been isolated[8]. The spiro-indans are structurally similar to synthetic compounds which potentiate estrogenic effects[9]. Four dihydrostilbenes have subsequently been reported to occur in *Cannabis*. The dihydrostilbenes are structurally similar to 4-{2-(4-hydroxyphenyl)ethyl}-phenol, which has weak estrogenic activity[10].

As part of our continuing investigation of compounds from *Cannabis* we wished to study the estrogenic effects of cannabispiran (**II**), dehydrocannabispiran (**III**) and β-cannabispiranol (**IV**). Preliminary studies of the estrogenic effects of cannabispiran, dehydrocannabispiran and β-cannabispiran were begun, but were hampered because ample amounts of these compounds were not available from plant extracts. Therefore, a program to synthesize cannabispiran and related compounds was begun.

The dihydrostilbene derivative of *Cannabis* (**I**) has been proposed as a biogenic precursor of cannabispiran[8]. It is also an intermediate in the total synthesis of cannabispiran[10] and, as such, has been successfully synthesized in g amounts. The subject of this paper is our evaluation of some aspects of the estrogenic effects of **I** in female rats.

Compound **I** was tested alone and in combination with diethylstilbesterol (DES) by measuring its effects on uterine weight in prepubescent female Sprague-Dawley derived rats. 4 groups of rats were tested in a 2×2 factorial design which was repeated for each of 3 test doses. The 4 groups were dosed as follows: 1. Test dose of **I**; 2. Vehicle control; 3. Test dose of **I** and 0.01 mg/kg of DES; and 4. 0.01 mg/kg of DES. The rats were given i.p. injections in corn oil on 3 consecutive days beginning when they were 23 days old. On the 4th day they were sacrificed, and their uteri were removed, freed from fat, blotted and weighed. Uterine ratios were calculated for each animal as uterine weight in mg per 100 b.wt. Uterine ratios were analyzed using 2×2 analysis of variance to determine the effects of compound **I** alone and its ability to potentiate the effects of DES. Compound **I** was tested at 10, 50, and 250 mg/kg.

Compound **I**, a non-cannabinoid, produced effects similar



Uterine ratios for groups of 8 prepubertal rats. Data expressed as mean ± SEM

Dose of **I**	**I**	Vehicle (control)	I + DES	DES (control)
10 mg/kg[a]	79 ± 16	78 ± 6	289 ± 14	242 ± 39
50 mg/kg[b]	79 ± 16	72 ± 12	331 ± 21	250 ± 18
250 mg/kg[c]	99 ± 16	72 ± 3	295 ± 18	258 ± 19

[a] Significant effect for DES ($p < 0.001$), nonsignificant effect for **I** and interaction. [b] Significant effects for DES ($p < 0.001$) and for **I** ($p < 0.01$), significant interaction ($p < 0.05$). [c] Significant effect for DES ($p < 0.001$) and for **I** ($p < 0.05$), nonsignificant interaction.

to those produced by estrogen. Compound **I** increased the uterine ratio reliably at 50 mg/kg and 250 mg/kg, but not at 10 mg/kg (table). The effects of **I** were much weaker than those of DES as is also true of 4-[2(hydroxyphenyl)-ethyl]phenol[10]. No lethality or overt toxic effects were seen in any group, but there was a loss of body weight during each experiment among the animals dosed with DES. In other testing in our laboratory, **I** has been given i.p. in male mice at doses up to 400 mg/kg without producing lethality or other observable toxicity.

The effects of compound **I** alone are not sufficient to account for the estrogenic properties attributed to *Cannabis*[4-6]. Preliminary testing with cannabispiran (**II**) revealed no significant increase in the uterine ratio at 10 mg/kg. Similar testing with dehydrocannabispiran (**III**) and β-cannabispiranol (**IV**) at 1 mg/kg also showed no significant uterine ratio increase. The increased estrogenic effect of compound **I** suggests that both cannabinoids and non-cannabinoids may be necessary to account for the estrogenic effects which are reported to result from the use of *Cannabis*.

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2 To whom correspondence should be addressed.
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Etamsylate as inhibitor of prostaglandin biosynthesis in pregnant human myometrium in vitro

L. Kovács and G. Falkay

Department of Obstetrics and Gynecology, University Medical School, Szeged (Hungary), 22 December 1980

Summary. The effects of etamsylate on prostaglandin (PG) biosynthesis in microsomes of pregnant human myometrium in vitro have been determined, and compared with those of indomethacin. Both drugs inhibited PG biosynthesis, indomethacin being the more potent inhibitor of the two. Etamsylate inhibited synthesis of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2, and thromboxane B_2; increasing the concentration of etamsylate increased the inhibition of synthesis. It is suggested that etamsylate has no anti-cyclo-oxygenase activity, but acts by inhibiting the activity of prostacyclin synthetase, endoperoxide reductase, endoperoxide isomerase, and thromboxane synthetase.

Etamsylate (diethylammonium 1,4-dihydroxy-3-benzenesulfonate) is a hemostatic drug that has been shown to be effective in reducing menstrual blood loss in intrauterine-device menorrhagia[1,2], and to inhibit prostaglandin (PG) biosynthesis in the rabbit iris-ciliary body and kidney medulla[3]. PGs are implicated in the mechanism of parturition in many animal species, including man[4], and there is increasing evidence that they may play an important part in the onset and progression of labor[5,6]. That several drugs can influence the biosynthesis of PGs in the pregnant human myometrium has been demonstrated in this laboratory[7-9].

It is only recently that human myometrial tissue has been shown to synthesize prostacyclin (PGI_2) and thromboxane A_2 (TXA_2), substances that are important in the regulation of vascular tone and thereby in the regulation of blood pressure. PGI_2 and TXA_2 are unstable; they break down spontaneously and quantitatively to 6-oxo-$PGF_{1\alpha}$ and TXB_2[10]. These findings prompted this preliminary study of the effect of etamsylate on PG biosynthesis in the pregnant human myometrium in vitro. The effect of etamsylate was compared with that of indomethacin, a commonly used inhibitor of PG biosynthesis.

Materials and methods. Etamsylate (Dicynone®) was obtained from OM Laboratories, Meyrin/Geneva, Switzerland. Myometrial samples from pregnant women were obtained by excision from the edge of the surgical incision in lower uterine segment caesarean sections. Details of the procedure and of assay of PG-synthetase activity for application to human myometrial tissue have been published[7]. Briefly, the myometrial strips were homogenized with 0.1 M Tris HCl buffer (pH 7.8) and the microsomes separated by ultracentrifugation. The assay mixture (2.0 ml) contained 0.1 M Tris HCl (pH 7.8), 2.0 mM L-adrenaline, 2.0 mM glutathione, [1-^{14}C] arachidonic acid (0.1 μCi; 10 μM), different concentrations of etamsylate (0.1, 1.0, 5.0, 10.0, 100.0 mM) and of indomethacin (Chinoin Pharmaceutical Ltd.,; 0.1, 1.0, 5.0, 10.0 μM) and 2.5-3.5 mg of microsomal protein. After incubation at 37 °C

Inhibition by etamsylate and indomethacin of prostaglandin synthesis in microsomes of pregnant human myometrium

PG products	Etamsylate I_{50} (mM)	Indomethacin I_{50} (μM)
6-oxo-$PGF_{1\alpha}$	2.88	0.121
$PGF_{2\alpha}$	0.50	0.131
PGE_2+TXB_2	5.44	0.144

PG synthesis was measured as described in the text. I_{50} = concentration (μM or mM in final dilution) producing 50% inhibition. I_{50} were calculated from 10 points of a concentration curve, using regression analysis when the transformations were ln [y/(100-y)] = b lgx + const. for etamsylate and ln (100-y) = b lgx + const. for indomethacin.