OESTROGENIC AND ANTI-OESTROGENIC ACTIVITIES OF SOME HEX-2-ENES RELATED TO DIETHYLSTILBOESTROL

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Some members of the stilboestrol series have been reported to show significant anti-oestrogenic activity and to act as true competitive inhibitors of oestrogen at the ‘receptor site’ in the vagina (Emmens & Cox, 1958; Emmens, Cox & Martin, 1959). However, the compounds investigated also possessed oestrogenic activity. We have synthesized a series of nuclear methylated compounds related to diethylstilboestrol (Clark & O’Donnell, 1965) in an attempt to find true competitive anti-oestrogens which possess no oestrogenic activity. The present communication describes the investigation of three of these compounds for oestrogenic and anti-oestrogenic activity.

The compounds were examined for oestrogenic activity by the Allen–Doisy vaginal smear test in ovarietomized mice, with both s.c. and intravaginal routes of administration. 3,5-Bis-(3,5-dimethyl-4-hydroxyphenyl)hex-2-ene (I) produced no oestrogenic response at s.c. doses up to 1 mg. and intravaginal doses up to 0·25 mg. 3-(3,5-Dimethyl-4-hydroxyphenyl)-4-(4-hydroxyphenyl)hex-2-ene (II) and 3,4-bis-(3-methyl-4-hydroxyphenyl)hex-2-ene (III) were assayed by a four-point quantal response assay, with 20 mice per dose group and s.c. administration of the test compounds and standard oestrogen in arachis oil solutions. Compound II had 0·015 (fiducial limits (P = 0·95) 0·0072–0·033) and compound III 0·2 (fiducial limits (P = 0·95) 0·16–0·25) times the activity of diethylstilboestrol. By the intravaginal route the median effective doses were 4·0 μg. and 0·005 μg. respectively for compounds II and III (cf. diethylstilboestrol, 0·006 μg.).

Ovariectomized mice were also used in tests for anti-oestrogenic activity. Vaginal smears were used as the index of change but the 0, 1, 2 method of scoring described by Emmens & Cox (1958) was adopted. The compound under test and standard oestrogen were administered in one solution, using 25% aqueous propylene glycol for compounds II and III and, because of its insolubility, 50% aqueous propylene glycol for compound I.

No inhibition of diethylstilboestrol-induced vaginal cornification by compound II at dose levels up to 0·1 μg. (2·5% intravaginal oestrogen ED50), was found. Compound III did not inhibit oestradiol-induced vaginal cornification at doses of 2·5 × 10−4 and 25 × 10−4 μg. (5% and 50% intravaginal ED50). Compound I at a dose of 25 μg. produced significant inhibition of the oestradiol-induced vaginal cornification.

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response (Table 1) when the criteria of scoring positive responses adopted by Biggers & Claringbold (1954) were strictly adhered to, i.e. the absence of leucocytes was regarded as the important criterion for a positive smear. Compound I did not prevent some keratinization of the epithelial cells but it appeared to interfere with the mechanism by which oestrogens prevent leucocytes from appearing in the vaginal smear.

Table 1. Effect of compound I on the intravaginal response to oestradiol in ovarietomized mice

All doses intravaginal; ten mice/group; scoring system: 0 = no reaction, 1 = one positive smear, 2 = two positive smears.

<table>
<thead>
<tr>
<th>Dose of compound I (µg.)</th>
<th>Score per group of ten mice. Dose of oestradiol (µg. x 10^-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-75  1-5  3  6</td>
</tr>
<tr>
<td></td>
<td>6  9  12  16</td>
</tr>
<tr>
<td></td>
<td>25  0  1  9  5</td>
</tr>
</tbody>
</table>

Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>D.F.</th>
<th>Sum of squares</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestradiol</td>
<td>3</td>
<td>8-85</td>
<td>5-36</td>
<td>0-01-0-001</td>
</tr>
<tr>
<td>Compound I</td>
<td>1</td>
<td>9-80</td>
<td>11-82</td>
<td>&lt; 0-0001</td>
</tr>
<tr>
<td>Interaction</td>
<td>3</td>
<td>1-80</td>
<td>1-09</td>
<td>&gt; 0-2</td>
</tr>
<tr>
<td>Residual</td>
<td>72</td>
<td>39-40</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>59-85</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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REFERENCES