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OESTROGEN POTENTIATING ACTIVITY OF PHENYL 2-(2-HYDROXYL INDEN-3-YL) PROPIONLACTONE, A COMPOUND WITH DEAROMATIZATION CHARACTER

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The synthesis of phenyl 2-(2-hydroxyl inden-3-yl) propionlactone (3) has been described. An explanation of its NMR spectrum has been postulated, where the aromatic nature of one of its phenyl group could have transitionally been lost. This compound resembles stilboestrol in its molecular dimension and potentiates its activity. However it exhibits no oestrogenic activity. It also gives some protection against leptazol convulsions.

Key words: Oral contraceptives; Oestrogen therapy; Fertility control.

INTRODUCTION

Oral contraceptives have been in use for over two decades and have been proved to be the most effective, popular and reversible methods for fertility control. The adverse reactions associated with this oestrogen therapy are serious and well documented. Thrombo-embolish, stroke, mycordial infarction hepatic tumours, gall bladder disease, hypertension, haemorrhages, alteration of developing cells of bonemarrow, endometrial carcinoma, malignant hypertension and breast cancers have all been reported in the literature [1-8].

Recently a World Health Organisation scientific group [1] has confirmed an earlier report [2] which stated that pill users under 25 years of age are at a high risk for breast cancer. Vessey *et al.* [3] and others [4] have reported that long-term use of the pill doubles the risk of cervical cancer. Thus there is an urgent need to find an effective and safer non steroidal antifertility agent. Recently it has been shown [5] that several non-steroidal compounds can be effectively used as contraceptives agents. Qazi *et al.* prepared a series of spiro and tricyclic compounds [6-10]. Most of these compounds are structurally similar to stilboestrol and have no oestrogenic activity, and yet potentiate stilboestrol activity on the immature uterus of the rodent [11-14]. An attempt has been made in this work to prepare a compound (2) which is similar to compound (1) but with one extra phenyl group. Compound (1) has been reported inactive as anticonvulsant [15]. Its inactivity might be due to an unfavourable partition coefficient to the extent that the compound did not reach the site of action. The synthesis of the proposed compound (2) with one more phenyl might result into a favourable partition coefficient and thus become pharmacologically active as anticonvulsant which could be screened for oestrogenic activity.

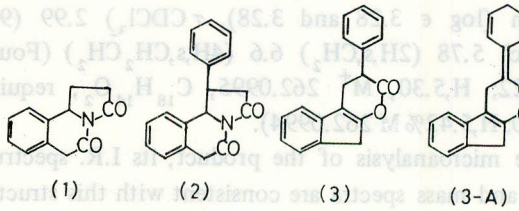
MATERIALS AND METHODS

Melting points were determined using an electrothermal melting point apparatus and are uncorrected. IR spectra were run as (Nujol mulls) on Perkin-Elmer 273 model and UV spectra with a Pye Unicam SP 800 spectrophotometer. NMR spectra were recorded on a Varian HA-100 spectrometer using tetramethylsilane as internal standard. Mass spectra were produced by the physicochemical measurement unit, Harwell, and microanalyses were performed by the School of Pharmacy, London.

Indan-2-one. It was prepared by the performic acid oxidation of indene, m.p. 56° (lit. 57-58° [16].

2-Morpholinylindene. It was prepared by Bloomquist and Moriconi's method; m.p. 196° (lit. 197-198° [17].

Ethyl tropate. Tropic acid (10 g, 0.06 mol) was refluxed with absolute ethanol (40 ml) and sulphuric acid (0.5 ml) for 16 hr. After cooling the mixture, the excess of ethanol was distilled, the residue was poured into the cold water (30 ml) and extracted with ether (3 x 100 ml). The combined extracts were washed with sodium hydrogen carbonate solution twice and finally with distilled water; it was dried over sodium sulphate. On evaporation it gave a yellowish oil, which was fractionally distilled to yield 9.1g ((77%), b.p. 102-105°, 0.3mm Hg, ^{n}D1.5186, υmax 3450 (OH) and 1750 cm^{-1} (Ester C=O).



(1) (2) (3) (3-A)

Ethyl-3-chloro-2-phenylpropionate. Ethyl tropate (3.5g, 0.018 mol) was refluxed with thionyl chloride (5 ml, 0.03 mol) in sodium dried ether (30ml) for 1 hr. On fractional distillation it gave (3.55g, 94%) a yellowish oil; b.p. 120-122°/3mm Hg.

1-Phenyl-2-(2-hydroxylinden-3-yl) propionolactone. Ethyl 3-chloro-2-phenylpropionate (10.9g, 0.051 mol) was refluxed with 2-morpholinylindene (11.0g, 0.054 mol) in dimethylformamide (100 ml) for 4 hr. After cooling to room temperature 2 N hydrochloric acid (50 ml) was added and the mixture was further refluxed for 2 hr. The mixture was allowed to cool and was extracted with ether (3 x 150ml). The combined extracts were washed with 2N hydrochloric acid (30ml) twice and finally with water (200ml). It was dried over sodium sulphate for 4 hr and on evaporation of the solvent an oily product was obtained. Fractional distillation gave (4.05g, 30%) a red oil, b.p. 184-190°/10 mm Hg. The oil solidified on standing in the refrigerator for two days. It was crystallised from absolute alcohol to give colourless crystals, which changed to light yellow after three days at room temperature, m.p. 172° The NMR spectrum showed that one of the aromatic proton could have transitionally been lost by deconjugation. This compound was further recrystallised by dissolving in a minimum amount of boiling ethyl alcohol (30 ml) and by adding sodium dried di-ethyl ether until the resulting solution became turbid. On cooling in the refrigerator for 6 hr. the product was obtained as small white crystals, m.p. 173.5°. The purity of this compound was further confirmed by thin layer chromatography where the compound gave one spot only. Di-ethyl ether/benzene (2:10) was used as developing solvent (Rf_{100} = 65). The above evidence showed that only one isomer could be present. The compound was subjected to acid hydrolysis with 50% hydrogen chloride acid for 5 hr and extracted with chloroform three times. It was dried over sodium sulphate. On evaporation it gave a thick oil product, which was fractionally distilled to afford a product, which on recrystallisation from ethanol gave a colourless solid, m.p. 56° and υmax 1750 (C=O) cm^{-1}. The m.p., mixed m.p. and I.R. spectrum of this product to be showed it indan-2-one. This indan-2-one was obtained by the acid hydrolysis of the lactone (3). Compound (3) gave spectrum (υ max) (Nujol) 1760 cm^{-1} (C=O, Lactone), λ max (ethanol) 273 and 280 nm (log ϵ 3.28 and 3.28), τ $CDCl_3$) 2.99 (9,m, aromatic) 5.78 (2H,s,CH_2) 6.6 (4H,s,CH_2CH_2) (Found, C, 82.22; H,5.30, M^+ 262.0995, $C_{18}H_{14}O_2$, requires, C, 82.50; H,5.42% M 262.0994).

The microanalysis of the product, its I.R. spectrum, its UV and mass spectra are consistent with this structure (3). Furthermore, TLC also confirms one isomer of compound (3).

Pharmacology. Two types of pharmacological studies were made on compound (3), viz., its anticonvulsant and oestrogenic activities.

Anticonvulsant activity. In order to detect anticonvulsant activity, a procedure prescribed by Swinyard *et al.* [18] was chosen. Mice in groups of 10 received injections of leptazol (100mg/kg) at a period of one or two hr after the injection of compound (3). The anticonvulsant activity of the compound was assessed by its ability to antagonise the effects of leptazol convulsions. The subsequent effects of leptazol were observed as reported by Qazi [19-21]. The results obtained were then quantified using a seizure severity score [22].

Table 1. Anticonvulsant activity of compound (3) with phenobarbitone.

Drug	Time of administration (hr)	Reduction in group seizure score (%)
Phenobarbitone	1	65.5
(10 mg/kg i.p.)	2	34.0
Compound (3)	1	42.2
(300 mg/kg i.p.)	2	30.3

The anticonvulsant activity of compound (3) was more persistent in duration than phenobarbitone and might have contributed by its rigid structure. But its activity was significantly less than that of phenobarbitone. Even the latter was used in a smaller amount. No further tests of anticonvulsant activity were carried out with compound (3).

Oestrogenic activity. Oestrogenic activity was assessed by a bioassay procedure using the immature mouse uterus [23]. Female albino mice (ICI-CFLP Strain) aged 22-26 days were allowed free access to drinking water and 41B cube diet throughout. The experiments were performed at a temperature of 23° ± 1° and at a relative humidity of 60-70%. Animals received daily intraperitoneal (i.p.) injections at 10.00 hr for three successive days ensuring that concomitant doses of agents were administered at sites which were removed from each other. 24 hr after the last injection, the mice were sacrified by cervical dislocation and the whole body as well as uterine weights were determined. The results were expressed as uterine ratios calculated by using the formula:

$$\text{Uterine ratio} = \frac{\text{Uterine weight (mg)}}{\text{Body weight (g)}} \times 100$$

Table 2. Oestrogenic activity of compound (3) alone and with stilboestrol (0.01 mg/kg).

Group	Treatment	Wt. of mouse (g) mean ± SE	Wt. of uterus (mg) mean ± SE	Uterine ratio mean ± SE
1.	Arachis oil, 10mg/kg	12.5 ± 0.8	13.0 ± 0.7	104 ± 4
2.	Compd. (3), 0.1mg/kg	11.8 ± 0.6	12.8 ± 0.8	108 ± 6[c]
3.	Compd. (3), 10 mg/kg	12.0 ± 0.6	13.5 ± 0.6	112 ± 8[b]
4	Stilboestrol, 0.01mg/kg A constant amount of stilboestrol (0.01 mg/kg) is used.	13.5 ± 0.8	37.2 ± 0.9	275.5 ± 12[a]
5	Stilboestrol + Compd (3) 0.1mg/kg.	13.4 ± 0.7	42.5 ± 0.8	317 ± 13[a]
6.	Stilboestrol + Compd (3) 1mg/kg.	13.6 ± 0.9	44.2 ± 0.8	325 ± 18[a]
7.	Stilboestrol + Compd (3) 10 mg/kg.	13.0 ± 0.5	43.4 ± 0.4	334 ± 15[a]

a Significant ($P > 0.001$); b Significant ($P > 0.05$); c Not significant ($P < 0.05$); When compared to arachis oil control Mouse (means + SE = Standard error); A group of 10 mice was used in each experiment.

Compound (3) produced some increase in uterine ratio as compared with the control vehicle (arachis oil) as shown in Table 2. This may have been indicative of weak oestrogenic activity. This effect however was small compared with that produced by only a low dose of stilboestrol (0.01/mg/kg) which gave nearly three fold increase in uterine ratio. Combined administration of increasing doses of compound (3) with a fixed dose of stilboestrol produced a marked dose-related potentiation of the overall oestrogenic effect on uterine ratio (Table 2). This effect showed a close linear relationship.

DISCUSSION

Compound (3) was obtained during an attempted condensation of ethyl chloro-2-phenylpropionate with morpholinylindene, where the enolic form of the resulting compound was further cyclised to give the lactone (3). The IR spectrum of compound (3) gave an absorption at 1760 cm^{-1} (C=O) and 1670, 1568 cm^{-1} (C=C), which is consistent with 1,2-dihydropyran-5-one. The microanalysis and the mass spectral molecular weight were consistent with this structure. But certain features in the NMR spectrum were not consistent with the structure (3). The eight and half aromatic protons which appeared as a multiplet at τ 2.98 a singlet for a methylene group at τ 5.78 and a singlet for two equivalent methylene groups at τ 6.6. This NMR spectral evidence would support structure (3-A), where the aromatic structure of one phenyl ring has transitionally been lost by deconjugation. But this structure is not supported by IR spectrum in which the carbonyl peak would be expected to be at a much lower wave number. Although it is not common, but the migration of double bonds has been reported earlier [24]. It could be due to a mobile proton, but this possibility of structure (3-A) is ruled out, since TLC indicates the presence of one isomer only. Repeated recrystallisation gave compound (3) which is consistent with its microanalysis IR, UV, NMR and mass spectrum. Even HPLC of (3) gave electrochemical behaviour similar to resembling compounds [25-26]. Compound (3) on acid hydrolysis gave a known compound indan-2-one, which was further confirmed by its m.p., mixed m.p. and IR spectrum. All this could explain that (3) is in fact the oxidative product of two molecules of indan-2-one.

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