s, 1-H). Anal. ($C_7H_7N_3O_2$) C, H, N.

6-Aminopyrazolo[4,3-*c*]pyridin-4(5*H*)-one (2). A mixture of 20 ml of anhydrous NH_3 and 550 mg (3.3 mmol) of 7 was heated 90 h in a steel bomb at 110 °C. Evaporation of the NH_3 gave a residue which was extracted with several portions of hot MeOH. The combined extracts were evaporated to give 50 mg (9%) of pure 2: gradual decomposition over 250 °C; λ_{max}^{pH1} 253 nm (ϵ 7100); $\lambda_{max}^{pH7and11}$ 272 nm (ϵ 13 000); R_f (cellulose TLC developed in 5% aqueous NH_4HCO_3) 0.33; NMR (Me_2SO-d_6) δ 3.73 (br s, 1-H, HOD), 5.41 (s, 1-H), 5.78 (s, 2-H), 7.82 (s, 1-H), 10.5 (br s, 1-H). Anal. ($C_6H_6N_4O \cdot 0.5H_2O$) C, N; H: calcd, 4.43; found, 4.98.

Acknowledgment. We wish to thank Dr. R. W. Sidwell, Dr. L. A. Allen, and Mr. J. H. Huffman for providing the results of antiviral testing and Mr. Ed Banta and Ms. Milda Alta for technical assistance.

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Estrogen Potentiating Activity of Two Spiro Compounds Having Approximately Similar Molecular Dimensions to Stilbestrol

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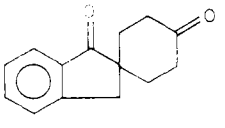
Pharmacological investigation of members of a series of synthetic spiro derivatives with similar molecular dimensions to stilbestrol revealed that two compounds, spiro[cyclohexane-1,2′-tetralin]-1,4′-dione and spiro[cyclohexane-1,2′-indan]-1,4′-diol, exhibited a marked ability to potentiate stilbestrol at doses which had no intrinsic estrogenic activity. It is postulated that such compounds may be of use in reducing the side effects associated with estrogen therapy.

It has previously been reported that one member (1) of a series of estrogenically inactive spiro derivatives with similar molecular dimensions to pharmacologically active estrogens potentiated the effects of stilbestrol on the immature mouse uterus.[1] This paper describes two further compounds, spiro[cyclohexane-1,2′-tetralin]-1,4′-dione (**3a**) and spiro[cyclohexane-1,2′-indan]-1,4′-diol (**4**), which were produced as a result of extending the series. They are of particular interest in view of a marked increase in potency compared with the compound originally described.

Chemistry. 2,2-Bis(β-ethoxycarbonylethyl)tetralone (**2a**) was prepared by a Michael condensation between ethyl acrylate and α-tetralone. Ring closure was then accomplished by means of a single-step modification of the Dieckmann reaction to give spiro[cyclohexane-1,2′-tetralin]-1,4′-dione (**3a**).

A similar sequence of reactions starting with α-indanone afforded spiro[cyclohexane-1,2′-indan]-1,4′-dione (**3b**) which was then reduced using sodium borohydride to give spiro[cyclohexane-1,2′-indan]-1,4′-diol (**4**).



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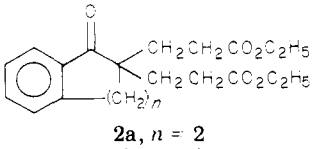

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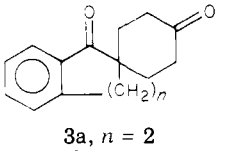
b, n = 1



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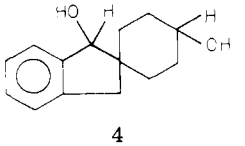

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Pharmacological Activity. In order to quantify estrogenic activity, a bioassay procedure based on the method first described by Rubin et al.[2] was employed. This method utilizes the increase in uterine weight produced by both synthetic and naturally occurring compounds possessing estrogenic activity in immature mice. It is both sensitive and precise and yields easily quantifiable data from simple objective measurements. Tests were carried out to detect any estrogenic activity of the spiro derivatives under investigation and also to estimate their ability to potentiate or antagonize the effects of a standard estrogen (stilbestrol).

Compound **4** and to a lesser extent **3a** produced a small although significant ($p < 0.001$) increase in the uterine ratio compared with arachis oil controls. This effect was, however, minimal when compared with that produced by a very much smaller dose (0.01 mg/kg) of stilbestrol. Furthermore, the response tended to fall off following a 100-fold increase in the dose and this may indicate an inhibitory effect at higher concentrations (Table I).

In contrast to the very small intrinsic estrogenic activity, both derivatives exhibited a marked ability to potentiate stilbestrol. Furthermore, this effect was produced at dose levels which alone had no significant estrogenic effect (Table II). Compound **3a** was the more potent in this respect and extending the dose range to encompass much smaller doses revealed that as little as 1 μg/kg produced a twofold increase in the stilbestrol response. This effect was even more marked at 10 μg/kg but began to diminish at doses in excess of this.

The adverse reactions associated with estrogen therapy are well documented and include thromboembolism, stroke, myocardial infarction, hepatic tumors, gall bladder disease, hypertension, and endometrical cancer. The type of compound described here is of interest since it may allow smaller doses of estrogens to be used clinically with a consequent reduction in the incidence and/or severity of these side effects.

At the present time the mechanism by which the spiro derivatives bring about their estrogen potentiating effects

Table I. Estrogenic Activity of Spiro Derivatives 3a and 4

Group	Treatment	Wt of mouse (g), mean ± SE	Wt of uterus (mg), mean ± SE	Uterine ratio, mean ± SE
1	Arachis oil, 0.1 ml ip	12.0 ± 0.7	6.9 ± 0.7	57 ± 4
2	Compd 4, 0.1 mg/kg ip	9.3 ± 0.5	7.4 ± 0.5	80 ± 2[a]
3	Compd 4, 10 mg/kg ip	11.4 ± 0.5	9.7 ± 0.3	86 ± 3[b]
4	Compd 3a, 0.1 mg/kg ip	12.8 ± 0.4	10.0 ± 0.2	78 ± 2[a]
5	Compd 3a, 10 mg/kg ip	10.7 ± 0.5	6.9 ± 0.4	64 ± 2[c]
6	Stilbestrol, 0.01 mg/kg ip	9.4 ± 0.5	17.5 ± 0.7	179 ± 14[a]

[a] Significant ($p < 0.001$). [b] Significant ($p < 0.05$). [c] Not significant ($p > 0.05$) when compared with arachis oil control.

Table II. Estrogenic Potentiation in Mice by Spiro Derivatives 3a and 4

Group	Treatment	Wt of mouse (g), mean ± SE	Wt of uterus (mg), mean ± SE	Uterine ratio, mean ± SE
1	Stilbestrol, 0.01 mg/kg	11.7 ± 0.6	21.1 ± 0.6	167 ± 6
2	Compd 4, 0.01 mg/kg, + stilbestrol, 0.01 mg/kg	10.9 ± 0.6	24.5 ± 1.4	225 ± 8[a]
3	Compd 4, 10 mg/kg, + stilbestrol, 0.01 mg/kg	11.8 ± 0.7	29.2 ± 2.0	247 ± 10[a]
4	Compd 3a, 0.001 mg/kg, + stilbestrol, 0.01 mg/kg	9.8 ± 0.3	29.9 ± 0.6	306 ± 9[a]
5	Compd 3a, 0.01 mg/kg, + stilbestrol, 0.01 mg/kg	9.9 ± 0.5	37.1 ± 1.2	377 ± 15[a]
6	Compd 3a, 0.1 mg/kg, + stilbestrol, 0.01 mg/kg	10.4 ± 0.4	30.7 ± 0.7	293 ± 5[a]
7	Compd 3a, 10 mg/kg, + stilbestrol, 0.01 mg/kg	12.4 ± 0.4	36.5 ± 1.1	293 ± 2[a]

[a] Significant ($p < 0.001$) when compared with stilbestrol (0.01 mg/kg) group.

must be speculative and will be the subject of further investigation. It could involve an increase in receptor sensitivity to estrogens, or, alternatively, the concept of silent receptors may be invoked.[3,4] It could then be argued that these compounds may occupy silent, i.e., pharmacologically inactive receptor sites that are also available to estrogenically active molecules. This in turn would allow a higher proportion of active receptor sites to be occupied with a resulting potentiation of estrogenic activity.

Experimental Section

Melting points were determined using a capillary apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer 357 spectrophotometer and UV spectra with a Pye Unicam SP800 spectrophotometer. NMR spectra were determined on a Varian HA-100 spectrometer using tetramethylsilane as an internal standard. Where analyses are indicated only by symbols of the elements, the results obtained for those elements are within ±0.4% of theoretical values. Elemental analyses were performed by Mr. G. Crouch, School of Pharmacy, University of London.

2,2-Bis(β-ethoxycarbonylethyl)tetralone (2a). Ethyl acrylate (20.0 g, 0.20 mol) was added to a well-stirred mixture of α-tetralone (14.6 g, 0.10 mol), Triton B (2 ml), and hydroquinone (2 mg) in dioxane (100 ml) at such a rate that the temperature was maintained at about 50 °C. After addition was complete the reaction mixture was stirred at 100–150 °C for a further 8 h, poured into H_2O (100 ml), and extracted with $CHCl_3$ (3 × 50 ml). The combined $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated in vacuo. The residue was distilled to afford the title compound as a viscous yellow oil (22.0 g, 63.5%): bp 204–206 °C (1.0 mm); IR (film) 1735 ($COOC_2H_5$), 1680 cm^{-1} (aromatic C=O). Anal. ($C_{20}H_{26}O_5$) C, H.

2,2-Bis(β-hydroxycarbonylethyl)tetralone. A suspension of **2a** (1.0 g) in 50% HCl (10 ml) and AcOH (5 ml) was allowed to reflux for 6 h and was then extracted with Et_2O. The ethereal extracts were dried (Na_2SO_4) and evaporated and the residue was recrystallized from Me_2CO–ligroine (bp 60–80 °C) to yield colorless needles: mp 152 °C; IR (Nujol) 1706 cm^{-1} (COOH). Anal. ($C_{16}H_{18}O_5$) C, H.

Spiro[cyclohexane-1,2′-tetralin]-1,4′-dione (3a). The above tetralone **2a** (17.3 g, 0.05 mol) was gradually added to a well-stirred suspension of metallic sodium (1.2 g) in dry toluene (100 ml) under an atmosphere of N_2 at 100 °C. After the vigorous reaction had subsided the mixture was extracted with $CHCl_3$ (3 × 50 ml). The combined, undried $CHCl_3$ extracts were evaporated and the residue was distilled in vacuo to afford a yellow oil, bp 183–187 °C (1 mm), which slowly crystallized from Et_2O as colorless needles (7.0 g, 61.4%): mp 72–73 °C; IR (Nujol) 1710 (six-membered cyclic C=O), 1680 cm^{-1} (aromatic C=O); NMR ($CHCl_3$) δ 8.05 (1 H, d, aromatic ring, J = 9 Hz, HC), 7.4 (3 H, m, aromatic ring), 3.05 (2 H, t, $ArCH_2$–), 2.35 (10 H, m, $-CH_2CCH_2CH_2COCH_2CH_2-$); UV (propan-2-ol) 295 nm (log ε 3.45). Anal. ($C_{15}H_{16}O_2$) C, H.

2,2-Bis(β-ethoxycarbonylethyl)indanone (2b). A reaction between ethyl acrylate (24.0 g, 0.24 mol) and α-indanone (38.0 g, 0.287 mol) as described for **2a** gave 48.0 g (52.0%) of 2,2-bis(β-ethoxycarbonylethyl)indanone: bp 186–188 °C (0.8 mm) [lit.[1] bp 190–195 °C (1.0 mm)].

Spiro[cyclohexane-1,2′-indan]-1,4′-dione (3b). A Dieckmann reaction upon **2b** (33.2 g, 0.1 mol) as described under **2a** gave 10.0 g (46.7%) of spiro[cyclohexane-1,2′-indan]-1,4′-dione: bp 168–170 °C (1.0 mm) [lit.[1] bp 156–158 °C (0.7 mm)].

Spiro[cyclohexane-1,2′-indan]-1,4′-diol (4). Sodium borohydride (1.8 g, 0.475 mol) was gradually added to a cooled solution of **3b** (6.0 g, 0.028 mol) in methanol (100 ml). After stirring for 0.5 h the solution was allowed to stand at room temperature for a further 4 h and then poured into H_2O (200 ml). The methanol was evaporated and the aqueous residue extracted with $CHCl_3$ (3 × 100 ml). The combined $CHCl_3$ extracts were evaporated to afford a pale yellow oil which solidified on cooling. Recrystallization from Me_2CO–ligroine (bp 40–60 °C) yielded colorless microneedles (5.0 g, 82.0%): mp 124 °C; IR (Nujol) 3300 cm^{-1} (OH). Anal. ($C_{14}H_{18}O_2$) C, H.

Pharmacological Results. Immature female albino mice (CFLP-ICI strain I) were random bred in our own laboratories and those animals between 23 and 25 days old were selected for experiment. They were distributed into groups of ten and placed in opaque polypropylene cages where they were allowed free access to drinking water and a 41B cube diet (Pilsburys Birmingham) throughout. All experiments were performed under constant environmental conditions and a laboratory temperature of 25 ± 1 °C and relative humidity of 60–70% were maintained.

The mice received either stilbestrol alone at a single dose of 0.01 mg/kg or together with various doses of the spiro derivative each day for three consecutive days. Further groups received the spiro derivatives alone in order to detect any intrinsic estrogenic activity within this class of compound. Control groups received arachis oil, 10 ml/kg. All compounds were administered in solution in arachis oil by intraperitoneal injection at 10.00 h. All animals were killed by cervical dislocation 24 h after the last injection and the body weights determined. The uteri were then carefully dissected out, freed from fat, blotted dry on filter paper,

and weighed. Finally, uterine ratios were calculated as uterine weight (mg)/body weight (g) × 100.

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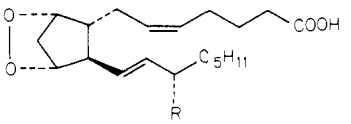
Communications to the Editor

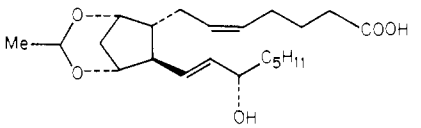
A Novel Prostaglandin Endoperoxide Mimic, Prostaglandin $F_{2\alpha}$ Acetal

Sir:

The prostaglandin endoperoxides PGG_2 and PGH_2 are known to be intermediates in the biosynthesis of PGE_2, $PGF_{2\alpha}$, PGD_2, and Thromboxane A_2.[1-4] The fact that PGG_2 and PGH_2 are extremely potent, but chemically labile, has prompted the synthesis of a number of stable analogues whose ring-system geometry approximates that of the endoperoxide.[5-8] Several of these analogues have been reported[6,7,9] to mimic the biological activity of PGG_2 and PGH_2. This communication describes the synthesis and biological properties of a novel endoperoxide mimic, $PGF_{2\alpha}$ acetal (1), whose ring geometry represents a departure from that found in the natural endoperoxides and their analogues.



PGG_2, R = OOH
PGH_2, R = OH



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Prostaglandin $F_{2\alpha}$ was allowed to react with neat acetaldehyde in the presence of 0.1% HCl for 1.5 h at 25 °C. The reaction mixture was treated with aqueous sodium bicarbonate to quench catalyst and the product 1 was regenerated with pH 3 buffer. Purification by column chromatography on silica gel furnished the desired acetal 1 as a mixture of two epimers 1a and 1**b**: yield 41%; two spots on TLC with R_f 0.53 and 0.49 [silica gel, 100 μm, double development in $CHCl_3$–EtOAc–HOAc (50:50:1)]. Anal. Calcd for $C_{22}H_{36}O_5$: C, 69.44; H, 9.54. Found: C, 69.40; H, 9.28. HPLC of the mixture using a 25 cm × 8 mm i.d. Micropak CN-10 column and 2% MeOH in $CHCl_3$ furnished purified 1a: IR ($CHCl_3$) 3500–3300 (OH), 1710 (acid C=O), 1340 cm^{-1} (OCHO, CH def); EIMS of the methyl ester m/e 394 (M^+). The NMR spectrum ($CDCl_3$) of the epimeric mixture prior to HPLC separation of 1a exhibited readily observable acetal methyl and methine proton absorptions as doublets and quartets, respectively. The methyl and methine resonances for the acetal group of the pure isomer 1a appear at δ 1.25 and 5.10, respectively, and those of 1**b** appear at δ 1.37 and 4.75, respectively. The NMR spectrum of the isomer mixture indicates an approximately 60:40 ratio for 1**a**:1**b** in the mixture.

Table I. Relative Potency of Prostaglandin $F_{2\alpha}$ Acetal (1a) on Various Smooth Muscle Preparations

	Relative contractile effects[a]		
	Gerbil colon[b] ($PGF_{2\alpha}$ = 1)	Rabbit aorta[c] (PGE_2 = 1)	Saphenous vein[d] ($PGF_{2\alpha}$ = 1)
1a	0.12 ± 0.01 (n = 3)	19 ± 4 (n = 3)	32 ± 7 (n = 6)
PGG_2	1.5[e]	80[e]	

[a] Based on the mean molar potencies ± SE. [b] Reference 18. [c] Reference 19. [d] Reference 20. [e] Data obtained from ref 10.

Preliminary experiments indicate that 1a is fairly stable in aqueous medium, as it was observed that close to 100% of the biological activity was retained when a Krebs solution (pH 8.2) of 1a was maintained at 4 °C for 1 week. Moreover, a saline solution of 1 stored at 25 °C for 24 h was estimated to be approximately 5% hydrolyzed.

Previous studies have indicated a spectrum of activities which are characteristic for the endoperoxides.[4,10] Prostaglandins G_2 and H_2 are potent inducers of platelet aggregation and, relative to the classical prostaglandins, are potent stimulators of vascular smooth muscle contractility but are generally less or equipotent stimulators of gastrointestinal tract smooth muscle. This profile of activity has been employed in the present study to evaluate 1**a**.

Epimer 1**a** was tested[11,12] for its ability to induce platelet aggregation in human, citrated platelet-rich plasma. At concentrations of 0.7–4 μg/ml $PGF_{2\alpha}$ acetal caused a small reversible wave of platelet aggregation which became complete and irreversible at concentrations of 5–10 μg/ml (n = 4). By comparison, PGG_2 produces irreversible aggregation at 100–250 ng/ml. Thus, 1**a** has approximately $^1/_{40}$–$^1/_{50}$th the potency of PGG_2. The effect of 1**a** on platelets is uninhibited by 10 μg/ml of the PG synthetase inhibitor,[13] indomethacin, suggesting that its primary action is direct and is not due to stimulation of endogenous prostaglandin synthesis. Finally, transmission electron microscopy of platelets exposed to 1**a** shows that its effect on platelet ultrastructure[14] is very similar to that of PGG_2.[15-17]

Additional evidence suggesting that 1**a** is acting as an endoperoxide mimic was obtained from its pattern of activity on three different smooth muscle preparations: the isolated gerbil colon,[18] rabbit thoracic aorta,[19] and canine lateral saphenous vein[20] (Table I). Prostaglandin $F_{2\alpha}$ acetal 1**a** caused concentration-dependent contraction of all three preparations and the pattern of relative potencies was qualitatively similar to that of PGG_2 (Table I). Epimer