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The oestrogenic and anti-oestrogenic properties of ring methyl-substituted stilboestrols

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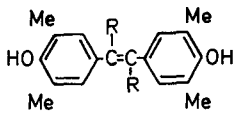
3,3′,5,5′-Tetramethylstilboestrol (I) and its α,α′-dimethyl- (II) and α,α′-diethyl- homologues (III) have been tested for oestrogenic and anti-oestrogenic activity. α,α′-Diethyl-3,3′,5,5′-tetramethylstilboestrol (III), by the uterine weight assay in immature mice, is $2{\cdot}1 \times 10^{-4}$ times as potent as an oestrogen as 17β-oestradiol [fiducial limits (0·95) = $1{\cdot}59 \times 10^{-4} - 2{\cdot}74 \times 10^{-4}$] but 3,3′,5,5′-tetramethylstilboestrol (I) and α,α′,3,3′,5,5′-hexamethylstilboestrol (II) were oestrogenically inactive at a dose of 0·8 mg. Compound III exhibited auto-inhibition of its own oestrogenic response at doses between 1·8 and 7·2 mg. None of the compounds tested inhibited the uterotrophic response to 17β-oestradiol. Compounds II and III, but not compound I, produced a highly significant inhibition of the vaginal cornification response to 17β-oestradiol when the test compounds (5 μg) and 17β-oestradiol were administered intravaginally in a single solution. It is suggested that the compounds II and III compete with 17β-oestradiol for the oestrogen receptor and that methyl groups positioned *ortho* to the phenolic hydroxyl group sterically interfere with the binding of the compounds to the oestrogen receptor, or with the initiation of the oestrogenic response, or both.

In the hope of producing anti-oestrogens which are effective when administered subcutaneously as well as locally, Clark & O'Donnell (1965a,b) synthesized and tested a series of ring methyl-substituted compounds related to diethylstilboestrol. Alkylation of the aromatic rings of di-*p*-hydroxyphenylalkanes and -alkenes, and ring A of 17β-oestradiol is known to reduce oestrogenic potency (Kaiser & Svarz, 1946; Shishido, Nozaki & Iwako, 1949; Niederl & Weiss, 1948; Iriarte & Ringold, 1958; Patton & Dmochowski, 1963), and reduced oestrogenic potency would allow a larger dose of the substance to be administered subcutaneously in tests for oestrogen antagonism. Clark & O'Donnell found that the introduction of four methyl groups, *ortho* to the phenolic hydroxyl groups in ψ-diethylstilboestrol, produced a compound with no oestrogenic activity in the Allen-Doisy test when 1 mg was given subcutaneously or 0·25 mg was given intravaginally to mice. No anti-oestrogenic activity was observed when the tetramethylated-ψ-diethylstilboestrol was administered subcutaneously but 25 μg inhibited the response to 17β-oestradiol when the two substances were administered intravaginally.

The observation by Emmens, Cox & Martin (1959) that in the α,α′-dialkylstilboestrols the highest anti-oestrogenic activity occurs in the dimethyl compound suggested that the examination of other ring tetramethylated stilboestrols would be of interest and we now report our studies with 3,3′,5,5′-tetramethylstilboestrol (I); α,α′,3,3′,5,5′-hexamethylstilboestrol (II); α,α′-diethyl-3,3′,5,5′-tetramethylstilboestrol (III) (Clark & O'Donnell, 1956b).



I R = H
II R = Me
III R = Et

METHODS

Preparation of solutions

(*a*) Solutions for subcutaneous injection were prepared immediately before each experiment. Aliquots of a stock ethanolic solution of 17β-oestradiol (1 mg/ml in 96% ethanol prepared every four weeks and stored at 4°) or volumes of a freshly prepared ethanolic solution of test substance (or both) were added to arachis oil to produce, after evaporation of the alcohol under nitrogen with slight warming on a water bath, the required dose of 17β-oestradiol or test compound in 0·15 ml arachis oil. Where systemic anti-oestrogenic properties of the compounds were to be examined, 17β-oestradiol and test compound were both added to the same volume of oil.

(*b*) Solutions for intravaginal injection were prepared in 2 or 4% aqueous Tween 80. Amounts of the ethanolic solutions of 17β-oestradiol or test compounds (or both) were added to undiluted Tween 80, and the alcohol evaporated off *in vacuo*, using a water pump. The solutions were then made up with warm distilled water to give the required doses in 0·02 ml of 2 or 4% aqueous Tween 80.

Uterine weight test

Oestrogenic properties of the compounds were assessed in randomly distributed groups of immature female mice (Tuck's No. 1 strain), each $\approx$12 g, injected subcutaneously with a range of doses of the compounds. The three-injection technique introduced by Rubin, Dorfman & others (1951) was used, each 'sub-dose' being given in 0·05 ml arachis oil at approximately 10 a.m. on each of three consecutive days. Animals were killed by cervical dislocation 24 h after the last injection and the uteri removed, freed of adhering connective tissue, and blotted before weighing on a Roller-Smith torsion balance. Actual uterine weights were used in calculations since no correlation between uterine weight and total body weight could be found.

Anti-oestrogenic activity was assessed by a comparison of the increase in uterine weight produced by two different doses of 17β-oestradiol alone with the same two doses of 17β-oestradiol each in combination with a range of non-oestrogenic doses of the test compounds.

Allen-Doisy test

Colonies of mice, ovariectomized at 6 weeks, were randomly distributed into groups and used at fortnightly intervals. Sensitivity was maintained by priming the mice every 6 weeks with 17β-oestradiol, 1 μg subcutaneously, in 0·05 ml arachis oil. An intravaginal two injection technique was used (Emmens & Cox, 1958) at approximately 10 a.m. on each of two consecutive days, each "sub-dose" being given in 0·01 ml of 2% aqueous Tween 80 for compounds II and III and a 4% solution for compound I because of its lower solubility. Vaginal smears were taken at approximately 10 a.m. and 5 p.m. on the 3rd day. Each mouse was scored as 0, 1 or 2 depending on whether neither, or one, or both smears indicated a positive oestrogenic response (Claringbold, 1956).

RESULTS

Uterine weight test

Neither test compound I nor II showed any oestrogenic activity at the doses tested (Table 1), which approached the limits of solubility, and which were much greater than the doses of 17β-oestradiol that produced an oestrogenic response. Compound III showed weak oestrogenic activity, being $2{\cdot}1 \times 10^{-4}$ times as potent as 17β-oestradiol [fiducial limits ($P = 0{\cdot}95$) = $1{\cdot}59 \times 10^{-4} - 2{\cdot}74 \times 10^{-4}$]; at higher doses (0·9–7·2 mg) auto-inhibition was observed.

The compounds in non-oestrogenic doses were inactive in antagonizing the effects of concurrently administered 17β-oestradiol.

Table 1. *Oestrogenic properties of the test compounds, subcutaneously administered in the uterine weight test in immature mice.*

Expt. No.	No. of mice per group	Total dose of test compound/mouse (mg)	Total dose of 17β-oestradiol per mouse (μg)	Mean uterine weight ± s.e. (mg)
1	15	3,3′,5,5′-Tetramethyl stilboestrol (I)		
		0·1		7·98 ± 0·50
		0·2		8·12 ± 0·43
		0·4		8·50 ± 0·57
		0·8		9·66 ± 0·63
			0·03	20·00 ± 1·04
			0·06	26·56 ± 2·58
		0·0	0·0	8·24 ± 0·81
2	10	α,α′,3,3′,5,5′-Hexamethyl stilboestrol (II)		
		0·1		9·43 ± 1·08
		0·2		8·68 ± 1·01
		0·4		8·29 ± 1·13
		0·8		10·56 ± 0·71
			0·03	12·62 ± 1·13
			0·06	19·67 ± 1·74
		0·0	0·0	8·08 ± 0·81
3	10	α,β-Diethyl-3,3′,5,5′-tetramethyl-stilboestrol (III)		
		0·9		44·67 ± 1·79
		1·8		41·21 ± 3·00
		3·6		36·01 ± 2·83
		7·2		33·98 ± 1·59
			0·045	23·69 ± 1·14
			0·09	38·12 ± 2·10
4	16	0·12		19·97 ± 1·11
		0·16		26·72 ± 1·93
		0·21		33·78 ± 4·10
		0·28		40·11 ± 2·89
			0·03	26·04 ± 1·39
			0·06	37·01 ± 2·18

Allen-Doisy test

Intravaginal administration, to ovariectomized mice, of the compounds II or III (5 μg) together with 17β-oestradiol ($1{\cdot}5 \times 10^{-4}$–$13{\cdot}5 \times 10^{-4}$ μg) demonstrated a highly significant inhibition of the vaginal cornification normally produced by 17β-oestradiol ($P < 0{\cdot}01 > 0{\cdot}001$ and $< 0{\cdot}001$ for II and III, respectively). Compound I was inactive (Table 2).

Table 2. *Anti-oestrogenic properties of the test compounds given intravaginally together with 17β-oestradiol.* (Scores are totals for groups of mice: scoring system 0, no reaction; 1, one positive smear; 2, both smears positive)

Expt. No.	No. of mice per group	Total dose of test compound (μg)	Total dose of 17β-oestradiol per mouse (μg × 10^{-4}) 1·5	4·5	13·5
			score		
		Compound I			
1	12	0	13	20	22
		5	13	21	24
		Compound II			
2	10	0	4	9	13
		5	0	2	8
		Compound III			
3	10	0	10	13	17
		5	1	5	11

DISCUSSION

As was found for ψ-diethylstilboestrol (Clark & O'Donnell, 1965a) the introduction of four methyl groups *ortho* to the phenolic hydroxyl groups into α,α′-dimethylstilboestrol and α,α′-diethylstilboestrol has markedly reduced the oestrogenic potency. Thus there was no increase in uterine weight with compound II at a dose some 30 times the oestrogenic dose of α,α′-dimethylstilboestrol, and compound III was only weakly oestrogenic (approx. 1/5000 the potency of 17β-oestradiol). The negative regression of the uterine weights with larger doses of compound III suggests auto-inhibition and is reminiscent of the report that MER-25 is weakly uterotrophic at 0·2–1 mg dose but not with a dose of 5 mg (Lerner, Holthans Jr., & Thompson, 1958). Compared with the structurally isomeric 3,4-bis-(3,5-dimethyl-4-hydroxyphenyl)hex-2-ene which Clark & O'Donnell (1965a) found to be non-oestrogenic in mice at 1 mg s.c., there is clearly an increase in oestrogenic potency when the double bond is shifted to form the stilbene. This is similar to the difference in potency amongst the analogous isomers of diethylstilboestrol (Wessely & Kleedorfer, 1939).

In parallel with the increase in oestrogenic potency with the shift in position of the double bond, there is an increase in anti-oestrogenic potency, a similar level of inhibition of the response to 17β-oestradiol being obtained with 5 μg of compound III instead of 25 μg for 3,4-bis-(3,5-dimethyl-4-hydroxyphenyl)hex-2-ene (Clark & O'Donnell, 1965a).

The hoped for increase in anti-oestrogenic potency on replacing the α-ethyl groups present in compound III by α-methyl groups (compound II) has not been realized, both compounds producing a similar degree of inhibition at similar doses. Furthermore, compound II is less active as an oestrogen antagonist than the parent α,α′-dimethylstilboestrol which Emmens & others (1959) found to effectively inhibit the vaginal response to 17β-oestradiol at 0·2–0·4 μg.

With the complete absence of both oestrogenic and anti-oestrogenic activity in compound I it must be concluded that in this type of compound the same structural features that endow the molecules with oestrogenic activity are also needed for oestrogen antagonism. It seems therefore that compounds II and III are producing their effect by combining with the oestrogen receptor, but at the doses at which antagonism is observed they are unable to stimulate the normal oestrogenic response.

The "auto-inhibition" exhibited by compound III in the uterine weight test may be the result of general toxicity or may be interpreted as evidence of two-point attachment to the oestrogen receptor, high doses resulting in over-saturation of the receptor sites so that many molecules can gain access to only a single point of attachment.

Although dissociation constants for the test compounds have not been determined, by analogy one can deduce from a comparison of the pairs 2,6-xylenol ($pK_a = 10{\cdot}58$) and phenol ($pK_a = 9{\cdot}90$) (Wheland, Brownell & Mayo, 1948); and 2,4,6-trimethylphenol ($pK_a = 10{\cdot}88$) and *p*-cresol ($pK_a = 10{\cdot}19$) (Sprengling & Lewis, 1953) that the presence of the two *o*-methyl groups reduces the dissociation of phenols. The reduction in oestrogenic activity occasioned by *o*-methyl substitution may be due to this decrease in acidity with a resultant decrease in the strength of hydrogen bonds between the test compounds and the oestrogen receptor. Alternatively the methyl groups may, by increasing the bulk of the molecules, sterically interfere with the processes involved in binding and initiation of the oestrogenic response at the receptor site. That the oestrogen-receptor interaction is very susceptible to steric inhibition is suggested by the findings of Patton & Dmochowski (1963) who observed that the presence of a n-propyl group at position 2 in oestrone produced a 3500 fold decrease in oestrogenic potency, whereas a 2-methyl group produced only a 30 fold decrease.

It is possible that the presence of the 4 ring-methyl groups may affect the uptake and distribution of the test compounds from their sites of injection. That this is, however, not a primary reason for the absence of oestrogenic activity in compounds I and II and the low oestrogenicity of compound III is suggested by the fact that, on intravaginal administration, 5 μg of compounds II and III exhibit anti-oestrogenic responses, i.e. they are non-oestrogenic at this dose, whereas the median effective dose, as an oestrogen, for α,α'-diethylstilboestrol is $3{\cdot}7 \times 10^{-4}$ μg (Emmens, 1942).

CHEMISTRY

The 3,3',5,5'-tetramethyl-α,α'-diethylstilboestrol used in the biological experiment was that described by Clark & O'Donnell (1965b). The α,α'-dimethyl homologue was prepared by an analogous series of reactions to those used for the diethyl compound, and the 3,3',5,5'-tetramethylstilboestrol by an essentially similar process which differed only in the reduction of the 3,3',5,5'-tetramethyldeoxyanisoin with lithium aluminium hydride before dehydration to the stilbene. That the 4,4'-dimethoxystilbenes possess the *trans* structure is indicated by their ultraviolet absorption spectra. The shape of the absorption curve for 4,4'-dimethoxy-3,3',5,5'-tetramethylstilbene is similar to that given by Laarhoven, Nivard & Havinga (1960) for 4,4'-dimethoxystilbene and the expected decrease in the long wavelength absorbance with α,α'-alkylation is observed. The absorbances of the free phenols shows a similar dependence on α-carbon alkylation.

The change in the ultraviolet absorption spectrum of compound I in alcoholic solution stored for 7 days at room temperature stresses the need for fresh solutions to be used in experiments. The hypsochromic shift of the peak at 305 nm to 292 nm and the marked decrease in intensity of the band is consistent with the conversion of *trans*-stilbene into *cis*-stilbene [cf. *trans*-stilbene λ_{max} 295 (ϵ 27 000), cis-stilbene λ_{max} 280 (ϵ 13 500) nm]. Since *cis*-diethylstilboestrol is only very weakly active compared with its *trans* isomer such isomerization could be expected to be disadvantageous for oestrogenic or anti-oestrogenic activity.

EXPERIMENTAL

Infrared spectra were recorded on a Perkin-Elmer Infracord Spectrophotometer, model 137 or model 257, nmr spectra on a Varian A60 spectrometer and ultraviolet spectra on a Unicam SP700 or SP800A.

Microanalyses are by Mr. J. A. Stewart of the Microanalytical Laboratory, University of Leeds.

α-(3,5-*Dimethyl*-4-*methoxyphenyl*)*propionic acid.* Diethyl 3,5-dimethyl-4-methoxyphenyl malonate (63 g) was alkylated in the usual way using sodium (4·9 g), dry ethanol (80 ml) and methyl iodide (33 g) to yield diethyl (3,5-dimethyl-4-methoxyphenyl)-methylmalonate (47 g), b.p. 134–137°/0·15 mm. (Found: C, 66·4; H, 7·9. $C_{17}H_{24}O_5$ requires C, 66·3; H, 7·8%). ν_{max} (liq. film) 1730vs (COOR), 1600w, 1490s, 1470s, 1450sh (aromatic ring), 1250vs (aralkyl ether), 1160vs, 1110vs, 1020vs, 875m, 865m (isolated aromatic-H) cm^{-1}.

The derived ester was hydrolysed with 10% alcoholic KOH and the isolated malonic acid decarboxylated by heating at 170° for 30 min under water pump vacuum. Recrystallization of the residue from 40–60° light petroleum yielded the required α-(3,5-*dimethyl*-4-*methoxyphenyl*)*propionic acid* (23 g), m.p. 81–82·5°. Found: C, 68·95; H, 7·5. $C_{12}H_{16}O_3$ requires C, 69·2; H, 7·75%. ν_{max} (KCl disc) 1700vs (COOH), 1595w, 1490s, 1460s (aryl ring), 1415s, 1375m, 1330m, 1300s, 1280m–s, 1220vs (aralkyl ether), 1150s, 1010s, 955m, 930m (OH deformation), 885m (isolated aromatic-H) cm^{-1}.

α-(3,5-*Dimethyl*-4-*methoxyphenyl*)*propionyl chloride.* α-(3,5-Dimethyl-4-methoxyphenyl)propionic acid (24 g) and thionyl chloride (56 g) when heated together under reflux for 3 h yielded the required acid chloride (23·5 g), b.p. 150–152°/12 mm.

3,3′,5,5′-*Tetramethyl*-α-*methyldeoxyanisoin.* Anhydrous, powdered aluminium chloride (15·2 g) was added slowly with stirring to a cooled (<5°) solution of 2,6-dimethylanisole (21 g) in dry carbon disulphide (80 ml) at below 5°. α-(3,5-Dimethyl-4-methoxyphenyl)propionyl chloride (23·5 g) was then run in slowly, with stirring at below 5°. The flask was then allowed to warm to room temperature and heated on a water-bath for 2½ h. The mixture was poured into crushed ice containing a little hydrochloric acid and the oily layer taken into ether. Distillation of the ethereal extract yielded an oil (26·7 g), b.p. 170–182°/0·01 mm which slowly crystallized. Recrystallization yielded the *required ketone*, m.p. 67–68·5°. (Found C, 77·2; H, 7·8. $C_{21}H_{26}O_3$ requires C, 77·3; H, 8·0%.) ν_{max} (liq. film) 1678vs (aryl ketone), 1595s, 1485vs, 1450s (aryl ring), 1415s, 1372m, 1337s, 1295s (alkyl ketone), 1235–1222vs (aralkyl ether), 1152vs (aryl ketone), 1100m, 1047m, 1012vs, 954m, 926m, 908m, 882s (isolated aromatic H), 793s, 770m cm^{-1}.

2,3-*Bis*-(3,5-*dimethyl*-4-*methoxyphenyl*)*butan*-2-*ol.* 3,3′,5,5′-Tetramethyl-α-methyldeoxyanisoin (28·4 g) in dry ether (100 ml) was added slowly with stirring to a Grignard reagent prepared from methyl iodide (49 g), magnesium turnings (3·35g) and dry ether (200 ml). The mixture was then heated under reflux on a water bath for 2 h and left overnight. The reaction mixture was poured into ice and dilute hydrochloric acid, the ethereal layer separated and the aqueous layer extracted with ether. The combined ethereal solutions were washed with water, 10% sodium bicarbonate solution, 10% sodium thiosulphate solution, and water, and dried ($CaSO_4$). Distillation of the ether gave crystals (28·5 g) m.p. 104–121°. Fractional recrystallization of a portion (2 g) from 96% ethanol yielded erythro-2,3-*bis*-(3,5-

dimethyl-4-methoxyphenyl)butan-2-ol (1·12 g), m.p. 125–128°. (Found: C, 76·9; H, 8·8. $C_{22}H_{30}O_3$ requires C, 77·2; H, 8·8%). ν_{max} (KCl disc) 3500s (OH), 1600w, 1488vs, 1453s (aryl ring), 1370s, 1224vs (aryl ether), 1145vs (alkyl ether), 1129s, 1020s, 1000s, 883m, 875m (isolated aromatic-H), 770m, cm^{-1}. The threo *isomer* (0·21 g), m.p. 110–112° (Found: C, 77·5; H, 8·95%) was obtained by recrystallization from ethanol of the viscous residue obtained by evaporation of the mother liquors from the *erythro* isomer. The infrared spectrum differs from that of the *erythro* isomer only in the relative intensities of the peaks at 1453, 883 and 875 cm^{-1}.

4,4′-*Dimethoxy-α,α′,3,3′,5,5′-hexamethylstilbene.* The unrecrystallized mixture of diastereoisomers of 2,3-bis-(3,5-dimethyl-4-methoxyphenyl)butan-2-ol (5 g) was heated to 130° under an air reflux, iodine (50 mg) added to the melt and the mixture maintained at 130° for 1½ h. After cooling the mixture was dissolved in ether and the ethereal solution washed with 10% sodium thiosulphate solution and water, and dried ($CaSO_4$). Evaporation of the ether gave a sticky solid residue (4·5 g) which was crystallized repeatedly from light petroleum (60–80°) to yield the required 4,4′-*dimethoxy-α,α′,3,3′,5,5′-hexamethylstilbene* (1·42 g) m.p. 117·5–119°. (Found: C, 81·5; H, 8·55. $C_{22}H_{28}O_2$ requires C, 81·4; H, 8·7%.) ν_{max} (CCl_4) 1590w, 1488vs, 1449s (aryl ring), 1418m, 1375m, 1323s, 1230vs (aryl ether), 1188m, 1163vs (alkyl ether), 1116w, 1095m, 1078w, 1022vs, 937w, 883s (isolated aromatic-H) cm^{-1}. λ_{max} (ethanol) 212·5 (ϵ 31 000), 245 (ϵ 14 570) nm. Nmr: τ 8·18 (2 × CH_3–C; singlet); τ 7·73 (4 × CH_3–Ar; singlet); τ 6·28 (2 × CH_3OAr; singlet); τ 3·2 (Ar–H; singlet).

4,4′-*Dihydroxy-α,α′,3,3′,5,5′-hexamethylstilbene.* 4,4′-Dimethoxy-α,α′,3,3′,5,5′-hexamethylstilbene (2 g) in dry ether (20 ml) was added to a Grignard reagent prepared from methyl iodide (8·5 g), magnesium turnings (1·43 g) and dry ether (50 ml). The ether was distilled and the residue heated at 160–170° (bath temperature) for 3 h. The reaction mixture was cooled, decomposed with ice and 2N hydrochloric acid, and extracted with ether. The ethereal extract was washed with water, 10% sodium thiosulphate solution, and water, and dried ($MgSO_4$). Distillation of the ether gave a residue (1·8 g). Recrystallization from 60% aqueous ethanol gave the *required phenolic stilbene* (1·34 g), m.p. 219–222° (decomp.). (Found: C, 80·9; H, 8·0. $C_{20}H_{24}O_2$ requires C, 81·05; H, 8·15%.) ν_{max} (KCl disc): 3340vs (OH), 1605m, 1485vs, 1442s (aryl ring), 1412s, 1390s, 1376s, 1367s, 1360sh, 1319vs (phenol), 1269m, 1225vs (phenol), 1167vs, 1092s, 1074m, 1030m, 990m, 948m, 897m, 874s (isolated aromatic-H), 758s, 731 cm^{-1}. λ_{max} (ethanol): 212·5 (ϵ 30 510), 250 (ϵ 13 730), shoulder at *ca* 280 (ϵ *ca* 7100) nm.

3,3′,5,5′-*Tetramethyldeoxyanisoin* was prepared in a manner analogous to that for the α-methyl homologue, using 3,5-dimethyl-4-methoxyphenylacetyl chloride (9·1 g), 2,6-dimethylanisole (8·75 g), anhydrous aluminium chloride (5·7 g) and dry carbon disulphide (*ca* 50 ml). The *required ketone* was obtained as a very viscous oil (6 g) which slowly crystallized over many months, b.p. (bath temperature) 195–200°/4 × 10^{-3} mm. (Found: C, 76·7; H, 7·65. $C_{20}H_{24}O_3$ requires C, 76·9; H, 7·7%.) ν_{max} (liquid film): 1678vs (aryl ketone), 1600s, 1490vs, 1449s (aryl ring), 1414s, 1379m, 1340sh, 1318vs, 1227vs (aryl ether), 1181m, 1148vs (alkyl ether), 1064m, 1014vs, 900m, 876m (isolated aromatic-H), 797m, 769m cm^{-1}.

1,2-*Bis-(3,5-dimethyl-4-methoxyphenyl)ethanol.* 3,3′,5,5′-Tetramethyldeoxyanisoin (6 g) in ether (100 ml) was reduced with lithium aluminium hydride (0·18 g) in the usual way to yield a solid which was recrystallized from ethanol to yield the *required alcohol* (4·1 g), m.p. 134–135°. (Found: C, 76·1; H, 8·2. $C_{20}H_{36}O_3$ requires C, 76·4;

H, 8·3%.) ν_{max} (KCl disc): 3400s (OH), 1600w, 1486vs, 1460sh (aryl ring), 1437m, 1420m, 1376m, 1344m, 1318m, 1300m, 1263m, 1222vs (aryl ether), 1183m, 1144vs (alkyl ether), 1072s, 1012vs, 888m, 876s (isolated aromatic-H), 851m, 768m cm^{-1}.

4,4′-Dimethoxy-3,3′,5,5′-tetramethylstilbene. 1,2-Bis-(3,5-dimethyl-4-methoxyphenyl)ethanol (4 g), glacial acetic acid (30 ml) and concentrated hydrochloric acid (7·5 ml) were boiled under reflux for 10 min, diluted with water (50 ml) and the solid product filtered off. Recrystallization from benzene gave the *required stilbene* m.p. 219–220. (Found: C, 81·3; H, 8·25. $C_{20}H_{24}O_2$ requires C, 81·0; H, 8·15%.) λ_{max} (ethanol): 212·5 (ϵ 24 220), 237 (ϵ 19 620), 244·5 (ϵ 15 024), 305 (ϵ 27 360), 318 (ϵ 26 910), shoulders at *ca* 230 (ϵ *ca* 17 700), *ca* 295 (ϵ *ca* 24 700) and *ca* 330 (ϵ *ca* 18 160) nm. After storage of the alcoholic solution for 7 days at room temperature λ_{max}: 216 (ϵ 24 600), 234 (ϵ 23 920), 292 (ϵ 13 320) nm. ν_{max} (KCl disc): 1600w, 1590w, 1485vs, 1450s (aryl ring), 1305s (trans α,β-disubstituted ethylene), 1220vs (aryl ether), 996vs (oop deformations of *trans* disubstituted ethylene), 881s (isolated aromatic-H) cm^{-1}.

4,4′-Dihydroxy-3,3′,5,5′-tetramethylstilbene. 4,4′-Dimethoxy-3,3′,5,5′-tetramethylstilbene (2 g) was demethylated with methyl magnesium iodide [prepared from methyl iodide (10·6 g), magnesium (1·6 g) and ether] in the usual way. Fractional crystallization of the product from absolute ethanol yielded the *required phenolic stilbene* (1·2 g), m.p. 239–241°. (Found: C, 80·55; H, 7·45. $C_{18}H_{20}O_2$ requires C, 80·6; H, 7·5%.) λ_{max} (ethanol): 213·5 (ϵ 25 940), 238 (ϵ 16 840), 311 (ϵ 28 510), 327·5 (30 460), shoulders at *ca* 246 (ϵ *ca* 12 870), and *ca* 300 (ϵ *ca* 25 300) nm. λ_{max} (ethanol) after 7 days storage at room temperature: 216 (ϵ 24 600), 234 (ϵ 23 920), 292 (ϵ 13 320) nm. ν_{max} (KCl disc): 3380–3410vs (OH), 1605s, 1487vs (aryl ring), 1322vs (*trans* disubstituted ethylene), 1233vs, 1210vs (aryl ether), 955s (isolated aromatic-H) cm^{-1}.

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