Estrogenic Thio-Analogs of the Dimethyl Ethers of Diethylstilbestrol, its Homologs, and Hexestrol

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The author and Y. Urushibara[1] have presented a hypothesis postulating that it is essential for a highly estrogenic substance to possess not only two groups carrying active hydrogen atoms, located at the optimum distance from one another,[2] but also a moderate thickness of the molecule. Estradiol is endowed *a priori* with such favorable structural features and hexestrol shares the fame of strong estrogens with the natural hormone due to its privilege of being a meso form, while diethylstilbestrol, which otherwise would have a planar structure, acquires its molecular thickness qualifying it for an estrogen of the same rank through the steric interference between the benzene rings and the ethyl groups, the former enforced to rotate to a moderate extent and the latter arranged in a coördinated manner. The steric interference and the rotation of the benzene rings caused by it have been deduced from a comparison of the absorption spectra of the dimethyl ethers of diethylstilbestrol and its lower and higher homologs with that of the unsubstituted 4, 4′-dimethoxystilbene on the assumption that the deterioration in the light absorption is associated with the loss in conjugation.

The substitution of the methoxy group or the methylthio group for the ethyl groups in hexestrol and diethylstilbestrol may cause no radical change in the shapes of the molecules. Thus, various oxygen and sulfur analogs in this line have been synthesized and proved to be estrogenic as reported from this laboratory.[3], [4], [5]

The present paper contains the following thio-analogs, derived from dimethyl ethers of diethylstilbestrol, its homologs, and hexestrol, by replacing the phenolic oxygen atoms with sulfur atoms. The methods of preparation of these substances are described in the Experimental Part.

4, 4′-Bis-(methylthio)-α, α'-dimethylstilbene,
$CH_3SC_6H_4C(CH_3)=C(CH_3)C_6H_4SCH_3$ (I)

4, 4′-Bis-(methylthio)-α, α'-diethylstilbene,
$CH_3SC_6H_4C(C_2H_5)=C(C_2H_5)C_6H_4SCH_3$ (II)

4, 4′-Bis-(methylthio)-α, α'-dipropylstilbene,
$CH_3SC_6H_4C(C_3H_7)=C(C_3H_7)C_6H_4SCH_3$ (III)

4-Methoxy-4′-(methylthio)-α, α'-diethylstilbene,
$CH_3OC_6H_4C(C_2H_5)=C(C_2H_5)C_6H_4SCH_3$ (IV)

3, 4-Bis-[*p*-(methylthio)-phenyl]-hexane,
$CH_3SC_6H_4CH(C_2H_5)CH(C_2H_5)C_6H_4SCH_3$ (V)

The steric arrangements of the groups constituting the molecular skeletons of these compounds are expected to be similar to those of the corresponding dimethoxy compounds, because the skeletons of the members of the present series are constructed in the same manner as those of the corresponding members of the dimethoxy series. The absorption data of the unsaturated members presented in Table 1 show that the steric interference and consequently the rotation of the benzene rings caused by it grow greater as the alkyl groups at the α, α'-positions become larger (I, II and III), although the maxima are displaced in the whole to longer wave lengths than in the dimethoxy series probably due to the influence of the methylthio groups. It is noticed that the absorption spectrum of substance IV, a combination of one half each of the corresponding dimethoxy and dimethylthio compounds, shows two maxima, one being located very close to the maximum (236 mμ) of the dimethoxy compound, namely, diethylstilbestrol dimethyl ether, and the other to that of the

Table 1

Substance	$\lambda_{max.}$ (mμ)	$\varepsilon_{max.} \times 10^{-4}$
I	271	3.1
II	266.5	2.7
III	265	2.5
IV	233	1.2
	263.5	2.0

(1) M. Ōki and Y. Urushibara, this Bulletin, **25**, 109 (1952).
(2) F. W. Schneler, *Science*, **103**, 221 (1946).
(3) Y. Urushibara and M. Ōki, this Bulletin, **23**, 35 (1950).
(4) Y. Urushibara and T. Takahashi, *ibid.*, **23**, 53 (1950).
(5) Y. Urushibara, M. Ōki, and R. Ikeda, *ibid.*, **25**, 66 (1952).

dimethylthio compound (II).

It is expected that the methylthio group, as well as the methoxy group, is demethylated *in vivo*, and thus two hydrogen bond-forming groups required for the development of estrogenic activity become available in these substances, although the distance between them may be a little greater than in the corresponding dihydroxy compounds, and the thiol group to be formed by demethylation may be subject to ready oxidation to a disulfide group *in vivo*. Estrogenic activities were evaluated by the vagina smear test with ovariectomized mice by subcutaneous injection of oil solutions in two portions. Substance I produced estrus in 50% of animals in the dosage of 300 γ. Substance II was fully active in 40 γ. Substance III showed activity, partially incomplete, in all the animals used in the dosage of 200 γ. Here, too, in the dimethylthio series, the medium member with a moderate molecular thickness shows the greatest activity. Substance IV was found very potent, being fully active in all the animals used in the dosage of 5 γ, while diethylstilbestrol dimethyl ether showed a minimum active dose of 2.5 γ by the same test method. Substance V showed an unexpectedly small potency, its minimum active dose being 200 γ. It may possibly be a racemic form instead of a meso form, which is also made probable by its low melting point and high solubility in organic solvents.

Experimental Part

The preparation of 4,4'-Bis-(methylthio)-α,α'-dialkylstilbenes

p-(Methylthio)-acetophenone, $CH_3SC_6H_4COCH_3$. To a mixture of 12.4 g. of thioanisole, prepared from thiophenol and dimethyl sulfate[6] and dissolved in 30 cc. of carbon disulfide, and 20.0 g. of aluminum chloride, 7.9 g. of acetyl chloride was gradually added with thorough cooling and stirring. The mixture was left to stand for 2 hours and then gently boiled for 30 minutes. After being treated with ice and hydrochloric acid (30 cc.), the mixture was extracted with benzene. The benzene solution was washed with 10% aqueous sodium hydroxide and with water, dried over calcium chloride, and distilled. *p*-(Methylthio)-acetophenone distilled over at 154-156°/12 mm. and was recrystallized from petroleum ether. Colorless prisms, m. p. 84°. Yield 10.5 g. (63% of the theory). Found: S, 19.36%. Calculated for $C_9H_{10}OS$: S, 19.29%.

The structure of the ketone was proved by its oxidation to *p*-methanesulfonylbenzoic acid, $CH_3SO_2C_6H_4CO_2H$. Namely, oxidation with potassium bichromate and 50% sulfuric acid gave a substance melting at 268-268.5° and containing S, 16.05%. *p*-Methanesulfonylbenzoic acid melts at 267-268°[7] and requires S, 16.02%.

p-(Methylthio)-acetophenone-hydrazone. A Mixture of 3.5 g. of *p*-(methylthio)-acetophenone dissolved in 5 cc. of hot alcohol and 2.7 g. of 72% hydrazine hydrate was refluxed for 5 hours. The crystalline product was recrystallized from benzene-petroleum ether. *p*-(Methylthio)-acetophenone-hydrazone was obtained in colorless leaflets melting at 96-96.5°. Yield 3.7 g. (97% of the theory). Found: N, 15.53; S, 17.55%. Calculated for $C_9H_{12}N_2S$: N, 15.54; S, 17.79%.

4,4'-Bis-(methylthio)-α,α'-dimethylstilbene (I). A suspension of 4.0 g. of *p*-(methylthio)-acetophene-hydrazone and 9.0 g. of yellow mercuric oxide in 100 cc. of petrolem ether was shaken for 8 hours at 22-23°. The resulting red solution was filtered from solids and sulfur dioxide was rapidly introduced, when yellowish crystals separated out. The solvent was distilled off, the remainder was dissolved in benzene, and the benzene solution was passed through an aluminum oxide column. From the colorless part of the column, 4,4'-(methylthio)-α,α'-dimethylstilbene was obtained in colorless needles melting at 132.5-133.5° after recrystallization from alcohol. Yield 0.3 g. Found: S, 21.39%. Calculated for $C_{18}H_{20}S_2$: S, 21.35%.

p-(Methylthio)-propiophenone, $CH_3SC_6H_4CO-C_2H_5$. Prepared in the same way as *p*-(methylthio)-acetophenone, using 9.3 g. of propionyl chloride instead of 7.9 g. of acetyl chloride. *p*-(Methylthio)-propiophenone distilled at 167-169°/14 mm. Recrystallized from petroleum ether, it was obtained in colorless prisms melting at 59-60°. Yield 12.2 g. (68% of the theory). Found: S, 17.95%. Calculated for $C_{10}H_{12}OS$: S, 17.79%. The structure was proved by its transformation into *p*-methanesulfonylbenzoic acid.

p-(Methylthio)-propiophenone-hydrazone. Prepared from 3.0 g. of *p*-(methylthio)-propiophenone and 1.0 g. of hydrazine hydrate, and recrystallized from benzene-alcohol. Colorless prisms, m. p. 75.5-76.5°. Yield 3.0 g. (94% of the theory). Found: N, 14.52; S, 16.73%. Calculated for $C_{10}H_{14}N_2S$: N, 14.42; S, 16.50%.

4,4'-Bis-(methylthio)-α,α'-diethylstilbene (II). A suspension of 4.0 g. of *p*-(methylthio)-propiophenone-hydrazone and 8.0 g. of yellow mercuric oxide in 50 cc. of petroleum ether was shaken for 6 hours at 16-18°, and the reaction mixture was treated in a manner similar to the case of 4,4'-bis-(methylthio)-α,α'-dimethylstilbene. A chromatographical separation followed by recrystallization from alcohol gave 4,4'-bis-(methylthio)-α,α'-diethylstilbene in colorless rhombic prisms melting at 132-132.5°. Yield 0.5 g. Found: S, 19.83%. Calculated for $C_{20}H_{24}S_2$: S, 19.52%. The same substance was obtained by catalytic hydrogenation of the reaction products with colloidal

(6) H. Gilman and N. J. Beaber, *J. Am. Chem. Soc.*, **47**, 1449 (1925).

(7) J. Walker, *J. Chem. Soc.*, **1945**, 630.

palladium in acetic acid followed by recrystallization from ethyl acetate.

A direct recrystallization of the products from alcohol gave *p*-(methylthio)-propiophenone-azine, m. p. 139-140°, identified with the specimen described below, and a small yield (0.01 g.) of a compound in prisms melting at 70-73° from the mother liquor. The latter is presumably *cis*-4, 4'-bis-(methylthio)-α, α'-diethylstilbene. Found: S, 19.30%. Calculated for $C_{20}H_{24}S_2$: S, 19.52%.

p-(Methylthio)-butyrophenone, $CH_3SC_6H_4CO\text{-}C_3H_7$. Prepared in the same way as *p*-(methylthio)-acetophenone, 10.7 g. of butyryl chloride being used. *p*-(Methylthio)-butyrophenone distilled at 168.5-171.5°/13 mm. Recrystallization from petroleum ether gave colorless needles melting at 69.5-70.5°. Yield 11.7 g. (61% of the theory). Found: S, 16.80%. Calculated for $C_{11}H_{14}OS$: S, 16.50%. The structure was proved by the formation of *p*-methanesulfonylbenzoic acid on oxidation.

p-(Methylthio)-butyrophenone-hydrazone. Prepared analogously from 9.5 g. of the above ketone and 4.5 g. of 72% hydrazine hydrate in 10 cc. of alcohol. Colorless needles, m. p. 71-72°. Yield 9.0 g. (90% of the theory). Found; N, 13.31; S, 15.35%. Calculated for $C_{11}H_{16}N_2S$: N, 13.45; S, 15.39%.

4, 4'-Bis-(methylthio)-α, α'-dipropylstilbene (III). Synthesized analogously to its lower homologs. Chromatographical separation with alumina and recrystallization from alcohol gave colorless prisms melting at 99-99.5°. Yield 0.2 g. from 4.0 g. of the hydrazone. Found: S, 18.22%. Calculated for $C_{22}H_{28}S_2$: S, 17.99%.

From the mother liquor of recrystallization a small amount of a viscous oil, presumably the *cis*-isomeride, was obtained, but not characterized.

The Preparation of 4-Methoxy-4'-(methylthio)-α, α'-diethylstilbene (IV)

The steps of synthesis are shown below schematically:

$$p\text{-}NO_2C_6H_4CH_2COCl + C_6H_5SCH_3 \rightarrow p\text{-}NO_2C_6H_4CH_2COC_6H_4SCH_3\text{-}p$$

$$\rightarrow p\text{-}NO_2C_6H_4CH(C_2H_5)COC_6H_4SCH_3\text{-}p$$

$$\rightarrow p\text{-}H_2NC_6H_4CH(C_2H_5)COC_6H_4SCH_3\text{-}p$$

$$\rightarrow p\text{-}HOC_6H_4CH(C_2H_5)COC_6H_4SCH_3\text{-}p$$
(not isolated)

$$\rightarrow p\text{-}CH_3OC_6H_4CH(C_2H_5)COC_6H_4SCH_3\text{-}p$$

$$\rightarrow p\text{-}CH_3OC_6H_4CH(C_2H_5)\text{-}C(C_2H_5)(OH)C_6H_4SCH_3\text{-}p$$

$$\rightarrow p\text{-}CH_3OC_6H_4C(C_2H_5)=C(C_2H_5)C_6H_4SCH_3\text{-}p$$

p-Nitrobenzyl-p-(methylthio)-phenyl-ketone, $NO_2C_6H_4CH_2COC_6H_4SCH_3$. To a solution of 6.2 g. of thioanisole in 50 cc. of carbon disulfide 15.0 g. of aluminum chloride was added. *p*-Nitrophenylacetyl chloride, prepared from 10.0 g. of *p*-nitrophenylacetic acid and 11.6 g. of phosphorus pentachloride,[6] was dropped into the mixture while the solvent was gently boiled. The mixture was boiled further 30 minutes to complete the reaction. The upper carbon disulfide layer was decanted off and the greenish-black viscous addition compound was decomposed with ice and hydrochloric acid (30 cc.). The yellowish fine granular product was collected, and washed well with water. (Sodium hydroxide cannot be used to remove aluminum compounds because the ketone is soluble in caustic alkali in purple color.) Recrystallization from acetone gave pale yellow crystals melting at 183-185°. Yield 12.2 g. (85% of the theory). Found: N, 4.73; S, 11.40%. Calculated for $C_{15}H_{13}O_3NS$: N, 4.88; S, 11.16%.

1-(p-Nitrophenyl)-propyl-p-(methylthio)-phenyl-ketone, $NO_2C_6H_4CH(C_2H_5)COC_6H_4SCH_3$. The above ketone (25 g.) was added in small portions to a hot solution of 1.2 g. of metallic sodium in 50 cc. of absolute alcohol, when an intensive violet color resulted. The mixture was heated on a water bath for 15 minutes, cooled slightly, mixed with 8.0 g. of ethyl iodide, and heated again for 2 hours. When the violet color almost disappeared, an alcoholic solution containing 0.6 g. of sodium ethoxide and then 2.0 g. of ethyl iodide were added. The mixture was further refluxed until the violet color completely disappeared (about 2 hours). The resulting light-brownish solution with crystals of sodium iodide in it was poured into 100 cc. of water. Alcohol was evaporated off under a reduced pressure and the remainder was extracted with ether. The ethereal solution was washed with aqueous sodium thiosulfate and then with water, and evaporated. The residue was distilled under a reduced pressure. A viscous orange oil distilled at 202-205°/0.02mm. Yield 11.0 g. (70% of the theory). On standing for a long time it solidified and was recrystallized from alcohol. Pale yellow needles, m. p. 64-65°. Found: N, 4.63; S, 10.06%. Calculated for $C_{17}H_{17}O_3NS$: N, 4.44; S, 10.17%.

1-(p-Aminophenyl)-propyl-p-(methylthio)-phenyl-ketone, $H_2NC_6H_4CH(C_2H_5)COC_6H_4SCH_3$. To a solution of 15.8 g. of the above ketone in 100 cc. of hot alcohol, 22.5 g. of granulated tin was added and 65 cc. of concentrated hydrochloric acid was gradually added in small portions. The mixture was refluxed until no violet coloration occurred on addition of a small portion to alcoholic sodium hydroxide (about 1.5 hours). On pouring the reaction mixture into 100 cc. of water a complex product separated out in a viscous brown oil soluble with difficulty even in hot water. After evaporation of alcohol *in vacuo*, the oily addition product and the upper hydrochloric acid layer were poured together into an excess of hot aqueous 10% sodium hydroxide, and the whole was boiled for a while to complete the decomposition of the addition product. A yellow viscous oil separated out and solidified on cooling. Recrystallized from benzene-petroleum ether,

(8) E. Wedekind, *Ann.*, **378**, 289 (1911).

the amino compound was obtained in colorless prisms melting at 100.5-102.5°. Yield 12.5 g. (81% of the theory). Found: N, 4.86; S, 11.56%. Calculated for $C_{17}H_{19}ONS$: N, 4.91; S, 11.23%.

1-(*p*-Methoxyphenyl)-propyl-*p*-(methylthio)-phenyl-ketone, $CH_3OC_6H_4CH(C_2H_5)COC_6H_4SCH_3$. The above amino compound (7.1 g.) and 20 cc. of 30% sulfuric acid were refluxed to a clear solution (about 15 minutes), which was poured on 100 g. of ice with vigorous stirring. To the resulting pasty mass 1.8 g. of sodium nitrite dissolved in 5 cc. of water was gradually added. After standing for 30 minutes the excessive nitrous acid was decomposed with urea. The diazonium sulfate solution was poured into 100 cc. of boiling 30% sulfuric acid in small portions while the mixture was kept boiling, and the whole was further boiled for a few minutes. A black viscous oil separated out, turning to a resinous mass on cooling. The aqueous layer was decanted off and the remainder was well washed with cold water. The phenol thus obtained could not be purified. The crude substance was dissolved in 25 cc. of 10% aqueous sodium hydroxide. (When 15% sodium hydroxide was used the sodium salt of the phenol could be dissolved in it with difficulty.) To the solution 4.0 g. of dimethyl sulfate was added with occasional shaking. The mixture was heated for 30 minutes on a water bath, then cooled, and extracted with benzene. The benzene extracts were dried over calcium chloride and evaporated. The methoxy compound distilled at 200-205°/0.15 mm. and solidified on cooling. Recrystallization from alcohol gave colorless needles melting at 76.5-78°. Yield 3.3 g. (44% of the theory). Found: S, 10.31%. Calculated for $C_{18}H_{20}O_2S$: S, 10.67%.

1-(*p*-Methoxyphenyl)-propyl-*p*-(methylthio)-phenyl-ethyl-carbinol, $CH_3OC_6H_4CH(C_2H_5)-C(C_2H_5)(OH)C_6H_4SCH_3$. A mixture of 3.0 g. of the above ketone dissolved in 10 cc. of warm benzene and a Grignard reagent prepared from 7.8 g. of ethyl iodide, 1.2 g. of magnesium, and 20 cc. of ether, was refluxed for 2 hours. Then the reaction mixture was treated with 50 cc. of 20% aqueous ammonium chloride and 50 g. of ice. The aqueous layer was extracted with benzene, and the combined benzene solution was dried over anhydrous sodium sulfate and evaporated. The residue gave a pale yellow oil distilling at 172-175°/0.14 mm. and boiling at 160-162°/0.10 mm. on redistillation. On standing, the distillate partially solidified and it was pressed on a porous plate. Recrystallization from alcohol gave colorless prisms melting at 75.5-77.5°. Yield 1.5 g. (45% of the theory). Found: S, 9.82%. Calculated for $C_{20}H_{26}O_2S$: S, 9.70%. The oily fraction contained perhaps a stereoisomeride with a lower melting point.

4-Methoxy-4'-(methylthio)-α, α'-diethylstilbene (IV). The above carbinol did not react with acetyl chloride and acetic anhydride.

The carbinol (0.5 g.) was fused with potassium bisulfate at 230-240° for 30 minutes. After cooling, the salt was washed out. On standing, the oily product partially solidified and was pressed on a porous plate. Recrystallization from alcohol gave colorless prisms melting at 107-108°. Yield 0.02 g. (4% of the theory). Found: S, 10.17%. Calculated for $C_{20}H_{24}OS$: S, 10.26%.

The Preparation of 3, 4-Bis-[*p*-(methylthio)-phenyl]-hexane (V)

***p*-(Methylthio)-propiophenone-azine,** $CH_3SC_6H_4-C(C_2H_5)=NN=C(C_2H_5)C_6H_4SCH_3$. A mixture of 30 g. of *p*-(methylthio)-propiophenone dissolved in 30 cc. of hot alcohol, 4.5 g. of hydrazine hydrate, and 7 cc. of acetic acid, was heated on a water bath, when suddenly a vigorous reaction took place and orange-colored crystals separated out. After completion of the reaction by heating further for 10 minutes, the reaction mixture was cooled and the crystalline product was collected. On recrystallization from benzene-alcohol the azine was obtained in yellow needles melting at 139.5-140°. Yield almost quantitative. Found: N, 8.16; S, 17.94%. Calculated for $C_{20}H_{24}N_2S_2$: N, 7.86; S, 17.99%.

3, 4-Bis-[*p*-(methylthio)-phenyl]-hexane (V). The above azine (1.0 g.) was suspended in a mixture of 20 cc. of methanol and 3 cc. of acetic acid, 0.1 g. of palladium chloride was added, and the mixture was shaken with hydrogen. Every time when the absorption of hydrogen slowed down, the evolved nitrogen was swept out from the hydrogenating apparatus, until no more hydrogen was absorbed. The solution filtered from the catalyst was diluted with water, when a pale yellow oil separated out and solidified on standing. Recrystallization from alcohol gave colorless plates melting at 62-63°. Yield 0.8 g. Found: S, 19.54%. Calculated for $C_{20}H_{26}S_2$: S, 19.40%.

The hydrogenation of the azine gave directly the ultimate product, and neither tetrahydro- nor dihydro-azine was isolated. It seems that the dihydro-azine is unstable and decomposed immediately to yield the dibenzyl compound and nitrogen.[9]

Ultra-Violet Absorption Spectra

The absorption spectra were recorded with a Beckman Model DU photoelectric quartz spectrophotometer in 95% ethanol solutions. The length of the solutions was 5 mm. Concentrations are given below:

Substance	Concentration
I	2.40×10^{-5} mol/l.
II	2.47
III	2.02
IV	2.76

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(9) Cf. H. Bretschneider, A. de Jonge-Bretschneider, and N. Ajtai, *Ber.*, **74**, 571 (1941); *Z.* Földi and G. von Fodor, *ibid.*, **74**, 589 (1941); B. R. Baker, *J. Am. Chem. Soc.*, **65**, 1574 (1943).

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