

Isomerization of α,α' -Diethylstilbestrol, Isolation and Characterization of the cis Isomer

Solutions of *trans*- α,α' -diethyl-4,4'-stilbenediol (DES) rapidly reach a cis-trans equilibrium in CHCl_3 , Et_2O , and C_6H_6 ; whereas in EtOH , CH_3COCH_3 , DMF, or DMSO, the rate is much slower. Temperature elevation accelerates the isomerization process. The equilibrium mixture consists of about 25% cis and 75% trans. Crystalline cis DES has been isolated for the first time. A 10% solution of an equilibrium mixture of DES in DMSO is diluted with an equal volume of H_2O ; the precipitate is

removed, and the filtrate diluted with a second volume of H_2O , yielding cis DES in 90 to 96% purity. Further fractional recrystallization from MeOH , H_2O gave a sample of 99%+ cis DES (mp 135-6° C). The ir, uv, and nmr spectra are consistent with a cis structure. The cis isomer is unstable in solution and may isomerize if allowed to stand. Caution should be used in formulating cis or trans DES since isomerization may lead to false conclusions.

The cis isomer of α,α' -diethyl-4,4'-stilbenediol (DES) has eluded isolation for the past 30 years. Dodds *et al.* (1939) synthesized *trans*- α,α' -diethylstilbestrol and found it to be one of the most active estrogens known. They reported isolating another isomer of DES which they called pseudodiethylstilbestrol or the ψ -isomer. This new isomer was assumed to be of the cis configuration. The first contradiction appeared when Wessely *et al.* (1940) found that on ozonolysis of the ψ -isomer dimethyl ether, acetaldehyde and α -ethyldeoxyanisoin were obtained. This experiment indicated that the true cis isomer had not been isolated, and the ψ -isomer or pseudodiethylstilbestrol was 3,4-bis(4-hydroxyphenyl)-2-hexane. Derkosch and Friedrich (1953) reviewed the subject of cis-trans isomerization of DES and attempted to isolate the free cis isomer (Figure 1). It had previously been shown by Wessely *et al.* (1940) that dipropionates of cis and trans DES could be prepared and separated. Derkosch isolated the dipropionate of cis DES and attempted both alkaline and acidic hydrolysis. The reaction was followed by observing the changes in the ultraviolet spectra. The cis isomer appeared to be rapidly isomerized in solution and only trans DES was ever isolated. It was concluded that cis DES was unstable, and its isolation did not appear to be possible.

The cis-trans isomerization of stilbenes has been studied in depth by Saltiel and Megarity (1969) and Gegiou *et al.* (1968). Most mixtures of stilbene isomers can be separated by conventional techniques such as recrystallization or chromatography. Kharasch *et al.* (1937) demonstrated the sensitivity of *cis*-stilbene to isomerization by such factors as temperature, light, and various catalyst(s). Ultraviolet- and γ -radiation have been shown by Malkin and Fischer (1962) and Fischer *et al.* (1966) to cause isomerization in stilbenes. Under certain conditions equilibrium mixtures of cis and trans stilbenes have been observed. Klasinc and Güsten (1969) and Bortolus and Cauzzo (1970) in their studies on thermal isomerization have shown that the ease of isomerization and point of equilibrium seems to be influenced by substituents in the α,α' positions and in some cases on the benzene ring.

EXPERIMENTAL

The equilibrium rates in several solvents are presented graphically in Figure 2. The temperature was held constant

at 25° C for all rates except those represented by broken lines which were 50° C. Under the conditions so far examined, the equilibrium appears to lie in the range 70 to 80% trans and 30 to 20% cis at ordinary temperatures. The rates were measured starting with 1% solutions of 100% trans DES and also with 1% solutions of 92% cis DES. The solvents used were reagent grade. No attempt was made to exclude air or light during the experiments.

The analytical method used to obtain the data of Figure 2 was developed by Rutherford (1970) of these laboratories. In principle it consists of forming trimethylsilyl ethers of the cis and trans isomers *in situ* by reacting with excess *N,O*-bis-(trimethylsilyl)acetamide (BSA). The resulting ethers, like other ethers and esters of DES, are highly stable and will not isomerize under ordinary conditions (Derkosch and Friedrich, 1953; Riezebos and Havinga, 1961; Walton *et al.*, 1943). By appropriate extraction and gas chromatography procedures, the cis and trans isomers of DES may be qualitatively and quantitatively measured.

All literature reports to date indicate that the only crystalline isomer of DES isolated has been the higher melting, less soluble trans isomer. After having determined some of the factors affecting the rate and extent of cis-trans isomerization, we have recently been able to isolate crystalline cis DES with purity of 99%.

A preferred technique for isolating cis DES consists of dissolving 10 g of trans DES in 100 ml of DMSO (reagent grade) and heating to reflux for 10 to 15 min. The solution is then diluted with 100 ml of H_2O and rapidly cooled to 20-25° C. The resulting precipitate of trans DES is removed by filtration and the clear filtrate diluted further with 300 ml of H_2O . This causes the precipitation of crystalline cis DES of 90 to 96% purity. It is collected on a filter and dried at room temperature. The yield is about 2 g. It may then be further purified to 99% cis isomer by recrystallization from methanol-water at room temperature or below. This procedure may be followed using other solvents such as ethanol, ethylene glycol, or DMF; the yield and purity of the cis isomer are lower. In all cases the purity of the isomer was determined by gas chromatography of the trimethylsilyl ether. Solutions of cis DES tend to convert back to the cis-trans equilibrium mixture if they are allowed to stand. The rate varies with the solvent, as seen in Figure 2. Elevation of the temperature causes a more rapid conversion.

The melting point of crystalline 99% cis DES determined on a Kofler-Reichert gradient hot bench was 135 to 136° C. At temperatures below 160°, the melt immediately resolidifies to the crystalline trans isomer which then melts at 169–70° C. The crystalline cis isomer appears to be stable at room temperature and can be kept for long periods of time. At 100° to 125° C, however, it is rapidly converted to the trans isomer, apparently without melting. When crystalline trans or cis DES are melted, the cis–trans equilibrium appears to be established within a few seconds.

The infrared absorption spectrum of cis DES (93%) differs only slightly from that of the trans isomer. It has a weak band at 900 cm⁻¹ (Nujol mull) not present in the trans spectrum. The trans isomer has weak bands at 808, 850 cm⁻¹ (Nujol mull) and 810, 850 cm⁻¹ (acetone) not found in cis DES.

Differences in the ultraviolet spectra of cis and trans DES are consistent with shifts seen by Riezebos and Havinga (1961) in other cis–trans stilbene pairs. The cis isomer shows a hypsochromic shift with a weaker extinction coefficient. In ethanol solution, the values for cis DES (99%) are λ_{max} 233 nm ($\epsilon = 13,195$), 260 nm ($\epsilon = 9291$), and 272 nm ($\epsilon = 8898$). For the trans isomer λ_{max} 240 nm ($\epsilon = 16,622$).

Comparisons of the nmr spectra of the cis and trans isomers are in agreement with their proposed configurations. An important difference is the upfield shift of the methyl protons in the trans spectrum. This may be accounted for by diamagnetic shielding from the top of the benzene ring. Molecular models indicate that the methyl groups in trans DES may lie directly over the benzene rings, while in the cis isomer the methyl groups are further away and the anisotropic effect is not seen. Thus, in the cis spectrum the methyl protons appear in the normal positions. Further

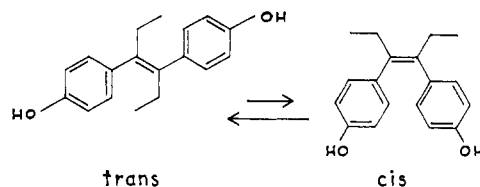


Figure 1. DES isomerization

shielding effects are seen in the spectrum of the cis isomer, where a shift to a higher field is seen for the aromatic protons. This effect may be due to the shielding of one ring by the other. The nmr spectra were obtained by dissolving the DES isomers in CD₃SOCD₃. Impurities in this solvent are seen at δ 2.5 (m) and 3.5 (s) for HCD₂SOCD₃ and H₂O, respectively. The trans isomer of DES shows the following proton signals: δ 0.73 (t, 6, $J = 7$ Hz, CH₃); and δ 9.27 (s, 2, OH) offset. The cis isomer has signals at: δ 0.9 (t, 6, $J = 6$ Hz, CH₃); δ 2.45 (q, 4, $J = 8$ Hz, CH₂); δ 6.6 (q, 8, $J = 9$ Hz, Ph); and δ 9.03 (s, 2, OH) offset. The δ values are in ppm with TMS as the internal reference. Nmr descriptions are: m = multiplet; s = singlet; t = triplet; q = quartet; J = coupling constant in Hz.

DISCUSSION

We have observed the more-or-less rapid isomerization of trans DES to a cis–trans equilibrium mixture. The rate at which equilibrium is established appears to depend upon a number of different factors, not all of which are clearly understood at this time. Some of the important factors that influence the isomerization are temperature, type of solvent, and presence of various other chemical substances. When DES is in solution, the nature of the

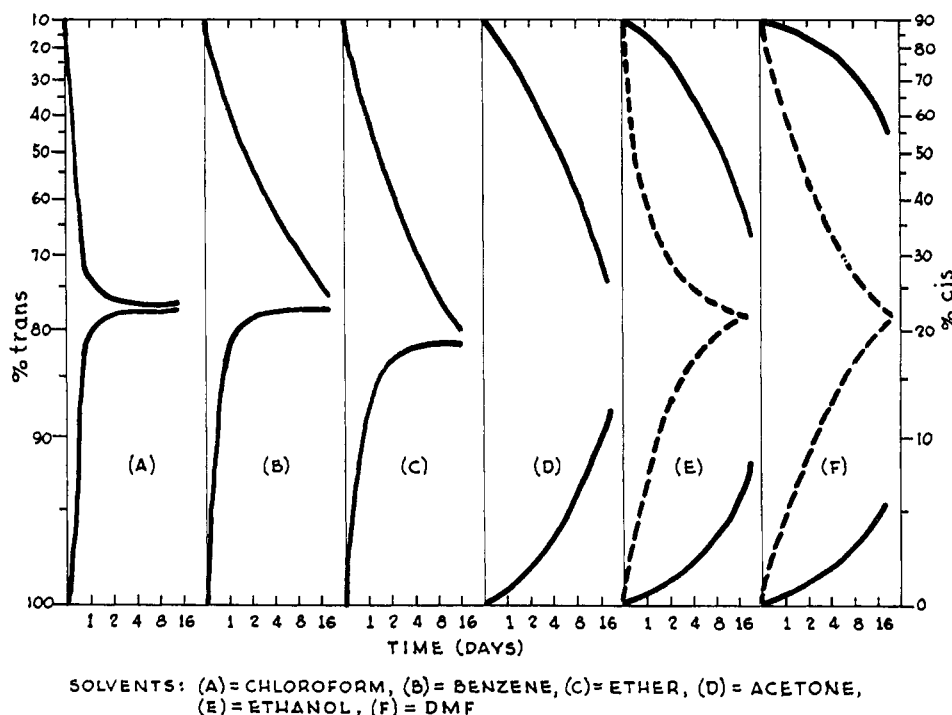


Figure 2. The influence of six different solvents (A = chloroform, B = benzene, C = ether, D = acetone, E = ethanol, F = DMF) on the rate of isomerization of both cis and trans DES are shown. The rate curves starting with trans DES are ascending, and the curves starting with 92% cis DES are descending. Both curves approach the equilibrium mixture (see text). The temperature remained at 25° C, except for the rates represented by broken lines which were at 50° C. One percent solutions of trans DES and 92% cis DES were used. At the various time intervals, aliquots of the solution were analyzed for the cis–trans ratio by silylation and subsequent gas chromatography (see text)

solvent has a profound effect upon the isomerization rate. In chloroform, benzene, or other, the rate is relatively fast; whereas, in acetone, methanol, ethanol, ethylene glycol, propylene glycol, polyethylene glycol, dimethyl-sulfoxide, or dimethyl formamide, it is slower. This effect differs from that found by Schulte-Frohlinde *et al.* (1962) in that chloroform caused the highest rates of isomerization. In chloroform solution, the equilibrium mixture is reached in a matter of minutes at the boiling point, but requires several hours at low temperatures.

We have found that the isomerization of trans DES to the cis-trans equilibrium mixture can be greatly inhibited. In fact, this isomerization can be essentially eliminated by the use of small quantities of appropriate stabilizers. Antioxidants such as ascorbic acid or hydroquinone constitute one class of stabilizers. The details of this work will be reported later.

Caution should be used in formulating experimental solutions or preparations of cis or trans DES since isomerization might lead to false conclusions. All formulations should be analyzed for cis-trans content both before and after the experiment is conducted. In certain formulations of DES used for growth promotion in ruminants, isomerization of the trans isomer to a cis-trans mixture has been found to occur. Addition of appropriate stabilizers inhibits isomerization of DES in such formulations. Preservation of the trans isomer is important since the cis isomer appears to have much less biological activity than the trans isomer. The relative biological activities in ruminants of cis and trans DES have been studied by Raun *et al.* (1970) of this laboratory.

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