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Separation of *cis* and *trans* Stilbenes by Application of the Chromatographic Brush Method

BY L. ZECHMEISTER AND W. H. MCNEELY

In the stilbene series the *cis*-isomers are usually prepared either by irradiation of the stable *trans*-compound¹ or by direct synthesis.² The stereoisomers have been separated or differentiated by fractional crystallization, distillation and hydrogenation methods, and use has also been made of molecular addition compounds. For the determination of the *cis* and *trans* components in mixtures among other methods use has been made of spectrophotometric measurements and the study of melting point curves.³

We find that a convenient and rapid procedure for the detection, purification, separation and estimation of stereoisomeric stilbenes is to be found in the so-called chromatographic brush method.⁴ After extrusion of the column, containing the invisible chromatogram, a narrow streak is made down the column with a brush which has been dipped into a 1% permanganate solution. Whenever the reagent crosses a zone containing *cis*- or *trans*-stilbenes it turns brown almost instantaneously. The zones thus located can be cut out and the isomers eluted after the streak has been shaved off.

In the present paper the application of this principle to stilbene, *p*-methylstilbene and *p*-methoxystilbene is described. In each case the *trans*-isomer possesses stronger adsorption affinity than the *cis*-form and is located near the top of the alumina column. After adequate development the two isomers are separated by a wide interzone. The procedure can be completed in ten to sixty minutes, with a total recovery of more than 90% when carried out with a quantity of starting material in a range of 10–500 mg. If the experiment

is performed at room temperature no measurable isomerization of the *cis*-compound seems to take place.

By this method samples of *cis*- or *trans*-stilbenes can be tested for possible contamination by the other isomer. If 30–100 mg. of a mixture is analyzed the limit of detection is 1–2%. The method may also prove useful for the study of *cis*-*trans* shifts under the influence of light⁵ and of other factors.

Finally, we may remark that the use of chromatography has revealed the presence of minor contaminants in commercial and in some recrystallized samples of stilbenes. These impurities are retained near the top of an activated alumina column where the adsorbate shows visible fluorescence in ultraviolet light. The main compound is easily washed into the filtrate by petroleum ether-benzene mixtures or by pure benzene. If the filtrate is sent through a fresh column, no fluorescing adsorbate appears. A great difference between the fluorescing power of non-chromatographed and chromatographed *p*-methyl- and *p*-methoxystilbene was observed when the crystals or 0.1% benzene solutions were inspected in ultraviolet light. The fluorescence of the purified samples was much weaker. No such difference was noticed in the case of stilbene except on the Tswett column. A preliminary chromatographic test of samples may be advisable if fluorescence spectra are to be studied.⁶

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Experimental

The following adsorbents were used: for the purification of commercial *trans*-stilbene, activated alumina ("Alorco," 150–200 mesh or minus 80 mesh); for the purification of synthesized *p*-methoxy- and *p*-methylstilbene, Super Filtrol (Filtral Corporation, Los Angeles) and activated alumina, and for the separation of *cis*-*trans* pairs, activated alumina. The petroleum ether had a boiling range 60–70°.

(5) J. Wislicenus, *Chem. Zentr.*, **72**, I, 463 (1901).

(6) B. Arends, *Ber.*, **64**, 1936 (1931), observed fluorescing contaminants in some of his *p*-methylstilbene samples. These were not removed by crystallization. No chromatographic treatment is mentioned.

(1) R. Stoermer, *Ber.*, **42**, 4865 (1909); R. Stoermer and L. Frigge, *Ann. Chem.*, **409**, 20 (1915); A. Smakula, *Z. physik. Chem.*, **B25**, 90 (1934); G. N. Lewis, T. T. Magel and D. Lipkin, *THIS JOURNAL*, **62**, 2973 (1940).

(2) Cf. e. g., E. Späth and K. Kromp, *Ber.*, **74**, 189 (1941); P. Ruggli and A. Staub, *Helv. Chim. Acta*, **19**, 1288 (1936); **20**, 37 (1937).

(3) G. B. Kistiakowsky and W. R. Smith, *THIS JOURNAL*, **56**, 638 (1934); C. Paal and H. Schiedewitz, *Ber.*, **63**, 766 (1930); T. W. J. Taylor and A. R. Murray, *J. Chem. Soc.*, 2078 (1938); Ch. C. Price and M. Meister, *THIS JOURNAL*, **61**, 1595 (1939); C. Weygand and Th. Siebenmark, *Ber.*, **73**, 765 (1940); C. Weygand and I. Rettberg, *ibid.*, **73**, 771 (1940); G. N. Lewis, *et al.*, see footnote 1.

(4) L. Zechmeister, L. Cholnoky and E. Ujhelyi, *Bull. soc. chim. biol.*, **18**, 1885 (1936); L. Zechmeister and O. Frehden, *ibid.*, **22**, 458 (1940).

All melting points are corrected and were taken in an electrically heated Berl block. The capillaries were introduced 10° below the melting point. The rise of temperature was 1–2° per minute.

For the inspection of chromatograms during development, a small portable ultraviolet lamp was used (light was excluded by covering the tube and the head of the observer with a black cloth), and for solutions or solids an Examalite Quartz Lamp, (Hanovia, Newark, N. J.). Irradiations were carried out by a mercury arc quartz lamp (4 amp. d. c., voltage drop 32 v.) in a hood cooled by ventilation. The distance between the lamp and the quartz test-tubes was 10 cm. Irradiations were discontinued at night.

cis- and *trans*-Stilbene.—The starting material was stilbene (Eastman Kodak Co.), m. p. 124–125°, from which a small quantity (<0.1%) of strongly fluorescent contaminants can be eliminated by filtering the benzene-petroleum ether (1:1) solution through activated alumina. The impurities are retained near the top while stilbene is washed into the filtrate. The melting point remained unchanged. The adsorbates⁷ of *trans*- or *cis*-stilbene purified in this way showed no visible fluorescence in ultraviolet light.⁸ For the preparation of the *cis*-compound 2.5 g. of stilbene in 35 ml. of benzene was irradiated for 200 hours. The pale yellow solution was kept in a cold room and samples were taken when needed.

A portion of this solution, containing 160 mg. of substance, was evaporated *in vacuo* at 35°, and in order to eliminate traces of benzene the evaporation was repeated twice after the addition of some petroleum ether. The residue (except for traces of yellow solids) was dissolved in 25 ml. of petroleum ether and poured onto an alumina column (17.5 × 1.7 cm.); the chromatogram was developed with 90 ml. of the solvent. Near the top a yellow by-product was retained, closely followed in some experiments by a minor fluorescent layer.⁹ The main section of the column was colorless and did not fluoresce visibly in ultraviolet light. The permanganate brush located a *trans*-stilbene zone (42 mm. broad) and below it, separated by a 6-mm. empty section, the *cis*-zone (48 mm. broad). The streak turned light brown within two seconds where it crossed the zones while the nearly empty interzones began to react after several minutes.¹⁰ Each isomer was eluted with dry ether and evaporated *in vacuo*.

The yield was 99.1 mg. of *trans*-stilbene (m. p. 123.5–124.5°, after one recrystallization from ethanol) and 51.8 mg. of *cis*-stilbene, the total recovery being 94% of the starting material. Before analysis the colorless, oily *cis*-compound (which can be crystallized at a lower temperature) was rechromatographed. Both samples were dried at 45° in high vacuum.

Anal. Calcd. for C₁₄H₁₂: C, 93.28; H, 6.72. Found (*cis*): C, 93.36; H, 6.65. Found (*trans*): C, 93.28; H, 6.78.

(7) We suggest that the term "Adsorbate" should designate the substance-adsorbent complex and not the adsorbed substance alone. (This nomenclature is now accepted in the German literature.)

(8) Cf. A. Winterstein and K. Schön, *Z. physiol. Chem.*, **230**, 146 (1934); K. W. Hausser, R. Kuhn and E. Kuhn, *Z. physik. Chem.*, **B29**, 417 (1935).

(9) On fluorescing of stilbenes cf. G. N. Lewis, *et al.*, footnote 1; H. Ley and H. Specker, *Z. wiss. Phot.*, **38**, 13 (1939).

(10) In blank experiments the column began to show a reaction with permanganate in several minutes.

The *cis* compound was further identified by addition of a trace of iodine and exposure to sunshine for a few hours. By this treatment it crystallized almost completely. A chromatographic analysis showed only a minor zone of unchanged *cis*-isomer while pure *trans*-stilbene was isolated in a yield of 78%.

In order to establish the limits of detection by brushing, artificial mixtures of *cis*- and *trans*-stilbene were chromatographed. It is possible to detect 2 mg. of *cis* in 75 mg. of *trans* on alumina (17.5 × 1.7 cm.) or 0.5 mg. of *trans* in 30 mg. of *cis* by using a smaller column (10.5 × 0.9 cm.). If the quantity of one of the isomers is below these limits, its location may be established roughly from that of the other isomer. By elution of the proper region with ether and analytical recovery by evaporation, an extension of the limits of detection can be obtained. The procedure is applicable to *p*-methyl and *p*-methoxy stilbene.

cis- and *trans*-*p*-Methylstilbene.—The *trans*-compound was prepared according to Meerwein and his associates,¹¹ however, the crude product was purified by chromatography instead of by tedious sublimations in high vacuum. The hydrochloric acid solution of 13.4 g. of *p*-toluidine was added, after diazotization, to 18.5 g. of cinnamic acid in 150 ml. of acetone. After the addition of 27.5 g. of sodium acetate and a solution of 6.7 g. of crystallized cupric chloride, the liquid was stirred at 20° until the evolution of gases ceased (about five hours). The volatile components were then removed by steam distillation, and the dark residue was extracted with 150 ml. of benzene. After filtration and removal of unchanged cinnamic acid by extraction with ammonia containing ammonium chloride, the solution was dried with sodium sulfate. It was diluted with 400 ml. of petroleum ether and filtered through a Superfiltrol column (27 × 7 cm.) which was washed with about 1 liter of a benzene-petroleum ether mixture (1:1) until a pale blue fluorescing zone reached the filtrate. The column showed numerous intensely colored layers which were discarded. The evaporation residue of the filtrate yielded 6.0 g. of *trans*-*p*-methylstilbene, m. p. 118.5–119.5°, on crystallization from alcohol.

Such a sample may be used directly for the preparation of the *cis*-isomer by irradiation, or it can first be rechromatographed on activated alumina in order to remove the fluorescing contaminant mentioned which appears near the top on developing with benzene-petroleum ether. After this treatment practically no visible fluorescence of the *cis*- or *trans*-form was observed in petroleum ether on the column.

For the preparation of *cis*-*p*-methylstilbene 450 mg. of the *trans*-isomer in 90 ml. of petroleum ether was irradiated for seventy-five hours and then developed on alumina (22.5 × 4.3 cm.) with 700 ml. of the solvent. On inspection and brushing the presence of the following zones was revealed (the figures on the left denote the width of the zones in millimeters)

5 by-product (yellow before brushing)
12 empty interzone
30 *trans*-*p*-methylstilbene (located by permanganate)
55 empty interzone
50 *cis*-*p*-methylstilbene (located by permanganate)
73 empty bottom zone

(11) H. Meerwein, E. Büchner and K. van Emster, *J. prakt. Chem.*, **152**, 237 (1939).

The isomers were isolated by eluting with dry ether, evaporating and drying *in vacuo*. The yields were 160 mg. of *trans*- and 268 mg. of oily *cis-p*-methylstilbene, the total recovery being 95%. For analysis the *trans*-compound was recrystallized from alcohol (m. p. 119.5–120°) and the *cis*-isomer rechromatographed as described. The samples were dried in high vacuum at 20°.

Anal. Calcd. for $C_{15}H_{14}$: C, 92.73; H, 7.27. Found (*cis*): C, 92.98; H, 7.50. Found (*trans*): C, 93.06; H, 7.33.

Eighteen mg. of oily *cis-p*-methylstilbene was isomerized in the sunshine with iodine. Crystallization began after fifteen min. but the exposure was continued for a few hours. On brushing the column did not reveal any unchanged *cis*-isomer while 16.4 mg. of the *trans*-form was recovered (m. p. 119.5–120° after one recrystallization). The yield was 91%.

The solution of an artificial mixture of 70.3 mg. of *trans*- and 56.2 mg. of *cis-p*-methylstilbene in 10 ml. of petroleum ether was developed on alumina (17.5 × 1.7 cm.) with 60 ml. of the solvent. This washing was completed in twenty-five min. Ten mm. below the top the *trans*-zone appeared (55 mm. wide), and after an empty section (30 mm.) the *cis*-zone followed (50 mm. wide). Elution with 65 ml. of ether and evaporation yielded 68.6 mg. of the *trans*-isomer (m. p. 118–119°, without recrystallization) and 54.4 mg. of the *cis*-compound. The total recovery was 97%. The samples were chromatographically homogeneous.

On an alumina column (10.5 × 0.9 cm.) 0.3 mg. of *trans-p*-methylstilbene can easily be detected in admixture with 25.3 mg. of the *cis*-form or on a larger column (17.5 × 1.7 cm.) 1.8 mg. of the *cis*-form in 98.8 mg. of the *trans*-compound.

cis- and *trans-p*-Methoxystilbene.—The synthesis of the *trans*-compound from diazotized *p*-anisidine and cinnamic acid was carried out according to Meerwein, *et al.*,⁹ the product was purified chromatographically as described for *p*-methylstilbene. By filtering a benzene solution through activated alumina a by-product can be removed, the adsorbate of which fluoresces much stronger than that of the methoxystilbene. The pure compound melted at 135–136° after recrystallization from methanol. For the preparation of the *cis*-isomer a solution of 730 mg. in 35

ml. of benzene was irradiated for 160 hours (shorter irradiation is also satisfactory). A portion of the solution containing 161 mg. of substance was evaporated. The residue was taken up with 2.5 ml. of chloroform and, after the addition of an equal volume of petroleum ether, chromatographed on alumina (17.5 × 1.7 cm.). On developing with 70 ml. of a petroleum ether–benzene mixture (3:1) the following chromatogram appeared

0.5 brownish top layer (visible before brushing)
5 empty interzone
1 bright yellow by-product (visible before brushing)
6 empty interzone
40 *trans-p*-methoxystilbene (located by permanganate)
15 empty interzone
47 *cis-p*-methoxystilbene (located by permanganate)
60 empty bottom zone

Each main zone was eluted with 65 ml. of ether and treated as described for the *p*-methyl compound. The yield was 58.5 mg. of the *trans*-isomer (m. p. 135–136°, after recrystallization from methanol) and 79.1 mg. of the *cis*-form (colorless oil), the recovery being 86%. The oil was rechromatographed before analysis.

Anal. Calcd. for $C_{15}H_{14}O$: C, 85.67; H, 6.72. Found: (*cis*) C, 85.76; H, 6.66; (*trans*) C, 85.31; H, 7.07.

2.2 mg. of the *cis*-isomer was detected in the presence of 95.4 mg. of the *trans*-form on alumina (17.5 × 1.7 cm.), and 1.5 mg. of *trans* in admixture with 86.6 mg. of *cis-p*-methoxystilbene.

Summary

The chromatographic brush method, with permanganate, has been used for the detection, separation and estimation of the *cis*- and *trans*-forms of stilbene, *p*-methylstilbene and *p*-methoxystilbene. In a mixture composed of two stereoisomeric compounds 1–2% of either form can be detected. The method can be used for the study of the interconversion of stilbene *cis*- and *trans*-isomers. Fluorescing contaminants can be removed by chromatography in ultraviolet light.

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