I. Contribution to the Stereochemistry of Stilbenes by Application of Chromatographic Methods

II. Separation of Some *cis*- and *trans*-Oximes by Means of the Chromatographic Brush Method

III. Chromatographic Isolation of Homopterocarpin and Vanillin from Red Sandalwood

IV. Model Experiments Directed Toward the Synthesis of Quinine

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William Harold McNeely

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Acknowledgment

I wish to express my sincere thanks to Professor L. Zechmeister and to Dr. E. Buchman for their personal interest in my work of the last three years. Their interest and suggestions have made this research a pleasure.

Introduction

Introduction

In the first few years of its development by Tswett[1] the chromatographic method was chiefly applied to the separation of colored compounds. When interest in this method became widespread after 1930, rapid advances were made in the chromatography of colorless compounds. In order to apply the chromatographic method to such compounds, various methods for the location of the zones of the "invisible chromatogram" were devised. Included in these methods were the use of ultraviolet light for fluorescent compounds,[4] empirical elution of various sections of the column, and "tagging" the colorless compound by reaction with a suitable colored substance and subsequent chromatography.[5] The fluorescence method is of limited applicability while the other two methods are rather awkward to apply. It was shown recently that the brushing of an invisible chromatogram with a suitable color reagent[6] can be used to locate and thus make possible the separation of many colorless substances. This procedure permits a convenient extension of the chromatographic method into the vast realm of colorless and non-fluorescent substances. The number of possible color reactions is large and some can be found for nearly any functional group.[7]

The introduction of the chromatographic brush method into inorganic analysis has opened a promising field. Zechmeister and Frehden[6] chromatographed aqueous solutions of metal ions on alumina. The ions were not well separated into distinct bands. However, by drawing a series of parallel streaks down the column with specific color reagents, each metal present in the mixture could be detected. For example, an aqueous solution of the nitrates of iron, copper, cobalt, and nickel was chromatographed. A solution of potassium ferrocyanide gave a blue color with iron and a brown color with copper. The cobalt was located by the bluish green color reaction with ammonium thiocyanide, while dimethylglyoxime gave a red coloration with nickel.

Several applications of the brush method have been made in vitamin chemistry. Zechmeister, Cholnoky and Ujhelyi[6] and later Willsteadt and With[8] used a chloroform solution of antimony trichloride ("Carr-Price reagent") to detect vitamin A on an alumina column. Willstaedt[9] found that vitamin B_1 coupled with diazotized 2,4-dichloroaniline to give a yellowish-red dyestuff. This reaction was utilized to detect, by the brush method, the vitamin B_1 adsorbed on alumina.

In the present investigation the brush method was utilized in order to obtain information on the adsorption behavior of some colorless stereoisomeric compounds in the stilbene and benzoin oxime series.[10] In addition, the method was applied in an investigation of naturally occuring colorless substances contained in red sandalwood.

Up to the present time nearly all information on the effect of _cis_ and _trans_ configuration on adsorption affinity has been obtained from a study of colored or fluorescing substances. In the field of the carotenoids where there is a possibility of a great number of _cis_- and _trans_-isomers[11], the form containing the all _trans_ configuration can possess either a stronger or a weaker adsorbability than its stereoisomers. Zechmeister, Le Rosen, Went and Pauling[12] recently reported the isolation of the naturally occuring prolycopene which presumably had a _cis_ configuration about 5 or 6 of the seven double bonds available for cis-trans changes. Catalytic isomerization of this compound in the presence of iodine, led to a mixture of stereoisomers. A poly cis-isomer in the γ-carotene series, pro-γ-carotene, investigated by Zechmeister and Schroeder[13] behaved similarly. According to Zechmeister and Escue[14] methylbixin isomerizes to a mixture of stereoisomers most of which are less strongly adsorbed than the all trans-form. Several isomers having a partial cis-configuration were observed by Le Rosen and Zechmeister[15] in the mixture obtained by the catalytic isomerization of diphenyloctatetraene. These isomers were less strongly adsorbed than the all trans-compound. However, in the azobenzene series all of the cis-compounds were found by Cook[16] to be more strongly adsorbed on alumina than the trans-isomers. In the stilbene and benzoin oxime series reported in this Thesis the trans-form is in every case more strongly adsorbed than the corresponding cis-isomer. Even in the case of cis- and trans-anisoin oximes where the stereoisomers have nearly the same melting point (123° and 125.5° respectively), the trans-form is more strongly adsorbed and the stereoisomers are easily separated on the column.

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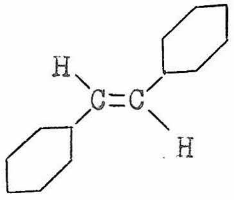
Part 1

Contribution to the Stereochemistry of Stilbenes by Application of Chromatographic Methods

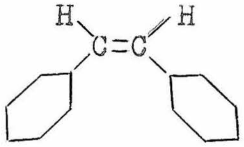
Methods of separation of stilbenes.- The preparation of *cis*-isomers in the stilbene series is usually carried out by irradiation of the *trans*-compound[1] or by direct synthesis.[2] Either method leads to a mixture of both isomers. These stereoisomers have been separated by fractional crystallization,[3] distillation[4] and by the use of molecular addition compounds.[5] Advantage has also been taken of the more rapid rate of hydrogenation of the *cis*-isomers to separate the mixtures.[6] For the determination of the *cis*- and *trans*-components in mixtures among other methods use has been made of spectrophotometric measurements[4] and melting point curves.[7]

We find that a convenient and rapid procedure for the detection, purification, separation and estimation of stereoisomeric stilbenes is to be found in the chromatographic brush method.[8] After extrusion of the column, containing the invisible chromatogram, a narrow streak is made down the column with a brush which has been dipped in a 1% permanganate solution. Wherever the reagent crosses a zone containing a *cis*- or *trans*-stilbene, it turns brown almost immediately. The zones thus located can be cut out and the isomers eluted after the streak has been shaved off.

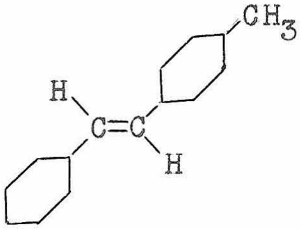
Analytical applicability of the chromatographic method.- In the present Chapter the application of this principle to a study of stilbene (Formulas I, II), p-methylstilbene (III, IV) and p-methoxystilbene (V, VI) is described. In each case the *trans*-isomer possesses stronger adsorption affinity than the *cis*-form and is located near the top of the activated alumina column. After adequate development the two isomers are separated by a wide interzone. The procedure can be completed



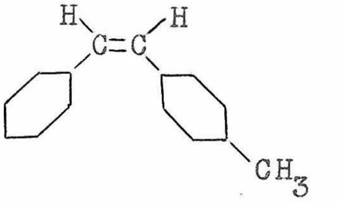
I. *trans*-Stilbene



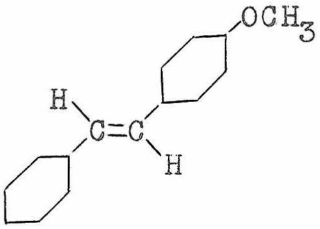
II. *cis*-Stilbene



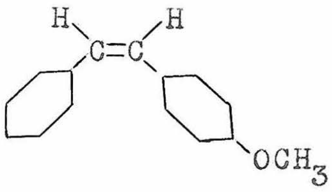
III. *trans*-p-Methylstilbene



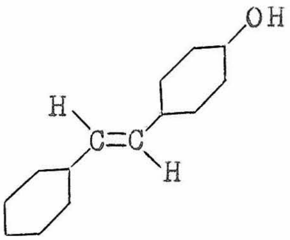
IV. *cis*-p-Methylstilbene



V. *trans*-p-Methoxystilbene



VI. *cis*-p-Methoxystilbene



VII. *trans*-p-Hydroxystilbene

Fig. I

Table 1.

Recovery of Stilbenes from Mixtures of the Stereoisomers

Substance	mg. Charged cis	trans	mg. Recovered cis	trans	Total % Recovery
Stilbene	160 (mixt.)		51.8	99	94
p-Methylstilbene	56.2	70.3	54.4	68.6	97
p-Methoxystilbene	161*		79.1	58.5	86

Table 2.

Limits of Detection by the Brush Method in Mixtures of Stereoisomeric Stilbenes

Substance	mg. cis	mg. trans	Remarks
Stilbene	2.5	74.5	cis detected
	1.1	75.	cis not detected
	30.	0.7	trans easily detected
p-Methylstilbene	1.8	98.8	cis detected
	25.3	0.3	trans detected
p-Methoxystilbene	2.2	95.4	cis detected
	0.9	87.1	cis not detected
	86.6	1.5	trans detected

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* This irradiation mixture contained a considerable quantity of yellow by-product.

in ten to sixty minutes, with a total recovery of more than 85% when carried out with a quantity of starting material in a range of 10-500 mg. Representative data are presented in Table 1.

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 analysed, the limit of detection by the brush method is 1-2% as shown in Table 2.

Adsorbents and starting materials.- Due to the moderate adsorption affinity of the stilbenes, a "strong" adsorbent is needed. "Alorco" activated alumina was found not only to adsorb the non-phenolic stilbenes from petroleum ether or benzene-petroleum ether mixtures, but to separate the cis- trans stereoisomers into bands with an empty interzone between. This activated alumina could also be used to remove fluorescent impurities from the stilbene samples. Neutral Filtrol proved to be a satisfactory adsorbent for trans-p-hydroxystilbene after a number of adsorbents were found to be unsuitable. Calcium carbonate was too weak while the phenolic group caused the stilbene to be too strongly adsorbed on activated alumina, calcium hydroxide and magnesia.

Both trans-p-methylstilbene and trans-p-methoxystilbene were prepared by coupling cinnamic acid with the corresponding diazotized para-substituted aniline.[9] Chromatographic purification of the crude product on Super Filtrol was found to be far more convenient than the usual sublimation in high vacuum. The by-products, instead of being obtained as a "dark tar", were found to separate on the column into a series of beautifully colored distinct bands. Chromatography offers a means of isolating practically all of the products of such a reaction in pure form and in relatively good yield.

For this investigation the cis-isomers of stilbene, p-methylstilbene and p-methoxystilbene were prepared by ultraviolet irradiation of the corresponding trans-compound. However, the irradiation of trans-p-hydroxystilbene failed to give appreciable amounts of the cis-isomer. The pure cis-isomers were isolated from the resulting cis-trans mixtures by the chromatographic method. Since there is little data in the literature on the best conditions for the irradiation of such compounds, the irradiation of trans-p-methylstilbene was carried out in two solvents and at several dilutions. The rate of formation of the cis-isomer was found to be about the same in benzene and petroleum ether. However, the rate increased considerably with dilution and was roughly proportional to the exposed cross sectional area of the quartz tubes. A 1/2% solution of trans-p-methylstilbene in petroleum ether was found to give a satisfactory rate of isomerization.

Fluorescence experiments.- These chromatographic experiments revealed the presence of minor fluorescent contaminants in recrystallized samples of commercial stilbene. The impurities are retained near the top of an activated alumina column where the adsorbate shows visible fluorescence in ultraviolet light. The purified 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 considerable amount of a brilliant blue fluorescent material* was removed from recrystallized trans-p-methylstilbene by chromatography on

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* B. Arends[10] observed fluorescing contaminants in some of his p-methylstilbene samples. These were not removed by crystallization. No chromotographic treatment is mentioned.

activated alumina. Benzene rapidly washed the p-methylstilbene into the filtrate while the fluorescent contaminants migrated downwards at a much slower rate. The adsorbate of this purified product on alumina did not fluoresce. Although a similar treatment of p-methoxystilbene removed a number of fluorescent impurities, the adsorbate of the purified product still possessed a pale blue fluorescence. 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. The chromatographed samples of stilbene, p-methylstilbene and p-methoxystilbene all fluoresced with approximately the same intensity.

A considerable literature has developed on the fluorescence spectra and absorption spectra of stilbenes.[11] Since some syntheses produce fluorescent contaminants which are difficult to remove by the classical methods, preliminary chromatographic tests of samples may be advisable if fluorescence spectra are to be studied.

Experimental*

Methods and materials.- The following adsorbents were used: for the purification of commercial trans-stilbene, activated alumina; for the purification of synthesized p-methoxy- and p-methylstilbene, Super Filtrol (Filtrol Corporation, Los Angeles) and activated alumina, and for the separation of cis-trans-pairs, activated alumina ("Alorco," 150-200 mesh or minus 80 mesh, Braun Corp., Los Angeles). The chromatographic tubes

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* The microanalyses were kindly carried out by Dr. G. Oppenheimer and Mr. G. Swinehart.

were manufactured by the Scientific Glass Apparatus Co., Bloomfield, New Jersey. The petroleum ether used had a boiling range of 60-70°. All melting points were taken in a Berl block and corrected for exposed stem.

For the inspection of chromatograms during development, a small portable ultraviolet lamp was used (light was excluded by means of 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., 32 volts drop) with a distance of 10 cm. between the lamp and the quartz test-tubes.

In the analytical experiments the following procedure was used to determine the weight of each recovered isomer. After the column had been extruded, brushed with potassium permanganate, and the reagent streak scraped off, the colorless zone (including a centimeter or two on each side) was cut out from the extruded column.* The adsorbent was eluted with commercial absolute ether on a sintered glass filter (Jena G3 or G4, or pyrex M or F) and the filtrate transferred with quantitative care to a tared standard taper boiling flask (usually 50 ml.). The flask was connected to a standard taper still head and evaporated to dryness on a water bath under reduced pressure. The residue was evaporated down once with a small amount of dry ether and then placed under full water pump vacuum for 15 minutes while maintaining the bath temperature at 45°. After the flask had been wiped dry with a lint free towel it was suspended near the balance by means of a weighed Nichrome wire for 15 minutes before weighing.

A blank on an activated alumina column (4 x 1.7 cm.) yielded only 0.2 mg. of material under the conditions of the experiments.

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* The exact boundaries of the zone inside the column could be found by gradually carving away each end while testing the column at intervals with the tip of the reagent brush. Most zones "cone" upwards. Where

<u>cis- and trans-Stilbene</u>

The starting material was stilbene (Eastman Kodak Co.), m.p. 124-125°, from which a small quantity ($< 0.1\%$) of strongly fluorescent contaminants was eliminated by filtering the benzene-petroleum ether solution (1:1) through activated alumina. The impurities were retained near the top while the stilbene was washed into the filtrate. The melting point remained unchanged. The adsorbates of <u>trans</u>- or <u>cis</u>- stilbene when purified in this way showed no visible fluorescence in ultraviolet light. For the preparation of the <u>cis</u>-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 at 5° and samples were taken when needed. This product as well as samples of pure <u>cis</u>-stilbene showed no appreciable re-isomerization after 6 months at this temperature.

<u>Isolation of cis-stilbene</u>.- A portion of the irradiated solution was evaporated in vacuo at 35°, and, in order to eliminate traces of benzene, the evaporation was repeated twice with small portions of petroleum ether. The residue was weighed and found to contain 160 mg. of material. This residue (except for traces of yellow solids) was dissolved in 25 ml. of petroleum ether and chromatographed on a column (17.5 x 1.7 cm.) of activated alumina. The invisible chromatogram was developed with 90 ml. of the solvent. After extrusion and brushing with a 1% aqueous solution of potassium permanganate** the presence of the following zones was revealed

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the column is small and the interzone large, it is unnecessary to cut away the extra adsorbent.

** The streak turned light brown within two seconds where it crossed the main zones while the nearly empty interzones began to react after several minutes. In blank experiments the column itself began to show a reaction with permanganate after several minutes.

(the figures on the left denote the width of the zones in millimeters):

0.5	by-product (yellow before brushing)
8.5	empty interzone
42	*trans*-stilbene (located by permanganate)
6	empty interzone
48	*cis*-stilbene (located by permanganate)
70	empty bottom section

Each isomer was eluted with 60 ml. of commercial dry ether and evaporated in vacuo. The yield was 99.1 mg. of *trans*-stilbene (m.p. 123.5-124.5°, after recrystallization from 2.6 ml. of hot ethanol) and 51.8 mg. of *cis*-stilbene. The recovery of the two isomers was 94% of the starting material. Before analysis the colorless, oily *cis*-stilbene (which can be crystallized below -30°*) was rechromatographed. Both samples were dried at 45° in high vacuum.

Anal.

2.732 mg. (*cis*) and 2.994 mg. (*trans*) subst: 9.347, 10.234 mg. CO_2;
1.624, 1.814 mg. H_2O

Calcd: $C_{14}H_{12}$: C, 93.28; H, 6.72

Found: C, 93.36, 93.28; H, 6.65, 6.78.

Isomerization of cis-stilbene.- A trace of crystalline iodine[12] was added to 16.1 mg. of chromatographed *cis*-stilbene in a small pyrex test tube. After 3 hours of exposure in bright sunshine the oil had crystallized. After 9 more hours of exposure the material was dissolved in 15 ml. of petroleum ether and chromatographed on a column (17.5 x 1.7 cm.)

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* For crystalline *cis*-stilbene *cf*. C. Weygand and I. Rettberg.[5]

of activated alumina. The chromatogram was developed with 75 ml. of the solvent, extruded and brushed.

0.5	visible yellow (iodine)
4.5	empty interzone
11	*trans*-stilbene (located by permanganate)
15	empty interzone
5	*cis*-stilbene (located by permanganate)
143	empty bottom section

After elution of the *trans*-zone with dry ether and analytical recovery, 12.6 mg. of *trans*-stilbene was obtained (m.p. 124-125°, after recrystallization* from 0.25 ml. of hot ethanol).

Artificial mixture.- An ether solution (20 ml.) of 5.2 mg. *cis*-stilbene and 1000 mg. *trans*-stilbene was evaporated in vacuo. Ten milliliters of a cold alcoholic extract of the residue was concentrated under vacuum to 1 ml. and filtered.** The combined filtrates of the mother liquor and a 0.5 ml. fresh extract of the *trans*-precipitate were evaporated to dryness. A petroleum ether extract (2 ml.) of this residue was chromatographed on a column (10.5 x 0.9 cm.) of activated alumina and developed with 18 ml. of petroleum ether. From the 25 mm. *cis*-stilbene band (located by permanganate) 4.0 mg. of the original 5.2 mg. was recovered. The substance was identified by isomerization to *trans*-stilbene with iodine in sunlight.

Limits of detection.- A solution of 2.5 mg. of *cis*-stilbene and 74.5 mg. of *trans*-stilbene (Eastman) in 8 ml. of petroleum ether was chromato-

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* For a description of the micro-crystallization equipment *cf*. L.C. Craig.[13]

** A micro-immersion filter stick was used.[13]

graphed on a column (17.5 x 1.7 cm.) of activated alumina. After development with 75 ml. of the solvent (during a total length of time of 24 minutes), extrusion, and brushing with 1% permanganate, the following zones were noted:

1	unknown
7	empty interzone
40	*trans*-stilbene
7	interzone
7	*cis*-stilbene (slowly turns tan)
113	empty bottom section

When 1.1 mg. of the *cis*-stilbene was mixed with 75 mg. of the *trans*-isomer and chromatographed, the *cis*-form could not be detected. Thus the limit of detection of *cis*-in *trans*-stilbene by the brush method described is about 2%.

In order to determine this limit for *trans*-in *cis*-stilbene, a solution of 0.7 mg. of *trans*-stilbene and 30 mg. of the *cis*-isomer in 2 ml. of petroleum ether was chromatographed on alumina (10.5 x 0.9 cm.). After development with 20 ml. of petroleum ether and brushing, the following zones appeared:

0.5	unknown
10	empty interzone
10	*trans*-stilbene (turns tan in 10 seconds)
40	empty interzone
44.5	*cis*-stilbene

Thus 2.3% of *trans*-stilbene in admixture with the *cis*-isomer could be easily detected.

If the quantity of one of the isomers is below the limits mentioned, 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 same procedure is applicable to p-methyl- and p-methoxystilbene.

cis- and trans-p-Methylstilbene

The trans-compound was prepared according to Meerwein and his associates[9]. However, the crude product was purified chromatographically instead of by tedious sublimation in high vacuum.

$$\mathrm{C_6H_5.CH{:}CH.CO_2H + ClN_2.C_6H_5 + NaOAc \xrightarrow[acetone]{CuCl_2} C_6H_5.CH{:}CH.C_6H_5.CH_3 + CO_2 + N_2 + NaCl + HOAc}$$

A mixture of 13.4 g. (0.125 mole) of p-toluidine (Merck C.P.), 50 g. of 25% HCl (0.385 mole) and 50 g. of ice was diazotized by the stepwise addition of a cold solution of 9 g. (0.13 mole) of sodium nitrite in 15 ml. of water during a period of 10 minutes. This solution of p-toluenediazonium chloride was added to a cold mixture of 18.5 g. (0.125 mole) of cinnamic acid (Eastman), 27.5 g. of sodium acetate (0.2 mole), and 150 ml. of acetone in a three-necked one liter flask. The flask was equipped with a mercury seal stirrer and a U tube bubble counter. After the addition of a solution of 6.7 g. of $\mathrm{CuCl_2.2H_2O}$ in 15 ml. of water the temperature was raised to 20°* while the mixture was stirred vigorously. After five hours the evolution of gases had nearly ceased. The acetone was then boiled off and the volatile components removed by 45 minutes of steam distillation. On cooling to about 50° the melt solidified to a

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* According to Meerwein[9] the reaction took 1.5 hours at 14-17°, but we found the evolution of gas to be very slow at that temperature.

dark, oily mass. The warm mixture was extracted with 100 ml. of benzene followed by 50 ml. more of the same solvent. The dark colored benzene extract was filtered free of cupric chloride. In order to remove unchanged cinnamic acid, the benzene solution was shaken six times with 50 ml. portions of a solution which was 2 molal with respect to ammonia and 0.5 molal with respect to ammonium chloride.* After two water washes the deep red benzene solution was dried over anhydrous sodium sulfate. The dry solution was diluted with 400 ml. of petroleum ether, filtered to remove a trace of precipitate, and then chromatographed on a Super Filtrol column (24 x 7 cm.). After developing with 900 ml. of benzene-petroleum ether (1:1) a pale blue fluorescent band (in ultraviolet light) of p-methylstilbene passed into the filtrate before any of the colored bands reached the bottom of the column. The appearance of the column was as follows:

16	dark brown
16	brown
23	orange
10	colorless interzone
5	red
15	orange
40	light orange
1	red
4	light orange
2	purple
20	colorless interzone
25	light yellow
63	empty bottom section

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* The addition of ammonium chloride prevents the formation of a bad emulsion at this stage.

All of these intensely colored layers were discarded. The colorless evaporation residue of the chromatographic filtrate yielded 6.0 g. (25%) of trans-p-methylstilbene (m.p. 118.5-119.5°), on recrystallization from 120 ml. of ethanol. This material was used directly for the synthesis of cis-p-methylstilbene. However, chromatography on activated alumina (discussed in the section on fluorescence, page 29) removes this blue fluorescent material[10] as an upper separate zone on developing with benzene-petroleum ether. After such treatment practically no visible fluorescence of either the cis- or the trans- form was observed in petroleum ether on the column.

cis-p-Methylstilbene.- Several different concentrations of trans-p-methylstilbene in benzene and petroleum ether were made up in order to determine the best conditions for the irradiation. These solutions were as follows: (1) 2.5 g. of trans-p-methylstilbene was dissolved in 35 ml. benzene and placed in a quart test tube (15.5 x 3.0 cm). (2) 500 mg. of p-methylstilbene was dissolved in 50 ml. of benzene and placed in a quartz test tube of the same size. (3) 500 mg. of p-methylstilbene was diluted to 100 ml. with petroleum ether and placed in two quartz test tubes (15.5 x 2.5 cm.). Each tube was stoppered with a tin foil covered cork and irradiated for 65 hours at a distance of 10 cm. from the lamp. Chromatography of a sample of each solution on a column (10.5 x 0.9 cm.) of activated alumina and brushing with 1% permanganate revealed the following approximate percentages of cis-p-methylstilbene formed: (1) 15%, (2) 30-35% and (3) 60%.

After 75 hours of irradiation 90 ml. of the petroleum ether solution (3) was chromatographed on a column (22.5 x 4.3 cm.) of activated alumina and developed with 700 ml. of the solvent. The duration of this operation was 65 minutes. Extrusion and brushing with permanganate revealed the presence of the following zones:

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 section

After elution of the *cis*- and *trans*-zones with 200 ml. and 160 ml. of dry ether respectively, each eluate was evaporated in vacuo. It was necessary to evaporate the *cis*-fraction with several portions of dry ether in order to reach constant weight. The yields were 160 mg. of crystalline *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 (colorless oil) was rechromatographed as described. The samples were dried in high vacuum at 20°.

Anal.

3.223 mg. (*cis*) and 2.421 mg. (*trans*) subst.: 10.982, 8.256
mg. CO_2; 2.159, 1.587 mg. H_2O

Calcd.: $C_{15}H_{14}$: C, 92.73; H, 7.27

Found: C, 92.98, 93,06; H, 7.50, 7.33.

Isomerization of cis-p-methylstilbene.- Eighteen mg. of oily *cis*-p-methylstilbene in a small stoppered pyrex test tube was isomerized in the sunshine in the presence of a trace of iodine. Crystallization began within

15 minutes, but the exposure was continued for a few hours. The crystals were dissolved in 2 ml. of petroleum ether and chromatographed on an alumina column (10.5 x 0.9 cm.). Development was carried out with 10 ml. of the solvent mentioned. On brushing, a 34 mm. band of the *trans*-compound was noted, but no unchanged *cis*-isomer was found. After elution with 15 ml. of dry ether 16.4 mg. of the trans-form was recovered (m.p. 119.5-120°, after recrystallization from 0.25 ml. of ethanol). The yield was 91%.

Artificial mixture.- A solution of a 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 x 1.7 cm.) with 60 ml. of the solvent. This washing was complete in 25 minutes. After extrusion and brushing the following zones were noted:

10	empty top section
55	*trans*-p-methylstilbene
30	empty interzone
50	*cis*-p-methylstilbene
30	empty bottom section

After elution of each zone with 65 ml. of dry ether and analytical recovery, 68.6 mg. of the *trans*-isomer (m.p. 118-119°, without recrystallization) and 54.4 mg. of the *cis*-compound were obtained. The total recovery was 97%. The samples were chromatographically homogeneous.

Limits of detection.- A solution of 0.3 mg. *trans*- and 25.3 mg. *cis*-p-methylstilbene in 2 ml. of petroleum ether was developed on a column (10.5 x 0.9 cm.) with 10 ml. of the solvent. After extrusion and brushing, the following zones were noted:

5	empty top section
3	trans-p-methylstilbene (turns tan within 60 seconds)
30	empty interzone
45	cis-p-methylstilbene (turns tan instantly)
22	empty bottom section

Thus 1.2% trans- in cis-p-methylstilbene was detected. Similarly 1.8 mg. of cis- in 98.8 mg. of the trans-compound was detected on a larger column (17.5 x 1.7 cm.) of activated alumina.

p-Hydroxystilbene

Trans-p-hydroxystilbene was prepared according to the method of Pfeiffer and Sergiewskaja[14] and Herwitt and associates.[14] A mixture of 20 g. (0.164 mole) of p-hydroxybenzaldehyde, 20 g. (0.147 mole) of phenylacetic acid, and 4 ml. of piperidine was heated on an oil bath, maintained at 150°, for five hours.

$$C_6H_5.CH_2.CO_2H + O{:}CH.C_6H_4.OH \xrightarrow{\text{piperidine}} C_6H_5.CH{:}CH.C_6H_4.OH + H_2O + CO_2$$

The reaction mixture was extracted with these 75 ml. portions of hot, 1-molal sodium carbonate to remove the major portion of unreacted p-hydroxybenzaldehyde. After each extraction the hot mother liquor was sucked off by means of an immersion filter. The orange residue was dissolved in 600 ml. of hot 1.5 N sodium hydroxide solution.* After an hour's standing with occasional scratching, crystallization of the sodium salt began. The beaker was allowed to stand overnight to complete crystallization. The white plates were filtered out and washed with 100 ml. of water on the

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* A much smaller volume would dissolve the product, but the large volume mentioned is needed to keep the sodium salt of p-hydroxybenzaldehyde from precipitating.

filter. The crystals were suspended in one liter of boiling water while 30 ml. of 6 N hydrochloric acid was slowly added. This resulted in the formation of a voluminous white precipitate of p-hydroxystilbene. After boiling for an additional 30 minutes, the mixture was cooled and filtered. The product was recrystallized from a mixture of 40 ml. of ethanol and 28 ml. of water. The yield was 2.25 g. (white plates, m.p. 188.5-189.5°).

Chromatography of trans-p-hydroxystilbene.- A solution of 746 mg. of *trans*-p-hydroxystilbene in 100 ml. of chloroform was developed on an alumina column (22.5 x 4.3 cm.) with 800 ml. of benzene-ethanol (50:1). After extrusion and brushing with 1% permanganate the following zones were noted:

14	unknown (tan in 1 minute)
80	*trans*-p-hydroxystilbene (tan in 1 second)
100	unknown (tan in 1 minute)
1	unknown (visible yellow before brushing)
30	empty bottom section (tan in several minutes)

The 80 mm. *trans*-zone was eluted with 350 ml. of ethanol-ether (1:1).

This material was chromatographed twice more on activated alumina. Each time the same sequence of zones appeared. From this it was concluded that some column-catalysed reaction was taking place. Therefore the use of activated alumina as an adsorbent for p-hydroxystilbene was abandoned.

A number of substances were tested as adsorbents for p-hydroxystilbene. Super Filtrol adsorbed the compound, but 1% permanganate reacted with the empty column so rapidly that it was difficult to locate the main zone. Calcium hydroxide (Shell) adsorbed p-hydroxystilbene very strongly even when washed with benzene-acetone (20:1). Calcium carbonate (Merck) would not adsorb the *trans*-compound from a chloroform-petroleum ether (1:1) solution. Basic magnesium carbonate (Kahlbaum) also had a very weak adsorptive power for the compound. Powdered magnesia (Micron Brand, Braun Corp., Los Angeles) adsorbed p-hydroxystilbene so strongly that even pure acetone would not more the zone. Finally, Neutrol Filtrol (Filtrol Corp., Los Angeles) was found to be an excellent adsorbent for p-hydroxystilbene. This material should be a good adsorbent for other simple phenols also.

Irradiation of trans-p-hydroxystilbene.- A solution of 600 mg. of *trans*-p-hydroxystilbene in 120 ml. of benzene was placed in two stoppered quartz test tubes (15.5 x 3.0 cm.) and irradiated for 80 hours. A portion of the solution (1.5 ml.) was then diluted with an equal volume of petroleum ether and chromatographed on a column (10.5 x 0.9 cm.) of Neutrol Filtrol. The colorless chromatogram was developed with 15 ml. of chloroform-petroleum ether (1:1), extruded and brushed with 1% permanganate:

50	empty top section (turns tan very slowly)
12	*trans*-p-hydroxystilbene (turns tan instantly)
21	empty interzone (turns tan very slowly)
3	unknown (turns tan slowly)
19	empty bottom section (turns tan very slowly)

The 3 mm. zone may be due to a trace of the *cis*-isomer. Since, at most, only a trace of the *cis*-isomer appeared to be formed during the irradiation, further experiments with p-hydroxystilbene were abandoned.

cis- and trans-p-Methoxystilbene

The synthesis of the *trans*-compound was carried out according to Meerwein and his associates[9]. However, the crude product was purified chromatographically instead of by sublimation.

$$\mathrm{C_6H_5.CH{:}CH.CO_2H + ClN_2.C_6H_4.OCH_3 + NaOAc \xrightarrow[acetone]{CuCl_2} C_6H_5.CH{:}CH.C_6H_4.OCH_3 + N_2}$$
$$\mathrm{+\ CO_2 + NaCl + HOAc}$$

A mixture of 15.4 g. (0.125 mole) of p-anisidine (Eastman), 50 g. (0.385 mole) of 25% hydrochloric acid and 50 g. of ice was diazotized by the addition during a period of 10 minutes of a cold solution of 9 g. (0.13 mole) of sodium nitrite in 15 ml. of water. The diazonium chloride solution was added to a well stirred cold mixture of 18.5 g. (0.125 mole) of cinnamic acid (Eastman), 27.5 g. of sodium acetate (0.2 mole) and 150 ml. of acetone in a three-necked one liter flask. The flask was equipped with a mercury seal stirrer and a U-tube bubble counter. After the addition of 6.7 g. of $CuCl_2.2H_2O$ in 15 ml. of water the temperature was maintained at 14-17° for 1-1/4 hours while stirring vigorously. After the acetone had been distilled off, the oily residue was steam distilled for one hour. The remaining dark tarry residue was extracted with 150 ml. and then twice with 50 ml. of hot benzene. This treatment left a crystalline residue of salt in the flask. The combined benzene extracts were filtered and then extracted with 75 ml. portions of an aqueous solution, 2 molal with respect to ammonia and 0.5 molal with respect to ammonium chloride. After six extractions, the benzene phase was given two water washes, dried over anhydrous sodium sulfate and evaporated to dryness. The crude yield was 12.7 g.

A portion of the material was purified by sublimation as suggested by Meerwein. After 12 hours 3.6 g. of the <u>trans</u>-compound had been sublimed onto a cold finger at a pressure of 0.05 mm. and a bath temperature of 110°. The sublimate was yellow instead of colorless.

In the chromatographic purification* 2 g. of the crude material was dissolved in 65 ml. of chloroform. The solution was diluted with 150 ml. of petroleum ether and chromatographed on a Super Filtrol column (16.5 x 4.3 cm.). The chromatogram was developed with benzene-petroleum ether (1:1 by volume, about 450 ml.) until the lowest colored zone reached the bottom of the column. The appearance of the column was as follows:

14	deep purple
20	dark red
10	red
3	purple
5	colorless interzone
12	reddish-brown
5	colorless interzone
15	violet
40	colorless interzone
41	maroon

The column with its numerous colored zones was discarded. Recrystallization of the evaporated filtrate residue from 75 ml. of methanol yielded 0.73 g. of _trans_-p-methoxystilbene (colorless flat rhomboids, m.p. 135-136°).

cis-p-Methoxystilbene.- A solution of 730 mg. of _trans_-p-methoxystilbene in 35 ml. of benzene was placed in a stoppered quartz test tube and irradiated for 160 hours (shorter irradiation is also satisfactory). For the chromatographic separation of the _cis_- and _trans_-isomers 7.6 ml. of the irradiated solution was evaporated to dryness. The residue weighed

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* In the purification of crude _trans_-p-methoxystilbene the procedure used in the synthesis of trans-p-methylstilbene should be followed. The dry benzene extract of the crude material should be diluted with petroleum ether and chromatographed directly on one or perhaps two Super Filtrol columns (27 x 7 cm.).

161 mg. It was taken up in 2.5 ml. of chloroform, and, after the addition of an equal volume of petroleum ether, chromatographed on an alumina column (17.5 x 1.7 cm.). On developing with 70 ml. of a petroleum ether-benzene mixture (3:1) and brushing, the following chromatogram appeared:

0.5	brownish top later (visible before brushing)
5	empty interzone
1	bright yellow by-product (visible before brushing)
6	empty interzone
40	<u>trans</u>-p-methoxystilbene (located by permanganate)
15	empty interzone
47	<u>cis</u>-p-methoxystilbene (located by permanganage)
60.5	empty bottom zone

Each main zone was eluted with 65 ml. of dry ether and recovered as previously described. The yield was 58.5 mg. of the <u>trans</u>-isomer (m.p. 135-136°, after recrystallization from 4 ml. of methanol) and 79.1 mg. of the <u>cis</u>-form (colorless oil), the recovery being 86%. The oil was rechromatographed before analysis.

<u>Anal.</u>

4.755 mg. (<u>cis</u>) and 3.371 mg. (<u>trans</u>) subst.: 14.770, 10.538
mg. CO_2; 2.833, 2.130 mg. H_2O

Calcd.: $C_{15}H_{14}O$: C, 85.67; H, 6.72

Found.: C, 85.76, 85.31; H, 6.66, 7.07

<u>Isomerization of cis-p-methoxystilbene.</u>- A trace of crystalline iodine was added to 20.3 mg. of the <u>cis</u>-isomer in a small pyrex test tube. Crystallization began after 15 minutes exposure to the sun's rays, but the

irradiation was continued for 6 hours. After dissolving the crystals in 0.5 ml. of chloroform the solution was diluted with 1 ml. of petroleum ether and chromatographed on a column (10.5 x 0.9 cm.) of activated alumina. The chromatogram was developed with 15 ml. of petroleum ether-benzene (3:1), extruded and brushed:

0.5	iodine (visible yellow)
11	empty top section
35	*trans*-p-methoxystilbene
15	empty interzone
20	*cis*-p-methoxystilbene
23.5	empty bottom section

After elution of the *trans*-zone with 12 ml. of dry ether and analytical recovery, a yield of 12.1 mg. of *trans*-p-methoxystilbene was obtained (m.p. 134-135.5°, without recrystallization).

Limits of detection.- A mixture of 2.2 mg. of the *cis*-p-methoxystilbene in 95.4 mg. of the trans-isomer was detected on an alumina column (17.5 x 1.7 cm.). When the developed chromatogram of a mixture of 0.9 mg. of *cis*- with 87.1 mg. of the *trans*-isomer was brushed, the *cis*-isomer could not be detected. In testing the limit of detection of *trans*- in *cis*-, 1.5 mg. of *trans*- was detected in admixture with 86.6 mg. of *cis*-p-methoxystilbene.

Fluorescence experiments

trans-Stilbene.- A solution of 250 mg. of *trans*-stilbene (Eastman) in 6 ml. of benzene was diluted with 6 ml. of petroleum ether and chromatographed on an activated alumina column (10.5 x 1.7 cm.). After developing with 50 ml. of petroleum ether-benzene (1:1) the fluorescence of the column appeared as follows in ultraviolet light:

3 pale blue fluorescence
102 non-fluorescent

The trans-stilbene zone was washed into the filtrate with 40 ml. of benzene. On rechromatographing the filtrate on a similar column, no fluorescence of the trans-stilbene adsorbate on activated alumina was observed. The stilbene zone was again washed into the filtrate with benzene and recovered by evaporation of the solution.

In order to examine the fluorescence of the solid, a few mg. of powdered crystals were placed between thin microscopic slides and viewed under the large ultraviolet lamp. Both the purified and non-purified stilbene fluoresced blue. No difference in intensity could be detected.

The fluorescence of the solutions was examined by inspecting 10 ml. of the benzene solution (1 mg. of substance per ml.) in a quartz test tube under the large ultraviolet lamp. The benzene solutions of both chromatographed and non-chromatographed stilbene had a very faint blue fluorescence.

trans-p-Methylstilbene.- A solution of 250 mg. of the trans-isomer (recrystallized from alcohol, m.p. 119-120°) in 3 ml. of benzene was diluted with 6 ml. of petroleum ether and developed on a column (17.5 x 1.7 cm.) of activated alumina with 50 ml. of petroleum ether-benzene (1:1). A minor brownish fluorescent zone was noted at the top. The rest of the column was nearly filled with a brilliant blue fluorescence. The filtrate was immediately rechromatographed on a similar column. No fluorescence of the trans-p-methylstilbene adsorbate on alumina was observed. The trans-isomer was washed into the filtrate with benzene and recovered.

This chromatographed sample fluoresced blue in the solid state. However, the non-chromatographed solid emitted a very brilliant blue fluorescence with an intensity many times that of the purified sample.

The benzene solution (1 mg. per ml.) of the chromatographed sample showed only a very faint bluish fluorescence. A solution of the crude product fluoresced even in sunlight. Under the ultraviolet lamp a very brilliant blue fluorescence was observed.

trans-p-Methoxystilbene.- A solution of 175 mg. of the trans-isomer (recrystallized from methanol, m.p. 135-136°) in 15 ml. of benzene-petroleum ether (1:1) was developed on an activated alumina column (10.5 x 1.7 cm.) with 75 ml. of benzene-petroleum ether (1:1). Ultraviolet light made the following fluorescent chromatogram visible:

1	pale blue
4	yellowish-brown
2	bright blue
100	pale blue (trans-p-methoxystilbene)

On developing with pure benzene the p-methoxystilbene zone moved rapidly into the filtrate, while the upper zones migrated down at a much slower rate. Eventually the 2 mm. bright blue zone split into a brilliant white upper and blue lower layer. Part of the filtrate was rechromatographed on a similar column. The p-methoxystilbene adsorbate still fluoresced pale blue. Fluorescent contaminants were missing, except for a trace of the brownish fluorescing zone.

In the solid state the chromatographed trans-p-methoxystilbene retained only a moderate bluish fluorescence while the non-chromatographed sample exhibited a brilliant bluish fluorescence. The chromatographed samples of stilbene, p-methylstilbene and p-methoxystilbene all fluoresced with approximately the same intensity.

In solution (1 mg. per ml. of benzene) the non-chromatographed p-methoxystilbene exhibited strong bluish fluorescence, while the purified sample retained a faint blue fluorescence.

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Part II

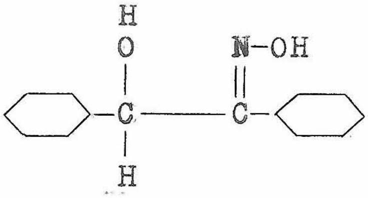
Separation of Some *cis*- and *trans*-Oximes by Means of the Chromatographic Brush Method

Brush Method.- Due to the difference in adsorption affinities of many stereoisomers, the chromatographic method has been of great aid in the separation of these compounds.[1] In the present Chapter this difference in adsorptive power is made use of in an investigation of the chromatographic behavior of some colorless cis- and trans-oximes. The location of the zones of the invisible chromatogram was made possible by use of the brush method recently reported by Zechmeister, Cholnoky and Ujhelyi.[2]

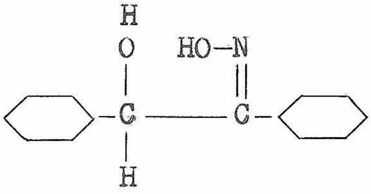
According to Feigl[3], a solution of trans-benzoin oxime (α or anti) (Formula I), or of trans-anisoin oxime (III), gives a stable deep-green complex[5] (V) when treated with ammoniacal copper sulfate. The analogous complex obtained with cis-benzoin oxime (β or syn) (Formula II) is brown.[4] This brown cis-benzoin oxime copper salt is soluble in ammonia and less stable than the trans-oxime copper salt which is insoluble in this reagent.

These color reactions of the stereoisomeric oximes with cupric ammonia complex were made the basis for the brush method. If two stereoisomeric oximes capable of complex formation are present in solution, chromatography on a Neutrol Filtrol column shows that the trans-form is adsorbed near the top and gives a green brush reaction while the cis-isomer is located in a lower section and turns brown where the brush crosses it. Between the two layers an interzone is formed which is nearly free of substance.

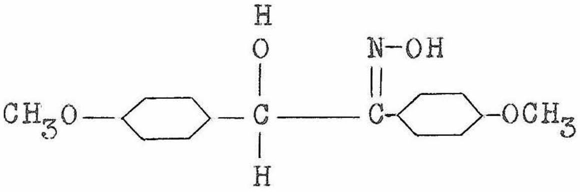
Adsorbents and starting materials.- A number of substances were investigated to determine their suitability as adsorbents. Both calcium carbonate and calcium hydroxide failed to adsorb the oximes from a ben-



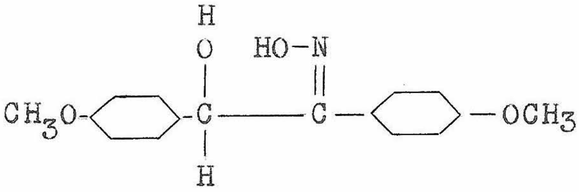
I. *trans*-Benzoin oxime



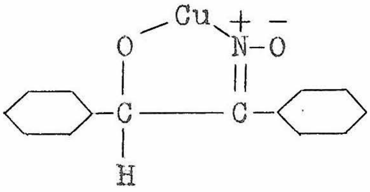
II. *cis*-Benzoin oxime



III. *trans*-Anisoin oxime



IV. *cis*-Anisoin oxime



V. Copper complex with *trans*-benzoin oxime[5]

Fig. II

zene solution. The stereoisomeric oximes were adsorbed on zinc carbonate, but no interzone was obtained. (In a preliminary work with Floridin* as adsorbent no interzone was obtained so the use of this adsorbent was abandoned.) A number of activated earths were found to separate the stereoisomeric oximes into two zones with a wide interzone between, but only the Filtrols were sufficiently free from oily impurities to permit quantitative work. Even the adsorbent chosen, Neutrol Filtrol, contained traces of oily impurities, but these were largely removed by preliminary extractions.

We were unable after many attempts to prepare chromatographically homogeneous cis-benzoin oxime by an earlier method.[6] Although the melting points of the samples obtained were close to the literature value, adsorption analysis revealed the presence of 18 to 50% of the trans-isomer in each case. The chromatographic method with Neutrol Filtrol as adsorbent was then used to prepare the pure stereoisomeric benzoin and anisoin oximes.

Analytical applications.- In order to determine the analytical applicability of the chromatographic separation of the stereoisomeric oximes, artificial mixtures of the isomers were chromatographed. After location of the zones by the brush method and elution and removal of the solvent, from 90 to 95% of each isomer was recovered. Representative data are presented in Table 3.

The limits of detection of one isomer in the presence of the other was determined by chromatographing a series of artificial mixtures. When working within the limits of 40 to 200 mg., 0.5 to 2% of either form can

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* Some qualitative experiments with Floridin have been described by G. Sólyom in his Thesis (Univ. Pécs, 1940).

be detected in the presence of the other by the brush method. These data are summarized in Table 4.

The ability to detect small quantities of one isomer in the presence of the other makes possible the study of the interconversion of the stereoisomeric forms under the influence of various factors. Werner[7] reported that cis-benzoin oxime is isomerized by refluxing in an alcoholic solution,

Table 3

Recovery of Oximes from Mixtures of the Stereoisomers

Substance	mg. Charged cis	trans	mg. Recovered cis	trans	trans*	Total% Recovery
Benzoin oxime	75	75	70.1	74.5	1.7	97.5
	1000 (Mixt.)		722	181	43	94.5
Anisoin oxime	75	75	68	69	0.8	92.0

* trans-form recovered from the interzone and formed by isomerization of the cis-oxime.

Table 4

Limits of Detection by the Brush Method in Mixtures of Stereoisomeric Oximes

Substance	mg. cis	mg. trans	Remarks
Benzoin oxime	0.4	40.	cis detected
	100.	2.0	trans detected
Anisoin oxime	1.6	200.	cis detected
	135.	1.2	trans detected

but our new chromatographic experiments demonstrate that the trans-compound also undergoes isomerization to a slight extent. Thus a measurable equilibrium exists when an alcoholic solution of the benzoin oximes is refluxed. Both cis- and trans-anisoin oximes partially isomerize when refluxed in alcohol. Trans-anisoin oxime, however, isomerizes to a greater extent under these conditions than trans-benzoin oxime.

Column catalysed isomerization.- The analytical applicability of the brush method is somewhat limited by a catalytic action of Neutrol Filtrol on some cis-oximes. If the rate of the chromatographic filtration is low and the duration of the experiment too long, the interzone which separates the cis- and trans-fractions contains small amounts of the trans-isomer formed on the column itself. The latter appears during the slow downward migration of the cis-oxime and is retained near the place of formation. In such a case the brush streak does not remain sky blue where it crosses the "interzone", but takes on a slightly greenish tint within several minutes. In contrast, the main portion of the trans-compound which was present in the original solution and is adsorbed near the top, instantaneously gives a well-defined dark green color on brushing. This difference allows quantitative experiments to be carried out. By mixing a filter aid with the adsorbent and thus speeding up the rate of filtration, the "column isomerization" amounts to less than 5% of the original quantity of cis-benzoin oxime and less than 1% of the cis-anisoin oxime.

Experimental

cis- and trans-Benzoin Oximes

Synthesis of trans-benzoin oxime.- Trans- (α or anti) benzoin oxime was prepared according to the data of Werner and Detscheff.[6] Eight grams (0.115 mole) of hydroxylamine hydrochloride (Eastman) was dissolved in 20 ml. of water and neutralized against litmus with a 26% aqueous sodium hydroxide solution (12.8 ml.). This neutralized solution was added to a suspension of 10 g. (0.047 mole) benzoin (Eastman) in 40 g. of 95% ethanol, refluxed for 45 minutes on a water bath, cooled, and freed from a little suspended matter by filtration. The slow addition of 125 ml. of water to the filtrate precipitated the oxime. After standing for 30 minutes to complete crystallization the crystals were filtered off and washed on the Buchner filter with 75 ml. of water. The yield of crude trans-benzoin oxime was 9.0 g. (84%) after drying in a dessicator over phosphorus pentoxide. This crude product was recrystallized twice from 160 ml. of dry ether as suggested by Werner and Detscheff.[6] However, the rate of solution was very slow, and it was necessary to concentrate the ether solution to 30 ml. before crystallization would start. (Absolute alcohol should be a more satisfactory recrystallization solvent than ether.) This recrystallization eliminates small amounts of the more soluble cis-isomer. The melting point of the trans-isomer was 150-151°.

Synthesis of cis-benzoin oxime.- Cis- (β or syn) benzoin oxime was also prepared by the method of Werner and Detscheff.[6] Although their procedure was repeated six times, chromatographically homogeneous cis-benzoin oxime could not be obtained. In fact, the cis compound was always contaminated with 30 to 60% of its trans-isomer.

The oximation of benzoin to the cis-isomer was carried out by Werner and Detscheff in an alkaline solution and with an unspecified excess of hydroxylamine hydrochloride. We have carried out a series of experiments with a varying excess of this reagent. These showed that the percentage of the trans-benzoin oxime in the crude product increased with an increasing excess of hydroxylamine hydrochloride. Therefore, the final procedure chosen was as follows: A solution of 3.0 g. (0.043 mole) of hydroxylamine hydrochloride (Eastman) in 20 ml. of water was made alkaline with 10.2 ml. of 26% sodium hydroxide (334 g./liter). This alkaline solution was added to a suspension of 5.0 g. (0.024 mole) of finely ground benzoin (Eastman, recrystallized from benzene) in 25.5 ml. of ethanol. The suspension was shaken continuously for 15 minutes while warmed by a water bath maintained at 50°. Within 15 minutes of shaking, practically all of the benzoin had dissolved. The solution was cooled, 200 ml. of water added, and any remaining benzoin filtered out. Carbon dioxide was bubbled through the solution until a permanent cloudiness appeared. The solution was allowed to stand overnight at room temperature in order to crystallize the oximes. (Further rapid addition of carbon dioxide throws out all of the oxime as a pinkish oil.) The next day the crystals were filtered out and washed on the filter with water. The dried crystals were extracted with 25 ml. of cold ether. After filtering off any undissolved trans-benzoin oxime, the ether solution was placed in a covered beaker and allowed to evaporate slowly during a period of two days at room temperature. Hard, slightly pinkish crystals of the ether addition compound of the oxime were formed as the solution went to dryness. The ether of crystallization was removed by warming in an oven at 35° for 6 hours. The melting point was 96-98.5° (the melt was slightly cloudy).

The melting point given by Werner and Detscheff was 99°. In spite of the close agreement of our melting point with that given by these authors, the product was shown by chromatography to contain 18% of the trans-isomer.

The chromatographic analysis and purification of the product is given on page 53 of this Thesis. If it is desired to prepare cis-benzoin oxime, the procedure developed for cis-anisoin oxime should be followed. That is, the trans- and cis-benzoin oxime mixture from the carbon dioxide precipitation (either oily or crystalline) should be dissolved in chloroform-benzene (1:1) and chromatographed directly.

After the pure cis- and trans-benzoin oximes had been prepared chromatographically, a melting point was taken on an artificial mixture containing 20% trans and 80% cis in order to check the close agreement between the melting point of the crude product and that of the pure cis-benzoin oxime. A mixture of 9.2 mg. of the trans-form with 37.3 mg. of cis-benzoin oxime was prepared by prolonged grinding in an agate mortar. This sample melted between 96 and 98° to a slightly cloudy melt and finally became homogeneous at about 116°. The cloudiness was difficult to detect with the naked eye, but easily seen with a 10 power telescope. Thus from the melting point alone it would be easy to mistake the mixture for pure cis-benzoin oxime.

Preliminary attempts were made to purify the cis-benzoin oxime by the usual methods in order to have the pure compounds available for the chromatographic separation experiments. A number of solvents were tested in attempts to recrystallize the cis-oxime. Toluene was found to remove most of the trans-isomer as well as colored impurities from very crude cis-benzoin oxime. For example, 4.15 g. of the crude cis-oxime was extracted with 15 ml. of toluene in a flask which was warmed by a bath maintained at 90°. The mixture was shaken for two minutes, cooled immediately to prevent appreciable isomerization, and chilled in an ice bath for 10 minutes. The trans-benzoin oxime that had not dissolved or that had crystallized out (0.7 g. in all) was removed by filtration. The filtrate was placed in a cold room overnight as the cis-oxime crystallized slowly. The next day 2.3 g. of hard crystals of the cis-oxime were isolated. However, the melting point indicated that some trans-oxime was still present.

Partitioning a mixture of the stereoisomeric benzoin oximes between alcohol and benzene by the addition of water to their alcoholic solutions enriched the benzene layer in the cis-isomer. Several successive partitions gave the nearly pure cis-compound, but considerable quantitaties (up to 50%) of the original cis-isomer were also lost. This demonstrated that partitioning could not be used as part of an analytical chromatographic procedure.

Selection of adsorbent.- A number of substances were investigated in order to determine their suitability as adsorbents for the separation of cis- and trans-benzoin oxime. Each material to be tested was packed in a chromatographic tube, size = 22.5 x 1.7 cm.* A solution of 150 mg. of cis-trans mixture in 35 ml. of chloroform-benzene (1:1) was then filtered

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* The chromatographic tubes were manufactured by the Scientific Glass Apparatus Co., Bloomfield, New Jersey.

through the column followed by 40 ml. of pure benzene. After extrusion of the column containing the invisible chromatogram, a narrow streak was made down the cylinder with a brush which had been dipped in the copper-ammonia reagent. The latter was prepared by diluting 0.15 moles of cupric sulfate and 4 moles of ammonia to one liter.

The substances giving no apparent adsorption were calcium carbonate (Merck, precipitated), calcium hydroxide (Merck), and Hyflo Super-Cel (Johns-Manville Co.). The reagent streak on zinc carbonate (Merck, precipitated) was green at the top and brown directly below. Thus there was a partial separation of the two stereoisomeric oximes, but no interzone had developed.

Fuller's Earth (Braun Corp., Los Angeles) and Lloyd's reagent (Eli Lilly and Co.) separated the oximes into an upper zone which gave a green color with the reagent (trans-benzoin oxime), an intermediate section which gave only a slightly positive test, and a lower zone which gave a dark brown color (cis-benzoin oxime). However, special experiments indicated that each adsorbent contained considerable quantities of extractable oily and solid impurities. For example, after 14 extractions of Fuller's Earth with benzene and alcohol-ether the extracts still contained appreciable amounts of a colored oil. Consequently the use of these adsorbents was abandoned. Neutrol Filtrol, Super Filtrol and Special Filtrol (Filtrol Corp., Los Angeles) were found to be far the best of any activated earths tested. They were white, contained only traces of oily impurities, and permitted excellent separation of the two isomers. Neutrol Filtrol was used in the subsequent experiments in order to avoid any possible complication with the slightly acidic Super and Special Filtrols.

Purification of Neutrol Filtrol.- A number of qualitative separations of cis- and trans-benzoin oxime were carried out with Neutrol Filtrol. Although the trans-isomer was easily crystallized after elution from the column, the traces of extractable oils made it difficult to obtain the cis-compound in crystalline form. When it was crystallized, the melting point was 4 to 8° low.

Due to this difficulty, the adsorbent was purified by preliminary extractions. Cold extractions with benzene and alcohol-ether or hot extractions in a soxhlet apparatus with acetone and benzene gave equally satisfactory results. In the first case 1250 g. of Neutrol Filtrol was extracted 7 times with benzene and 14 times with alcohol-ether (1:1). Each extraction was carried out in a 3 liter wide mouth glass stoppered bottle with 1.5 liter of fresh solvent. The adsorbent was extracted for 20 minutes on a shaking machine and then freed from solvent by suction on a Büchner funnel. The adsorbent was washed on the filter with 0.5 liter of solvent each time. After the last extraction, the damp adsorbent was placed in five 20 cm. evaporating dishes, dried in the sun to remove most of the solvent, and then heated in an oven at 90° for 4-5 hours. It was then mashed to a fine powder and bottled.

In the purification of the adsorbent by hot extraction 2500 g. of Neutrol Filtrol was extracted for 15 hours with 15 liters of acetone in a large metal Soxhlet apparatus. After removing the acetone, the extraction was continued with benzene for 10 hours. The drying procedure was as described.

The treated adsorbent is more active than the untreated since it is necessary to develop the benzoin oximes with 2% ethanol in benzene on the treated adsorbent while development with pure benzene is sufficient on the crude material.

Experimental technique.- In order to determine the amount of impurities extracted from the adsorbent under the conditions of the analytical experiments, a blank was run on the treated Neutrol Filtrol. A chromatographic tube (22.5 x 1.7 cm.) was filled by introducing the adsorbent through a powder funnel while the suction on the column was gradually increased to full water pump vacuum. After the tube had been filled to the desired level, the top was tamped lightly (tamping while the column is being formed results in a slow rate of filtration). Twenty-five ml. of chloroform-benzene (1:1) was sucked through the column. This was followed by 50 ml. of benzene-ethanol (50:1). The duration of the experiment was 70 minutes. After extrusion, a 40 mm. section in the upper part of the column was eluted with 40 ml. of absolute alcohol-ether (1:1) while the lowest 40 mm. was eluted with 40 ml. of ab. ether. Each eluate was evaporated and weighed under "standard conditions", see below. The weight of the dry residue from the upper section was 0.0015 g. while that from the lower section was 0.0014 g.

The "standard conditions" were as follows: The adsorbent was eluted on a sintered glass filter (Jena G3). The filtrate was transferred to a tared standard taper boiling flask (usually 50 ml.) with absolute ether, connected to a standard taper still head, and evaporated to complete dryness on a water bath under reduced pressure. The alcohol-ether residues were evaporated down three times with small amounts of ether, and then placed under full suction for 15 minutes while maintaining the bath temperature at 70°. The flask was disconnected from the still head, wiped dry with a lint free towel, and suspended near the balance for 15 minutes before weighing. In the case of the absolute ether eluates the treatment was the same except that the residue was evaporated down only once with ether and then dried at 45°.

Chromatographic purification of cis-benzoin oxime.- In order to purify the crude cis-benzoin oxime, 200 mg., prepared by the previously described method, was dissolved in 25 ml. of chloroform-benzene (1:1) and chromatographed on a 14 x 1.7 cm. column of treated Neutrol Filtrol. The flask was rinsed out with 5 ml. of benzene in several portions. The column was then developed with 75 ml. of benzene-ethanol (50:1) during a total length of time of 80 minutes. After extrusion and brushing with the reagent, the presence of the following zones was revealed (the colors given refer to the streak):

6	sky blue (empty top section)
12	dark green (trans-benzoin oxime)
42	light green (interzone, containing small amounts of trans)
13	dark brown (cis-benzoin oxime)
67	sky blue (empty bottom section)

After cutting out the three zones and shaving off the streak, each of the two trans-fractions was eluted with 40 ml. of absolute alcohol-ether (1:1) and the cis-fraction with the same volume of absolute ether. Evaporation and analytical weighing indicated the presence of 44 mg. of trans-benzoin oxime in the upper section, 20 mg. in the central "interzone" and 136 mg. of the cis-oxime in the lowest section. The crude trans-oxime melted at 132-133°, and after recrystallization from 0.4 ml. of absolute alcohol in a micro-centrifugal apparatus the melting point was raised to 147°-148°. After one recrystallization of the intermediate zone residue from 1.8 ml. of hot benzene and thorough drying, 14 mg. of the trans-oxime was obtained, melting at 144.5-145.5°. The oily cis-fraction was transferred to a small beaker with a few ml. of dry ether and evaporated to dryness in a carbon dioxide stream while scratching with a glass rod (white crystals, m.p. 98-99°.)

Heat isomerization of cis- and trans-benzoin oximes.- The chromatographic brush method offers a convenient means of studying cis-trans-isomerizations. For example, a solution of chromatographed cis-benzoin oxime (which had shown only a trace of the upper trans-zone) in 25 ml. of alcohol was refluxed for two hours, evaporated, and taken up in 10 ml. of chloroform-benzene (1:1). After chromatography on a 17.5 x 1.7 cm. column of treated Neutrol Filtrol, development with 70 ml. of benzene-ethanol (50:1), extrusion, and brushing, the following zones appeared:

7	sky blue (empty top section)
4	dark green (trans-benzoin oxime)
38	turns light green slowly (interzone, containing a small amount of trans)
5	dark brown (cis-benzoin oxime)
121	sky blue (empty bottom section)

After elution of an upper 15 mm. zone including the trans-benzoin oxime with 40 ml. of absolute alcohol-ether (1:1) and a 10 mm. region including the lower cis-zone, 11.2 mg. of the trans- and 29.3 mg. of unchanged cis-compound were isolated.

The isomerization of the trans-oxime was checked qualitatively. Two hundred mg. of chromatographed trans-benzoin oxime was divided into two portions. One portion was dissolved in 30 ml. of alcohol and refluxed for two hours. After evaporation under vacuum, the residue was taken up in 40 ml. of chloroform-benzene (1:1) and chromatographed on a 14 x 1.7 cm. column of Neutrol Filtrol. The column was developed with 80 ml. of benzene. The non-refluxed portion was chromatographed simultaneously. Brushing revealed the presence of approximately twice as much of the cis-isomer in the refluxed as in the non-refluxed portion. The amount of isomerization was estimated to be 1%.

Column catalysed isomerization of cis-benzoin oxime.- In the described experiments and other similar ones, the region between the upper green trans- and lower brown cis-zones was noted to give a light green test with the cupric-ammonia brush reagent. Since the interzone gave an oxime test with the reagent only in those experiments in which an appreciable amount of the cis-isomer was present and not in those in which only the trans-oxime was present, the interzone was apparently due to isomerization of the cis-benzoin oxime on the surface of the adsorbent. This was demonstrated quantitatively in the following two consecutive experiments. A solution of 129.2 mg. of chromatographed cis-benzoin oxime in 25 ml. of chloroform-benzene (1:1) was chromatographed on a 14 x 1.7 cm. column of treated Neutrol Filtrol. After development with 75 ml. of benzene-ethanol (50:1) (duration of experiment 100 minutes), extrusion, and brushing, the following zones were noted:

5	sky blue (empty top section)
3	light green (slightly darker than interzone)
57	light green ("interzone")
6	dark brown (*cis*-benzoin oxime)

After elution of the top two zones with 55 ml. of absolute ethanol-ether (1:1) and of the *cis*-zone with 40 ml. of absolute ether, evaporation and analytical recovery, 40 mg. of crystals originating from the interzone and 86 mg. of *cis*-benzoin oxime were obtained. This amounts to 30% and 67% of the starting material respectively. The total recovery was 97%.

The cis-benzoin oxime (86 mg.) from the above experiment was dissolved in 25 ml. of chloroform-benzene (1:1) and re-chromatographed as above. The same sequence of zones appeared. From the upper two zones 24 mg. (28%) of crystals was obtained while 61 mg. (71%) of unchanged *cis*-benzoin oxime was recovered. The total recovery was 99% of the starting material. After recrystallization of the 24 mg. of upper zone crystals from 1.3 ml. of benzene and careful drying (to remove benzene of crystallization), the melting point of the sample was near that of *trans*-benzoin oxime, viz. 146-147°.

The following experiment demonstrated that the "interzone" contained mostly *trans*-benzoin oxime mixed with a little of the *cis*-form. A solution of 14.5 mg. of interzone material in 3 ml. of chloroform-benzene (1:1) was chromatographed on a 9.5 x 0.85 cm. column of treated Neutrol Filtrol and developed with 15 ml. of benzene-ethanol (50:1) during 40 minutes. After brushing, the following zones were noted:

3	sky blue (empty top section)
10	dark green (*trans*-benzoin oxime)
30	sky blue (interzone)
2	dark brown (*cis*-benzoin oxime)
50	sky blue (empty bottom section)

A special experiment was carried out to determine whether or not the isomerization of the *cis*- to the *trans*-oxime was purely a time effect. A solution of 200 mg. of chromatographed *cis*-benzoin oxime in ether was divided into halves and both portions were evaporated to dryness. One portion was dissolved in chloroform-benzene (1:1) and chromatographed immediately while the second portion was allowed to stand at room temperature for four days in benzene-ethanol (50:1) and then for one day in chloroform-benzene (1: 1) before chromatographing. The upper *trans*-zone from each column was eluted and the extracted substance weighed. From the first chromatogram 5 mg. of material was recovered while the second yielded 6 mg. Consequently the time effect of the isomerization is negligible under the conditions described.

Since previous experiments indicated that the *trans*-oxime in the interzone was formed by a column-catalysed isomerization of the *cis*-isomer during its migration down the column, a filter aid was mixed with the treated Filtrol to speed up the rate of flow. A mixture of one part of Celite 535 or Hyflo Super-Cel (Johns-Manville Co.) to 5 parts of adsorbent was found to be satisfactory.

In order to demonstrate the catalytic role of the column material on the isomerization, a slow chromatogram (by means of little suction) and a rapid chromatogram of the *cis*-oxime were carried out on the Filtrol-

Celite mixture. A solution of 125 mg. of purified cis-benzoin oxime in 10 ml. of chloroform-benzene (1:1) was chromatographed on a 14.5 x 1.7 cm. column of treated Neutrol Filtrol-Celite 535 (5:1). (The flask was rinsed out with analytical care with 2.5 ml. of benzene.) The development was carried out with 50 ml. of benzene-ethanol (50:1) under weak suction so that the total duration of the experiment was 196 minutes. On brushing with the reagent the following zones were seen:

5 sky blue (empty top section)
67 light green (trans-benzoin oxime)
24 dark brown (cis-benzoin oxime)
50 sky blue (empty bottom section)

On elution of each zone and analytical recovery 35 mg. was recovered from the 67 mm. zone, while 82 mg. of unchanged cis-benzoin oxime was isolated from the 24 mm. zone. The extent of "column isomerization" was 28% in this case.

In a corresponding rapid experiment 125 mg. of pure cis-benzoin oxime from the same source was dissolved in 10 ml. of chloroform-benzene (1:1) and chromatographed on a 15.5 x 1.7 cm. column of treated Neutrol Filtrol-Celite 353 (5:1). Development was with 50 ml. of benzene-alcohol (50:1) under full water pump suction so that the duration of the experiment was only 22 minutes. On brushing the presence of the following zones was revealed:

2 sky blue (empty top section)
66 blue (traces of trans)
25 dark brown (cis-benzoin oxime)
62 sky blue (empty bottom section)

After elution the 66 mm.-zone yielded 6 mg. of material while 114 mg. of unchanged cis-benzoin oxime was recovered from the 25 mm.-zone. The extent of the "column isomerization" was only 5% in this rapid experiment.

Isolation of cis-benzoin oxime on a preparative scale.- The method developed for the diminishing of the "column isomerization" effect was then adapted to a large scale preparation of pure cis-benzoin oxime. For this purpose the solution of 1000 mg. of crude cis-oxime (page 42 of this Thesis) in 100 ml. of chloroform-benzene (1:1) was developed on a 19 x 3.2 cm. column of treated Neutrol Filtrol-Celite 535 (5:1) with 160 ml. of benzene-ethanol (50:1). The total duration of the experiment was 30 minutes. The following zones appeared:

5	sky blue (empty top section)
16	dark green (trans-benzoin oxime)
50	bluish green (small amounts of trans)
54	dark brown (cis-benzoin oxime)
65	sky blue (empty bottom section)

After cutting out the three zones and shaving off the streak, each of the two trans-fractions was eluted with 100 ml. of absolute alcohol-ether (1:1) and the cis-fraction with the same volume of dry ether. Evaporation and recovery indicated the presence of 181 mg. and 43 mg. of trans- and 722 mg. of cis-benzoin oxime, the total recovery being 95%. The melting point of the trans-compound was 147-148° (after recrystallization from 2 ml. of absolute ethanol). The oily cis-fraction was dissolved in a few ml. of dry ether and evaporated in a carbon dioxide current, at 25°. On rubbing with a glass rod as the solution went to dryness, the oxime crystallized (m.p. 99-99.5°).

Anal.

Calcd. for $C_{14}H_{13}O_2N$: N, 6.17.

Found (cis): N, 6.38. Found (trans): N, 6.15.

Artificial mixture.- In order to determine the suitability of the method for the separation of cis-trans-benzoin oxime mixtures, an artificial mixture of 75 mg. of each purified isomer was dissolved (with warming) in 20 ml. of chloroform-benzene (2:1) and chromatographed on a 17.5 x 1.7 cm. column of treated Neutrol Filtrol-Celite 535 (5:1). After development with 40 ml. of benzene-ethanol (50:1) (duration of experiment 20 minutes), the column was extruded and brushed:

2	sky blue (empty top section)
17	dark green (trans-benzoin oxime)
42	blue (interzone, traces of trans)
26	dark brown (cis-benzoin oxime)
88	sky blue (empty bottom section)

Fifty ml. of dry alcohol-ether (1:1) was used for the elution of the 17 mm.-trans-zone, 40 ml. of the same mixture for the interzone and 40 ml. of dry ether for the cis-compound. The recovery was 74.5 mg. and 1.7 mg. of trans- and 70.1 mg. of cis-benzoin oxime; the melting points were correct.

Limits of detection.- The limits of detection were established by a series of artificial mixtures as described above. In the presence of 40 mg. of trans-benzoin oxime 0.5 mg. of the cis-isomer can be detected by washing with 12 ml. of benzene-ethanol (50:1) on a 10.5 x 0.85 cm.

column. In the presence of 100 mg. of cis-, 2 mg. of the trans-compound was detected on a column of the size mentioned. Each experiment required from 20 to 25 minutes. Thus in a mixture composed of the two stereoisomers 1 to 2% of either form can be detected by the brush method. 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 an extension of the limits of detection may be obtained.

cis- and trans-Anisoin Oximes

The adsorbent used in these experiments was the treated Neutrol Filtrol described on page 45. In each case 5 parts of this adsorbent was mixed with 1 part of Celite 535.

The color reagent was the cupric-ammonia complex (page 44).

Synthesis of anisoin.- In preliminary experiments with crude cis- and trans-anisoin oxime mixtures, the "cis-isomer" split into two bands when developed with benzene-acetone (15:1). Since this may have been due to impurities in the starting material, special precautions were taken in the synthesis.

Two hundred grams of anisaldehyde (Eastman) was vacuum distilled through a two foot packed fractionating column at 49 mm. pressure. Eighty-five grams of anisaldehyde boiling within an 0.5° range was collected from the middle portion ($n_D^{20} = 1.5730$).

This sample was then condensed to anisoin in the presence of potassium cyanide.[11] A solution of 42 g. of anisaldehyde in 65 ml. of 95% alcohol and 20 ml. of water was placed in a flask on a steam bath and maintained under a nitrogen atmosphere. One-half of a solution of 16 g. of potassium cyanide in 25 ml. of water was added initially and the remainder in two equal portions after one and two hours respectively. After a total of three hours of refluxing the flask was removed from the steam bath and shaken continuously while allowed to cool. After 30 minutes crystals appeared. Crystallization was completed overnight in a cold room at 5°. The crystals were filtered off and washed on the filter with 25 ml. of 50% ethanol-water. After crystallization from ethanol-water (3:2) 14.3 g. of anisoin were obtained (m.p. 112-113°).

In spite of the correct melting point, the recrystallized product proved to be chromatographically heterogeneous. For purification 20 g. was dissolved in 0.8 liter of chloroform-benzene (1:1) and chromatographed on a 27 x 7 cm. column of treated Neutrol Filtrol-Celite. The development was carried out with 2.5 liters of benzene containing 0.5% ethanol. On extruding and brushing with 1% permanganate the presence of the following zones was revealed:

20	unchanged (empty top section)
90	tan (anisoin)
100	unchanged (interzone)
10	tan (impurity)
5	unchanged (empty bottom section)

The anisoin was eluted with 2 liters of absol. alcohol-ether (10:1) and the solution concentrated in a carbon dioxide stream to 50 ml. to effect crystallization. The product was recrystallized from 50 ml. of 95% ethanol (17 g., m.p. 112-113°).

<u>Anal.</u>

2.514 mg. subst.: 4.315 mg. AgI

Calcd.: $C_{16}H_{16}O_4$: OCH_3, 22.79

Found: OCH_3, 22.68

Synthesis of cis- and trans-anisoin oximes.- The oximation, carried out as described by Werner and Detscheff[9] for both *cis*- and *trans*-benzoin oxime gave a mixture of the stereoisomers. For example, 2.3 g. of hydroxylamine hydrochloride in 10 ml. of water (neutralized against litmus with 4.0 ml. of 26% sodium hydroxide) was added to 4.5 g. of anisoin in 20 ml. of ethanol. After 1-1/2 hours of refluxing, the liquid was diluted with 150 ml. of water and allowed to stand overnight. The oil was taken up with 300 ml. of benzene, dried with sodium sulfate, and chromatographed on a 22.5 x 4.3 cm. column. Development was with 500 ml. of benzene-ethanol (30:1). After extruding and brushing with the cupric-ammonia reagent, the presence of the following zones was revealed:

12	sky blue (empty top section)
73	dark green (*trans*-anisoin oxime)
56	bluish-green (trace of *trans*)
1	pink, without brushing (unknown)
35	dark brown (*cis*-anisoin oxime)
48	sky blue (empty bottom section)

The *trans*-compound was eluted with 450 ml. of dry alcohol-ether (1:1) and the *cis*-isomer with 250 ml. of dry ether. The solvents were removed in vacuo under an atmosphere of carbon dioxide.

The *trans*-compound appeared as an oil and was crystallized by dissolving it in about 50 ml. of benzene at 25° and scratching. The yield was 1.9 g. The colorless, short, quadrangular plates melted at 125.5°. The *trans*-oxime can also be crystallized from several milliliters of ether to give samples with the same melting point. If the *trans*-oxime is crystallized from hot benzene, it comes down as a soft mat of tiny needles containing benzene. It is then difficult to remove the benzene and obtain a sharp melting point.

The cis-compound came out in the form of crystals which contained ether. These were dissolved in 6 ml. of acetone at room temperature and evaporated rapidly with a carbon dioxide stream at 25°. The oily residue was evaporated again, after the addition of 2 ml. of benzene, by means of a carbon dioxide jet, dissolved in 10 ml. of cold benzene and scratched. Thin, elongated prisms appeared. (1.2 g., m.p. 121-122°). The same melting point is obtained by carefully drying the ether addition compound obtained after a recrystallization from several ml. of ether. The crystals develop a yellowish tint after a few days in a dessicator.

Anal.

2.941 mg. (cis) subst.: 7.195 mg. CO_2; 1.471 mg. H_2O

5.517 mg. (cis) and 5.611 mg. (trans) subst.: 0.257 ml. N (26°, 744 mm.), 0.258 ml. N (25°, 737 mm.)

Calcd.: $C_{16}H_{17}O_4N$: C, 66.88; H, 5.96; N, 4.88

Found: C, 66.76; H, 5.60; N, 5.08, 4.99.

The difference in the melting points of the two isomers is remarkably small. The configurations, however, are given by the Feigl color reaction with cupric-ammonia complex and by the relative positions of the two isomers on the column. In addition, the crystallization behavior of the two isomers is similar to that of the corresponding benzoin-oxime isomers. Each trans-compound forms a soft mat of needles containing tightly held benzene on recrystallizing from hot benzene and gives no ether addition product. On the other hand, each cis-isomer forms an ether addition product but no benzene addition compound. Each isomer was quite pure, showing the presence of less than 1% of the corresponding stereoisomer on rechromatographing.

<u>Artificial mixture</u>.- In order to determine the applicability of the method to the analysis and separation of *cis*- and *trans*-anisoin oxime mixtures, an artificial mixture of 75 mg. of each isomer was dissolved in 35 ml. of benzene by slight warming and developed on a 14 x 1.7 cm. column with 50 ml. of benzene-ethanol (30:1)[*]

10	sky blue (empty top section)
21	dark green (*trans*-anisoin oxime)
37	blue (interzone)
7	dark brown (*cis*-anisoin oxime)
65	sky blue (bottom section containing a faint yellow line, visible before brushing)

The *trans*-zone was eluted with 55 ml. of dry alcohol-ether (1:1) and evaporated under vacuum. Sixty-nine mg. of oil was obtained, which, after crystallization from 2 ml. of cold benzene, yielded 50 mg. of crystals melting at 125-125.5°. The cis-zone was eluted with 50 ml. of dry ether. After evaporating rapidly under "standard conditions" (page 46), 68 mg. of oil was obtained. Upon crystallization from 0.6 ml. of benzene (overnight), 56 mg. of crystals were isolated (m.p. 122.5-123.5°). The interzone yielded 0.8 mg. of substance after elution with 50 ml. of dry ether and analytical recovery. The total recovery was 92%.

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[*] The zones developed a faintly visible yellow color during the adsorption.

Limits of detection.- The limits of detection by the brush method were found to be 1.6 mg. of *cis*-anisoin oxime in 200 mg. of the *trans*-isomer or 1.2 mg. of *trans*- in the presence of 135 mg. of the *cis*-compound. The limit is thus 0.5 to 1% on the experimental scale indicated.

Heat isomerization of cis- and trans-anisoin oximes.- A solution of 140 mg. of *cis*-anisoin oxime was isomerized by refluxing for 1-1/2 hours in 40 ml. of ethanol. After removal of the solvent the residue was taken up in 35 ml. of benzene and chromatographed on a 13 x 1.7 cm. column. The latter was developed with 50 ml. of benzene-ethanol (30:1):

7	sky blue (empty top section)
6	green (*trans*-anisoin oxime)
45	blue (interzone)
10	dark brown (cis-anisoin oxime)
60	sky blue (empty bottom section)

Elution of the *trans*-zone with 40 ml. dry ethanol-ether and analytical recovery yielded 11.5 mg. of an oil. The latter was dissolved in 0.7 ml. of cold benzene, sucked through a filter stick, and crystallized in a micro crystallization vessel. After drying at 20 mm. and 55° for 15 minutes the melting point was 125-125.5°. After elution of the *cis*-zone with 75 ml. of dry ether and analytical recovery, 107 mg. of the oily *cis*-anisoin oxime was obtained. Upon crystallization from 1 ml. of benzene as described for the *trans*-isomer, 86 mg. of crystals were isolated (m.p. 122-122.5°). In a blank experiment (without refluxing) only 1.2 mg. of *trans*-anisoin oxime was obtained from the same amount of *cis*-anisoin oxime while 125 mg. of unchanged *cis*-isomer was recovered. The extent of isomerization by boiling was thus 8%.

In an isomerization experiment with trans-anisoin oxime 200 mg. dissolved in 50 ml. of 95% ethanol was refluxed for 1.5 hours. The solution was vacuum distilled to dryness, evaporated down with a small amount of benzene, and then taken up in 40 ml. of benzene (with warming). After chromatography on a 14 x 1.7 cm. column, development with 50 ml. of benzene-ethanol (30:1), and brushing, the presence of the following zones was revealed:

9	sky blue (empty top section)
40	dark green (trans-anisoin oxime)
37	blue (interzone)
1	dark brown (cis-anisoin oxime)

After elution of the trans-zone with 125 ml. of dry ethanol-ether (1:1) and analytical recovery, 185 mg. of oily trans-oxime was obtained. On crystallization from 0.6 ml. of ether and drying, 124 mg. of crystals were obtained (m.p. 125-125.5°). The cis-zone was eluted with 40 ml. of dry ether and evaporated under "standard conditions". The cis-oxime was obtained as 6.3 mg. of an oil. The oil was dissolved in 0.6 ml. of benzene, filtered through a filter stick, and evaporated to 0.1 ml. with a carbon dioxide jet. On scratching white crystals appeared. These were centrifuged and dried at 55° and 20 mm. for 15 minutes (m.p. 120-121°). In a simultaneous blank experiment (without refluxing) 1.6 mg. of the cis-oxime was recovered from the same amount of trans-anisoin oxime. The extent of heat isomerization was thus 2.5%.

Column catalysed isomerization of cis-anisoin oxime.- The catalytic influence of the adsorbent on isomerization during the chromatographic development was determined in a "slow" experiment. A solution of 125 mg. of cis-anisoin oxime in 30 ml. of benzene was chromatographed on a 15.5 x 1.7 cm. column and developed with 50 ml. of benzene-ethanol (30:1). By the use of very weak suction the experiment was made to last 3-1/4 hours. After brushing the presence of the following zones was noticed:

4	sky blue (empty top section)
48	light green (interzone containing the trans)
8	dark brown (unchanged cis-anisoin oxime)
94	sky blue (empty bottom section)

After elution of the interzone with 60 ml. of dry ethanol-ether (1:1) 13.7 mg. of the trans-isomer was obtained. Thus the "column isomerization" amounted to 11% in this case. In a corresponding "short" experiment the "column isomerization" was about 1%.

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Part III

Chromatographic Isolation of Homopterocarpin and Vanillin from Red Sandalwood

In 1832 Pelletier[1] began the chemical investigation of red sandalwood (a wood cultivated in the Philippines and East Indies) by isolating the red pigment santalin. Since that time a few colorless compounds have also been isolated, including santal, $C_{13}H_{10}O_5$, obtained first by Weidel[2] and later by Raudnitz and Perlmann;[3] homopterocarpin, $C_{24}H_{24}O_6$, and pterocarpin, $C_{20}H_{16}O_6$, isolated by Cazeneuse and Hugounenq.[4] In more recent years the two later compounds were isolated again, in attempts to determine their structure, by Dieterle and Stegemann,[5] Dieterle and Leonhardt[6] Raudnitz and Perlmann,[3] Späth and Schläger,[7] Leonhardt and Fay,[8] Leonhardt and Oechler[9] and by Mc Gookin, Robertson and Whalley.[10] Finally, in 1940, Späth and Schläger[11] reported the isolation of pterostilbene, $C_{16}H_{16}O_3$ from red sandalwood.

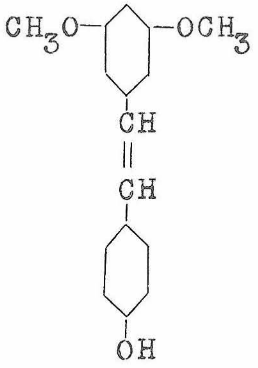
There has been considerable disagreement concerning the degree of purity and structure of some of the compounds of this group. Apparently repeated recrystallizations failed to give pure homopterocarpin since its melting point reported by earlier authors was 4 to 5 degrees below the melting point obtained by Späth and Schläger after a distillation in high vacuum. Only pterostilbene (Formula I) has been definitely clarified.[11] Späth and Kromp[12] subsequently confirmed this structure by synthesizing the compound. A probable structural formula for homopterocarpin (II) has been suggested by Späth and Schläger.[7]

It should be remarked that stilbene derivatives are not as rare in nature as it was supposed earlier. In 1930 Asahina and Asano[13] isolated a stilbene, "Hydrangeasäure" (III), from Hydrangea leaves. In 1939, Erdtman[14] obtained "pinosylvin" (IV) and its mono methyl ether (V) from silver pine wood while Kawamura[15] reported the isolation of the aglucon "Rhapontigenin" (VI) from rhubarb roots. In 1940 Takaoka[16] isolated "Resveratrol" (VII) and "Hydroxy-resveratrol" (VIII) from white hellebore.

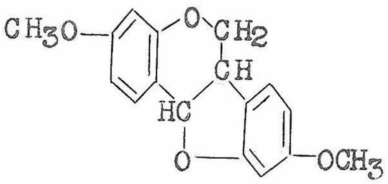
It can be seen (I - VIII) that most of these compounds are closely related, being mainly hydroxy or methoxy stilbenes. The chance isolation of stilbenes from these different classes of plants indicates that a more systematic search may be advisable.

Since we have shown (Chapter I) that chromatography can be of great use in the field of stilbenes, it was assumed that other stilbenes besides pterostilbene could be isolated from sandalwood by application of the brush method. Permanganate seemed to be a suitable reagent. However, due to National Defense Work it was necessary to discontinue this investigation prematurely. Thus the material presented is incomplete.

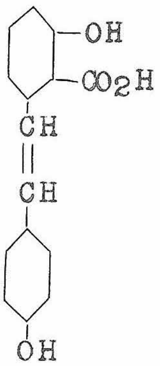
In the present investigation the isolation and chromatographic purification of homopterocarpin is described. Repeated crystallizations from methanol failed to remove all the impurities while a single chromatographic purification apparently gave a pure product. Furthermore, vanillin (HO)$(OCH_3)C_6H_3.CHO$, was isolated in small amounts by means of chromatography and identified as the 2,4-dinitrophenylhydrazone. Since vanillin frequently occurs in woods, it's isolation from sandalwood is not surprising. In addition, several other substances were obtained by chromatography, but crystallization attempts were unsuccessful in the short time available for this research.



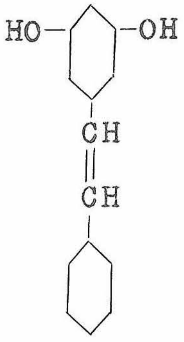
I. Pterostilbene



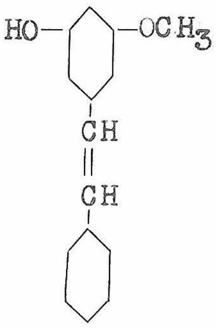
II. Homopterocarpin



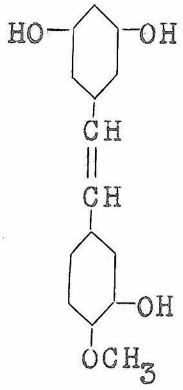
III. "Hydrangeasaure"



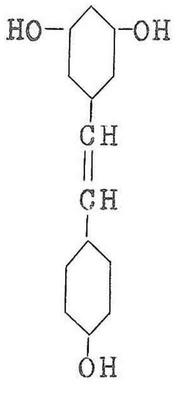
IV. Pinosylvin



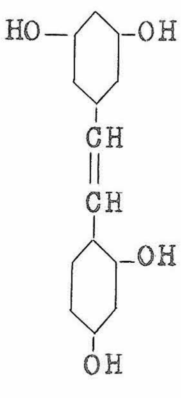
V. Pinosylvin-mono-methyl-ether



VI. Rhapontigenin



VII. Resveratrol



VIII. Hydroxy-resveratrol

Fig. III

Experimental

The red sandalwood was obtained from S. B. Penick and Company in New York. This was rasped "Red Saunders" of a U.S.P. grade.

In order to prepare the wood for the extraction, 775 g. was ground in an electric Wiley Mill (No.1) to 20 mesh size and then packed into a 2 liter percolator. The percolation was made continuous by placing the 2 liter receiving flask in a hot water bath and conducting the hot ether vapors through a side arm to the condensers above the percolator. The material was extracted with peroxide-free ether for 8 hours and then allowed to stand in contact with ether for 16 more hours. This procedure was continued for three days.

The first portions of the dark red extract showed a brilliant green fluorescence even in sunlight. After 36 hours the percolate coming down was nearly colorless although the wood still retained a red coloration.

The dark extract was concentrated in vacuo to about 200 ml. with the inlet connected to a carbon dioxide generator. The concentrate was decanted from red resins, which formed a coating on the flask walls, into 1 liter of carbon tetrachloride. A voluminous red precipitate of the pigment santalin intermixed with resins separated. In order to remove the latter more completely, the ether was distilled off. In addition the liquid was refluxed for 45 minutes to remove occluded colorless material from the precipitate. After cooling and filtering, the carbon-tetrachloride was removed by vacuum distillation. The tarry residue (25-30 g.) was taken up in 1 liter of ether and placed in a 2 liter Squibb separatory funnel for the following fractionation by means of alkali.

A preliminary extraction with weak alkali was carried out by allowing 1 liter of 0.5% aqueous KOH to drop through the ether solution. (Any agitation of the mixture would result in a bad emulsion). The dark aqueous extract was continuously drained from the bottom of the funnel directly into dilute hydrochloric acid in order to prevent oxidation of the phenolates. A tarry oil appeared; it was taken up in 800 ml. of benzene and dried over sodium sulfate (Fraction 1).

Following the initial weak alkali extraction a similar extraction was carried out with 450 ml. of 5% aqueous KOH. The last portion of the extract was nearly colorless. After acidifying as described above an oil appeared which was taken up in 1 liter of benzene and dried over sodium sulfate (Fraction II).

Fraction III was obtained from an additional extraction with a liter of 5% potassium hydroxide solution. The phenol- content was transferred to 1 liter of benzene by acidification. This fraction corresponds to that solution from which Späth isolated 3,5-dimethoxy-4'-hydroxy-stilbene by lengthy extractions.

Up to this point the procedure of Späth and Schläger[11] was followed except for some modifications.

<u>Isolation of homopterocarpin</u>.- The residual yellow ether solution containing a neutral portion (Fraction IV) was washed alkali free, dried, and evaporated completely in vacuo. Crystallization soon started in the viscous residue. The oily crystals were transferred to a small procelain mortar and rubbed with 75 ml. of cold methanol. Most of the oil went into solution while a voluminous crystalline precipitate appeared. The latter

was centrifuged and the liquid decanted. The crystals were stirred with 20 ml. of cold methanol in the same tube and centrifuged again. These operations were twice repeated. A yield of 7.0 g. of crude yellowish crystals was obtained (1% of the wood). The crystals contained some oil and were further purified by crystallization from methanol. Four and a half grams were stirred with 80 ml. of methanol for a few minutes at 45°. The solution was decanted from a little oil and filtered. On allowing the liquid to cool slowly, rosettes of very pale yellow needles formed. After standing in ice water for 1 hour, filtering, and drying, 2.03 g. of crystals were obtained. M.P. 81.5 - 83°. Microscopic examination revealed the presence of a few small plates among the predominating large needles.

Small scale experiments revealed that the material could be further purified by chromatography on activated alumina. Two hundred mg. were dissolved in 25 ml. of benzene and developed on a column (19.2 x 3.2 cm.) of "minus 80 mesh Alorco activated alumina" with 400 ml. of benzene. Since the adsorbent remained colorless and non-fluorescing, a 1% solution of permanganate was used as a brush reagent. In the following scheme the figures on the left denote the width of the zones (in mm.), while those on the right give the time (in min.), which is necessary for the permanganate streak to turn light brown:

17	nearly empty	15
20	by-product	3
15	" "	7
60	main zone	5
78	empty section	20

The main zone was eluted with 100 ml. of dry ether. On evaporation 165 mg. of colorless oil was obtained. This was dissolved in 5.5 ml. of methanol at 50°, sucked through a filter stick, and cooled in ice. Rosettes of colorless needles appeared. The crystals were collected in a micro centrifugal filtering vessel.[17] Since only 66 mg. was obtained, considerable material remained in the mother liquor. The melting point was 87.5-88.5° (corr.). The substance is very soluble in benzene or ether, moderately soluble in methanol, and insoluble in water and petroleum-ether (B.P. 60-70°). It has a saturated character, since it does not reduce permanganate at 20° in a dilute alcoholic solution. The properties and melting point of 88-89°[7] indicate that the compound is homopterocarpin (II).

<u>Anal.</u>

3.106 mg. subst.: 8.241 mg. CO_2; 1.619 mg. H_2O

4.887 mg. subst.: 10.34 ml. 0.02 N thiosulfate

Calcd.: $C_{17}H_{16}O_4$: C 71.80; H 5.68; OCH_3 21.82

Found: C 72.40; H 5.83; OCH_3 20.91

<u>Preliminary experiments on fractions I - III.</u>- In order to determine the composition of the first three fractions, small scale experiments were carried out. Fifteen ml. of the benzene solution from Fraction III was chromatographed on a column (17.5 x 1.7 cm.) of Neutrol Filtrol-Celite 535 (5:1) and developed with 60 ml. of benzene. No portion of the column fluoresced in ultra-violet light, and only a trace of visible maroon was noted near the top. On brushing with 1% permanganate the following zones were located:

3 turns the streak light brown rapidly (10-15 sec.)
18 " " " " " very slowly (15 min.)
8 " " " " " rapidly
14 " " " " " slowly (interzone)
17 " " " " " rapidly
115 " " " " " very slowly (empty)

One of the zones which rapidly decolorize permanganate should contain the stilbene of E. Späth and Schläger,[11] while the other two zones may contain additional stilbenes.

When a sample of Fraction II was developed on Neutrol Filtrol-Celite several red and blueish (visible) bands were formed. In addition, the permanganate streak revealed a broad zone which reacted in a few seconds with permanganate. This zone was probably heterogeneous since sections of the streak reacted at different rates.

Isolation of vanillin from red sandalwood.- An attempt was made to chromatograph directly the tarry residue from the carbon-tetrachloride evaporation before the alkali fractionation (see above). Two grams of this tar corresponding to 75 g. of wood was dissolved in 100 ml. of chloroform and, after dilution with 1 volume of benzene, developed on Neutrol Filtrol (27 x 7 cm.) with 1200 ml. of benzene containing 4% ethanol. Several colored zones appeared. On brushing with permanganate the following results were obtained:

22	maroon	(reacts rapidly with permanganate)
23	light red	(" " " ")
50	violet	(" " " ")
25	faint pink	(" " " ")
5	dark brown	(reacts instantaneously with permanganate)
4	light brown	(" " " ")
4	light blue	(" " " ")
147	colorless	(no reaction " ")

Many of these colors apparently are due to complex formation of phenols with the adsorbent. If the colorless compounds in the initial tarry residue are first filtered through calcium carbonate and the nearly colorless filtrate is rechromatographed on Neutrol Filtrol, the sequence of colored zones described above reappears.

After elution of the region around the 5 mm. zone with 300 ml. of alcohol-ether (1:1) and evaporation, 550 mg. of a light brown solid was obtained. A solution of 450 mg. of this material in 30 ml. of chloroform-benzene (1:1) was developed on Neutrol Filtrol (21 x 5.9 cm.) with 1600 ml. of benzene. The only visible zone was a 30 mm. dark brown one near the top, while 1% permanganate located the following zones:

16	(reacts instantly with permanganate)
31	(" slowly " ")
4	(" instantly " ")
160	(" very slowly)

The 16 mm. zone was eluted with 150 ml. of alcohol-ether (1:1) and yielded, after removal of the solvent in vacuo, a brown paste which slowly solidified. Crystallization attempts were unsuccessful.

Elution of the 4 mm.-zone with 120 ml. of alcohol-ether and evaporation yielded a yellowish oil with a strong odor of vanillin. In order to identify the latter, a solution of the oil in 6 ml. of 3 N.-hydrochloric acid was treated on a water bath with a warm solution of 50 mg. of 2,4-dinitrophenylhydrazine in 6 ml. of 3 N-hydrochloric acid.[18] The flocculent brick red precipitate was recrystallized from a hot dioxane-water solution (1:1) and yielded several milligrams of crystals. The m.p. was 260-262° (with decomposition) instead of 263-265°.

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Part IV

Model Experiments Directed Toward the Synthesis of Quinine

Summary

Part I

The chromatographic brush method, with permanganate as brush reagent, 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. Fluorescent contaminants can be removed by chromatography in ultraviolet light.

Part II

The chromatographic brush method, with an ammoniacal copper solution as color reagent, has been used for the detection and separation of cis- and trans-benzoin and anisoin oximes on Neutrol Filtrol columns. In a mixture composed of two stereoisomers, 1-2% of either form can be rapidly detected in the presence of the other. Some data concerning the interconversion of stereoisomeric oximes is given, and the influence of the adsorbent on the cis-trans shift is discussed.

Part III

A preliminary chromatographic investigation of some colorless constituents of red sandalwood has been carried out. The chromatographic isolation and purification of homopterocarpin and vanillin is described.

Part IV

Model experiments directed towards the synthesis of quinine have been carried out.

Propositions

Propositions

1. The catalysis of the isomerization of *cis*- to *trans*-ethylenes by different types of substances can be explained if two separate mechanisms are assumed to exist. One reaction path is followed in the presence of a substance such as BF_3 which is capable of combining with a pair of electrons and involves a carbonium ion intermediate. A different course is followed when the reaction is catalysed by a substance with an odd electron such as $Br^{\bullet}$ or $Na^{\cdot}$ and involves a free radical intermediate.

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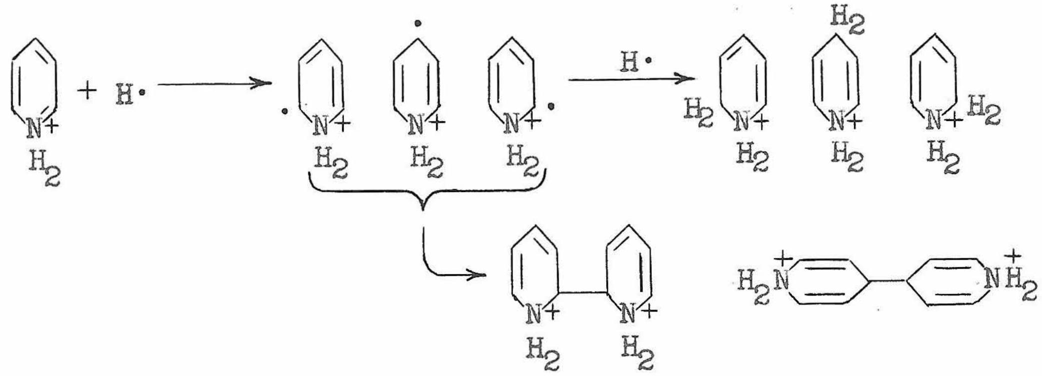
2. The electrolytic reduction of pyridine or alkyl substituted pyridines in an aqueous solution at low acid concentration results in the formation of large quantities of the corresponding α,α'- and γ,γ'-dipiperidyl. The reduction in an aqueous solution at high acid concentration gives good yields of the corresponding piperidine and only small amounts of dipiperidyls. These facts can be explained by a primary attack of atomic hydrogen on the nitrogen followed either by intermolecular condensation of the resulting free radical or by further reaction with atomic hydrogen. The dihydro-molecules are subsequently completely reduced.



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3. The ultraviolet irradiation of *trans*-p-methoxystilbene leads to a mixture containing more than 60% of the *cis*-isomer. A similar irradiation of *trans*-p-hydroxystilbene fails to give an appreciable quantity of of the *cis*-isomer. These facts can be explained by an isomerization mechanism involving $C_6H_5\text{-}CH_2\text{-}CH = C_6H_4 = O$ which is a tautomer of p-hydroxystilbene.

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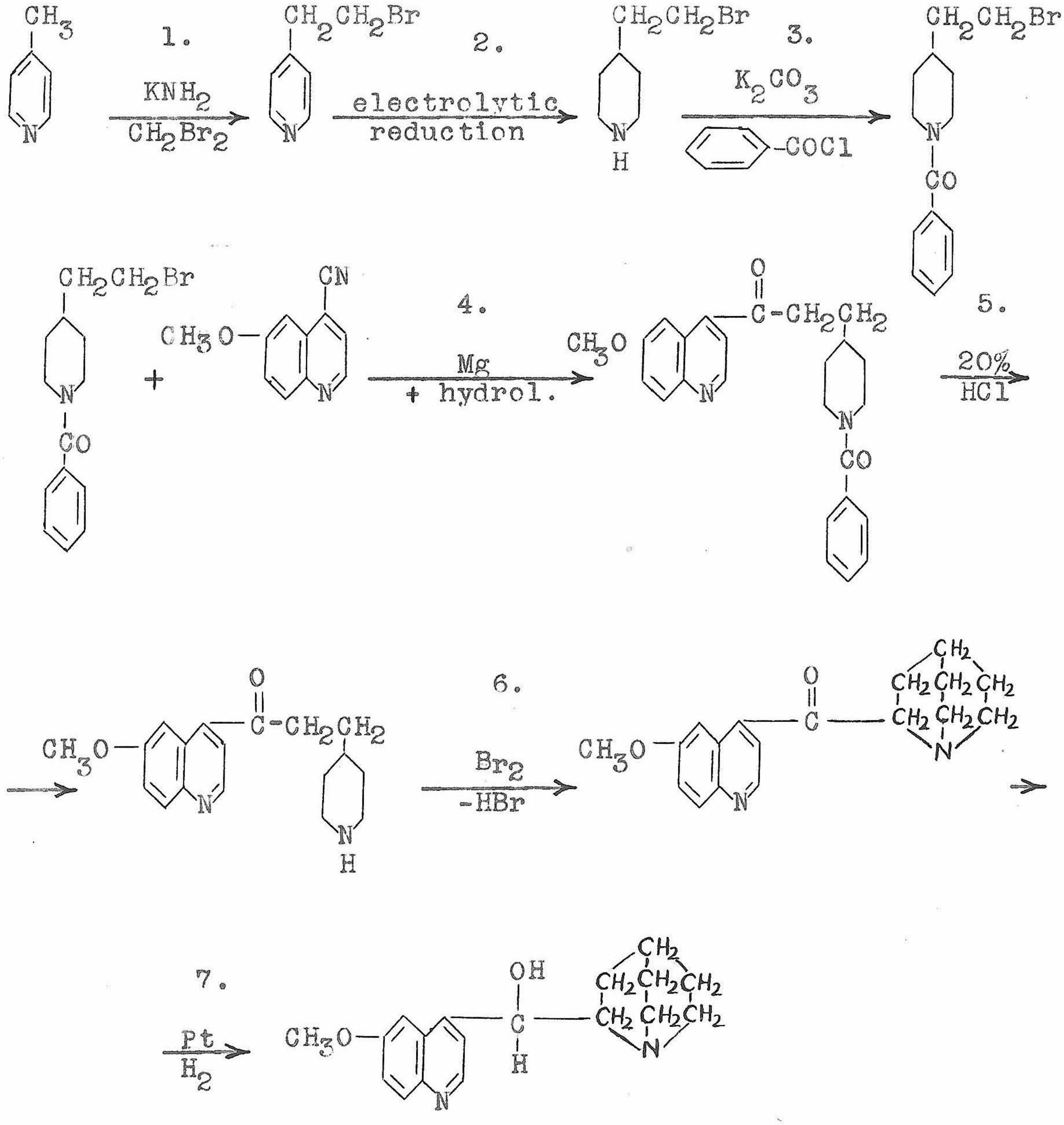
4. The following synthesis of 6'-methoxy-9-hydroxy-rubane is proposed:



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5. α and γ picolines react with the alkali amides to form colored salts. These salts can then be treated with alkyl halides to split out the alkali halide and lengthen the pyridine side chain.

Chichibabin has recently attempted to generalize this reaction to include all alkyl groups. However, he was unable to obtain satisfactory yields with compounds containing an active halogen. I propose that this reaction can be made a preparative method with such active halides under the proper conditions.

The failure to obtain the expected product with the group $XCHRCO_2C_2H_5$ is due to an anomolous reaction of the α ester group and not to the active halogen.

References:

A. E. Chichibabin, Bull. soc. chim., (5), 5, 436 (1938).

F. W. Bergstrom and W. C. Fernelius, Chem. Rev., 20, 413 (1937).

6. Recent evidence indicates that phenolic stilbenes may be quite widespread in plants. I propose that they may have fungicidal and insecticidal properties and thus aid in wood preservation.

The similarity in structure of these natural stilbenes isolated from different sources indicates a common biosynthesis.

References:

E. Späth and J. Schläger, Ber., 73, 881 (1940).

H. Erdtman, Ann., 539, 120 (1939).

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7. A systematic application of the chromatographic brush method to the isolation of colorless substances from natural products such as sandalwood and the conifers is desirable.

8. A commercial charging device designed to lengthen the life of radio "B" batteries was developed a few years ago. Since this recharging only lengthened the life of the dry cells by a factor of two or three times, it has been proposed that this "recharging" merely makes available part of the large amount of unused material in the cell.

I propose that under the proper conditions this is a "semireversible" process with an ampere hour efficiency of about 90%. The failure of a new dry cell to use more than 20% of the available material may be due to the formation of a coating of manganic hydroxide over the active manganese dioxide particles.

9. Discharged flashlight dry cells when not corroded or dried out can be recharged to give a second discharge substantially equivalent to the initial discharge. The current scarcity of flashlight batteries could be alleviated by recharging the "dead" cells. This would result in the saving of large quantities of zinc and manganese. The cost of the electricity used in the recharging is 0.01 cent per cell.

10. The installation of a few large pieces of equipment such as a 20 gallon glass-lined steam-jacketed kettle, a lead-lined reaction vessel, and "Lightnin" mixers would aid considerably in the preparation of synthetic chemicals in the organic chemistry department.