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## Chromatography of *cis* and *trans* Benzoin and Anisoin Oximes with Application of the Brush Method

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It was shown recently that the brushing of a chromatographic column with a suitable color reagent<sup>1</sup> can be used for the detection, separation and estimation of *cis*- and *trans*-stilbenes.<sup>2</sup> This was made possible by the differences in adsorption affinities of stereoisomers which have been observed in various classes of compounds.<sup>3</sup> The present paper describes the application of the same principle to an investigation of the chromatographic behavior of some *cis*- and *trans*-oximes. According to Feigl,<sup>4</sup> a solution of *trans*-benzoin oxime ( $\alpha$  or *anti*),  $C_6H_5CHOHC(:NOH)C_6H_5$ , gives a stable deep-green complex when treated with ammoniacal copper sulfate, while, according to Meisenheimer and Theilacker,<sup>5</sup> the complex obtained with the *cis*-isomer ( $\beta$ , *syn*) is brown. It is this behavior which has been made a basis for the application of the brush method. If two stereoisomeric oximes capable of complex formation are present in a 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 found, free of substance or containing only very little; no such interzone appears on any other adsorbent tested, including Floridin.<sup>6</sup>

The behavior of the two anisoin oximes,  $CH_3O-C_6H_4CHOHC(:NOH)C_6H_4OCH_3$  is analogous.

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

(2) L. Zechmeister and W. H. McNeely, *THIS JOURNAL*, **64**, 1919 (1942).

(3) A. Winterstein and G. Stein, *Z. physiol. Chem.*, **220**, 247 (1933); A. H. Cook, *J. Chem. Soc.*, 876 (1938); A. H. Cook and D. G. Jones, *ibid.*, 1309 (1939); A. H. Cook, D. G. Jones and J. B. Polya, *ibid.*, 1315 (1939); L. Zechmeister, O. Frehden and P. Fischer Jørgensen, *Naturwiss.*, **26**, 495 (1938); L. Zechmeister and P. Tuzson, *Ber.*, **72**, 1340 (1939); L. Zechmeister, L. Cholnoky and A. Polgár, *ibid.*, **72**, 1678 and 2039 (1939); L. Zechmeister, A. L. Le Rosen, F. W. Went and L. Pauling, *Proc. Nat. Acad. Sciences*, **27**, 468 (1941); H. H. Strain, *THIS JOURNAL*, **63**, 3448 (1941); H. H. Strain, "Leaf Xanthophylls," Carnegie Inst. of Washington, No. 490 (1938).

(4) F. Feigl, *Ber.*, **56**, 2083 (1923); F. Feigl, G. Sicher and O. Singer, *Ber.*, **56**, 2294 (1925).

(5) J. Meisenheimer and W. Theilacker, in K. Freudenberg's "Stereochemie," F. Deuticke, Leipzig, 1933, p. 1019.

(6) A series of experiments with Floridin has been described by G. Solyom in his Thesis (Univ. Pécs, 1940). This adsorbent has been now abandoned as the use of Neutrol Filtrol is far more satisfactory. The earlier experiments were carried out in collaboration with Dr. O. Frehden.

If 40–200 mg. of a mixture of two stereoisomers is analyzed by the procedure described, 1–2% of either form can be detected. This allows a study of their interconversion under the influence of different factors. It was known that *cis*-benzoin oxime is isomerized by refluxing an alcoholic solution,<sup>7</sup> but our chromatographic experiments demonstrate that the *trans*-compound also isomerizes to a slight extent.

The analytical applicability is somewhat limited by the 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 migration of the *cis*-oxime and is retained near the place of the conversion. In such a case the brush streak does not remain 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.

Under the conditions applied, the "column isomerization" amounts to less than 5% of the original quantity of *cis*-benzoin oxime and less than 1% of *cis*-anisoin oxime.

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### Experimental

Neutrol Filtrol (Filtrol Corp., Los Angeles) was used as an adsorbent. A trace of oil was removed either by hot extractions with benzene and then acetone, or by numerous extractions with benzene and alcohol-ether (1:1) at room temperature, with mechanical shaking. The adsorbent was dried at 90° for four hours. These operations increase the adsorptive power, but decrease the rate of filtration. Therefore, 17% Celite (no. 535) or Hyflo Super-Cel (Johns-Manville Co.) was used as a filter aid.

The color reagent was prepared by diluting 0.15 mole of copper sulfate and 4 moles of ammonia to one liter. The

(7) A. Werner, *Ber.*, **23**, 2333 (1890).

melting points are corrected and were taken in an electrically heated Berl block.

*cis*- and *trans*-Benzoin Oxime.—The oximes were prepared according to the data of Werner and Detschiff.<sup>8</sup> In repeated experiments, however, we were unable to obtain chromatographically homogeneous samples. Adsorption analysis revealed the presence of, for example, 1% of the *cis*-isomer in the *trans*-compound and 30% or more of the *trans*-isomer in the *cis*-compound. The latter when purified in this way showed the m. p. 99° as given by the authors mentioned. The *trans*-compound melted at 151°.

In order to purify *cis*-benzoin oxime, a solution of 1 g. in 100 ml. of a chloroform-benzene mixture (1:1) was poured on a column (19 × 3.2 cm.) and developed within thirty min. with 160 ml. of benzene containing 2% alcohol. After brushing with the reagent, the presence of the following zones was revealed (the colors given refer to the streak,<sup>9</sup> the figures on the left denote the width of the zones in millimeters)

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 alcohol-ether (1:1) and the *cis*-fraction with the same volume of dry ether. The adsorbent was removed on a sintered glass funnel. Evaporation and analytical weighing 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 ethanol. The oily *cis* fraction was dissolved in a few ml. of dry ether and evaporated in a carbon dioxide current at 25°. On scratching, it crystallized out (m. p. 99–99.5°).

*Anal.* Calcd. for C<sub>14</sub>H<sub>13</sub>O<sub>2</sub>N: N, 6.17. Found: (*cis*) N, 6.38. Found: (*trans*) N, 6.15.

(a) An artificial mixture of equal parts (75 mg.) of *cis*- and *trans*-benzoin oxime was dissolved in 20 ml. of chloroform-benzene (2:1) and developed with 40 ml. of benzene containing 2% ethanol on a column (17.5 × 1.7 cm.) within twenty min.

2 sky blue (empty top section)  
17 dark green (*trans*-benzoin oxime)  
42 blue (trace 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 *trans*-zone, 40 ml. of the same mixture for the interzone and 40 ml. of 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.

(b) The limits of detection were established by experiments of the type (a). In the presence of 40 mg. of *trans*-benzoin oxime 0.4 mg. of the *cis*-isomer can be detected by washing with 12 ml. of benzene plus 2% ethanol (column: 10.5 × 0.9 cm.). In the presence of 100 mg. of *cis*-, 2 mg. of the *trans*-compound was detected on a larger column

(8) A. Werner and Th. Detschiff, *Ber.*, **38**, 69 (1905); *cf.* L. Malatesta, *Gazz. chim. ital.*, **68**, 319 (1938).

(9) In some cases the *cis*-zone showed a pale yellow color before brushing. This color darkens in air.

(17.5 × 1.7 cm.). Each experiment required twenty to twenty-five minutes.

(c) A solution of 50 mg. of chromatographed *cis*-benzoin oxime was refluxed in 25 ml. of alcohol for two hours, evaporated and taken up in 10 ml. of chloroform-benzene (1:1). The adsorption analysis showed two equally broad oxime zones from which 11.2 mg. of the *trans*- and 29.3 mg. of unchanged *cis*-compound were isolated in pure state. A similar experiment with *trans*-benzoin oxime showed only 1% isomerization.

(d) Two 125-mg. samples of chromatographically pure *cis*-benzoin oxime were dissolved in 10 ml. of chloroform-benzene (1:1). Each solution was developed on a column (17.5 × 1.7 cm.) with 50 ml. of benzene containing 2% alcohol. The development in one experiment was completed in twenty-two minutes using full suction. The development of the other column, carried out with very little suction, required three and one-fourth hours. In the first case the chromatogram showed a 66-mm. section (above the unchanged *cis* zone) which gave a very weak reaction for *trans* and contained 5.9 mg. of this isomer; 114.4 mg. of *cis*-compound was recovered.

In the slow experiment the 67-mm. upper zone gave a definite brush reaction for the *trans*-oxime and yielded 34.6 mg. of this compound formed by isomerization; 82.3 mg. of the starting material was recovered unchanged. The extent of isomerization during chromatography was 5 and 28%, respectively, in the rapid and slow experiment.

*cis*- and *trans*-Anisoïn Oxime.—Anisaldehyde (Eastman Kodak Co.) was fractioned *in vacuo*; it was then condensed to anisoïn in the presence of potassium cyanide.<sup>10</sup> In spite of the correct melting point (112–113°) of the recrystallized product it proved to be chromatographically heterogeneous. For purification, 20 g. was dissolved in 0.8 liter of chloroform-benzene (1:1) and developed on a column (27 × 7 cm.) with 2.5 liters of benzene containing 0.5% ethanol. On brushing with 1% permanganate the anisoïn was located in a 90-mm. zone near the top while a minor compound adsorbed below was discarded. The anisoïn was eluted with 2 liters of alcohol-ether (10:1) and the solution concentrated in a carbon dioxide stream to 50 ml. The product was recrystallized from alcohol (17 g., m. p. 112–113°).

*Anal.* Calcd. for C<sub>10</sub>H<sub>10</sub>O<sub>4</sub>: OCH<sub>3</sub>, 22.79. Found: OCH<sub>3</sub>, 22.68.

The oxidation carried out as described by Werner and Detschiff<sup>8</sup> for benzoin oxime gave a mixture of both stereoisomers. For example, 2.3 g. of aqueous hydroxylamine hydrochloride (neutralized against litmus) was added to 4.5 g. of anisoïn in 20 ml. of ethanol. After one and one-half hours of refluxing, the liquid was diluted with 150 ml. of water and allowed to stand overnight. The oil was dissolved in 300 ml. of benzene, dried with sodium sulfate, developed with 500 ml. of benzene containing 3% alcohol on a column (22.5 × 4.3 cm.) and brushed<sup>9</sup>

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

(10) M. Böslér, *Ber.*, **14**, 323 (1881); R. Stierlin, *ibid.*, **22**, 376 (1889).

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

The *trans*-oxime 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 *cis*-compound came out in the form of crystals which contained ether. These were dissolved in 6 ml. of acetone at room temperature and rapidly evaporated with a carbon dioxide stream at 25°. The oily residue was evaporated with 2 ml. of benzene, dissolved in 10 ml. of cold benzene and scratched. Thin, elongated, colorless prisms appeared (1.2 g., m. p. 121-122°).

*Anal.* Calcd. for  $C_{16}H_{17}O_4N$ : N, 4.88. Found: (*cis*) N, 5.08; (*trans*) N, 4.99.

The difference in the melting points of the two isomers is remarkably small. The configurations, however, are given by the location on the column and by the color reactions. Chromatography showed the presence of less than 1% of the *cis*-compound in the *trans*-sample and *vice versa*.

(a) A mixture of 75 mg. of each isomer was dissolved in 35 ml. of benzene by slight warming, developed on a column (14 × 1.7 cm.) with 50 ml. of benzene containing 3% abs. alcohol, and brushed:

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

After elution with 55 ml. of the solvent mentioned, 69.2 mg. of *trans*-oxime (m. p. 125-125.5°, after crystallization from cold benzene) and 68.3 mg. of *cis*-oxime (m. p. 122.5-123.5°, after crystallization) were isolated, the total recovery being 92%. The interzone yielded 0.8 mg. of the *trans*-compound.

(b) It was found in similar adsorption experiments that 1.6 mg. of *cis*-anisoin oxime can be detected in the presence of 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 0.5-1%.

(c) A solution of 140 mg. of *cis*-anisoin oxime in 40 ml. of ethyl alcohol was refluxed for one and one-half hours. After removing the solvent and chromatographing as described in expt. (a), a 6-mm. zone was located above the main zone by brushing. This contained 11.5 mg. (8%) of the *trans*-compound, formed by isomerization. A parallel experiment carried out with *trans*-anisoin oxime yielded 2.5% of formed *cis*-isomer.

(d) A solution of 125 mg. of pure *cis*-anisoin oxime in 30 ml. of benzene was developed in three and one-fourth hours on a column (17.5 × 1 cm.) with 50 ml. of benzene containing 3% absolute alcohol. From the interzone 13.7 mg. of the *trans*-isomer was isolated. The extent of the "column isomerization" amounted to 11% in this case while the corresponding figure for a short experiment is about 1%.

### Summary

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 are given, and the influence of the adsorbent on the *cis-trans* shift is discussed.

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## The Configuration of Organic Coordination Compounds of Nickel, with Especial Reference to Bis-formylcamphor-ethylenediamine-nickel

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Evidence is accumulating from various groups of investigators which may ultimately furnish an answer to the question implied in Pauling's<sup>2</sup> statement that "factors which determine whether the diamagnetic square or the paramagnetic tetrahedral configurations will be assumed by a nickel complex cannot be stated precisely." The present paper seeks to add to the evidence results of three different types: first, the magnetic susceptibilities of nine compounds; second, the absorption spectra of four of these compounds; and third, the

*relation between* the adsorption spectrum and rotatory dispersion of a single one of the compounds.

**Magnetic Susceptibility.**—Determination of magnetic susceptibility is probably the most generally used method for the study of the configuration of the quadricovalent nickel complexes, since diamagnetism is associated with the square coplanar configuration and no unpaired electrons, while paramagnetism is associated with the tetrahedral configuration and two unpaired electrons. Pauling<sup>3</sup> has given the most satisfactory explanation for this change in the number

(1) Taken in part from the M.A. Thesis of M. Z. Magee and from the Honors Thesis of E. Sheffield.

(2) Pauling, "Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1939, p. 112.

(3) Pauling, *ibid.*, p. 111.