[CONTRIBUTION NO. 1838 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

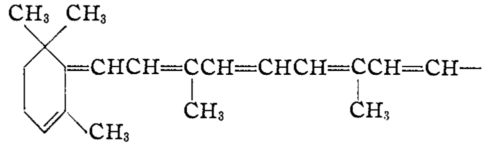
A Study of Some *cis–trans* Isomeric Dehydro-β-carotenes

BY L. ZECHMEISTER AND L. WALLCAVE

RECEIVED MAY 25, 1953

Dehydro-β-carotene samples obtained from β-carotene constitute mixtures of stereoisomers from which two main crystalline *cis* forms were isolated. The interconversion of members of this stereoisomeric set was studied by thermic, photochemical and catalytic methods. The mixtures thus obtained contain less of the all-*trans* compound than in the case of carotenes. One of the observed *cis* dehydro-β-carotenes showed a degraded spectrum. No distinct *cis*-peak phenomena were observed in this "retro" system.

Dehydro-β-carotene, $C_{40}H_{54}$, first isolated under the name of "isocarotene" by Kuhn and Lederer[1] has, according to Karrer *et al.*,[2] the constitution indicated by the formula below. This hydrocarbon represents a "retro" type of structure[3] containing double bonds which connect the rings with the main aliphatic chain, and a single bond in the center of the chromophore.



$^1/_2$ dehydro-β-carotene

The number of "unhindered" stereoisomers formed by rotation around the four "unhindered," purely aliphatic double bonds is 10; however, in case the two double bonds linked to the cyclohexene rings should also undergo spatial rearrangements, this number would increase to 36. The number of all possible *cis–trans* forms, hindered and unhindered,[4,5] would then be 2080.

We found that samples obtained following Kuhn and Lederer's directions[1] are chromatographically heterogeneous and contain, in addition to the all-*trans* compound and several minor isomers, two main crystallizable *cis* forms, termed neo-A and -D, whose combined amount exceeds by far that of the all-*trans* isomer. While the authors just mentioned gave the m.p. 180–181°, our once-recrystallized dehydro-β-carotene preparations melted somewhere between 170 and 180°. As shown in Table I, pure all-*trans*-dehydro-β-carotene, isolated by chromatography from such mixtures, melts at 192–193° in accordance with a m.p. reported by Karrer.[2]

The spectral maxima of the stereoisomers observed in the present study are listed in Table II. Since, according to earlier observations,[5] one *trans* → *cis* rearrangement shifts the extinction maxima (in hexane) by 3 to 5 mμ toward shorter wave lengths, we propose that neo-dehydro-β-carotene-A is a mono-*cis* and neo-D a di-*cis* compound. The minor isomers listed in Table II represent, respectively, di-, tri- and perhaps tetra-*cis* configurations (for neo-J see below).

TABLE I

SOME CONSTANTS OF STEREOISOMERIC DEHYDRO-β-CAROTENES, $C_{40}H_{54}$

Stereoisomer ⟶		All-*trans*	Neo-A	Neo-D
M.p., °C.		192–193	161	177–178
Visually-observed spectral max. in hexane, mμ		504, 474	500, 470	497, 467
$E^{mol}_{1cm} \times 10^{-4}$ at γ_{max} (Beckman), mμ)		16.6 (471)	16.0 (467)	15.6 (464)
Carbon, %	Calcd.	89.83	89.83	89.83
	Found	89.91	89.70	89.66
Hydrogen, %	Calcd.	10.17	10.17	10.17
	Found	9.95	10.22	10.21

TABLE II

SPECTRAL CHARACTERISTICS OF STEREOISOMERIC DEHYDRO-β-CAROTENES LISTED IN THE SEQUENCE OF DECREASING ADSORPTION AFFINITIES (BECKMAN)

Stereoisomer	Maxima in hexane, mμ	$E^{mol}_{1cm} \times 10^{-4}$ at λ_{max}
All-*trans*	502, *471*, 445	16.6
Neo-A	497, *467*, 442	16.0
Neo-B	494, *463*, 439	15.5
Neo-C	491, *461*, 436	15.5
Neo-D	494, *464*, 440	15.6
Neo-E	487, *457*, 433	14.2
Neo-F	489, *459*, 434	14.5
Neo-G	482, *453*, 430	13.4
Neo-H	479, *452*, 429	11.3
Neo-J	*449*	10.3

When compared with some well-known carotenoids, the dehydro-β-carotenes occupy the following relative positions on the lime–Celite column: lycopene (top); all-*trans*-dehydro-β-carotene; its *cis* isomers A–J; and β-carotene. Stereoisomers adsorbed above the all-*trans* compounds ("neo-β-carotene U type"[6]) did not appear in our chromatograms. All the dehydro-β-carotenes observed are very strongly retained on activated alumina and require benzene–hexane mixtures (with at least 50% benzene) for satisfactory migration.

The respective configurations of all-*trans*-dehydro-β-carotene and of the two crystalline *cis* forms show very similar stabilities when submitted to refluxing, melting or illumination under the conditions specified below. While the carotenes retain about half of their molecules in the all-*trans* configuration when catalyzed with iodine (in light), the corresponding figure in the dehydro-β-carotene set is as low as 20%, *i.e.*, much lower than the sum of the neo forms A and D (50%).

---

[1] R. Kuhn and E. Lederer, *Ber.*, **65**, 637 (1932).

[2] P. Karrer, K. Schöpp and R. Morf, *Helv. Chim. Acta*, **15**, 1158 (1932); P. Karrer and G. Schwab, *ibid.*, **23**, 578 (1940).

[3] W. Oroshnik, G. Karmas and A. D. Mebane, THIS JOURNAL, **74**, 295 (1952).

[4] L. Pauling, *Fortschr. Chem. organ. Naturstoffe*, **3**, 203 (1939).

[5] L. Zechmeister, *Chem. Revs.*, **34**, 267 (1944).

[6] A. Polgár and L. Zechmeister, THIS JOURNAL, **64**, 1856 (1942).

In spite of this preponderance of *cis* configuration in some stereoisomeric quasi-equilibria the extinction curves are remarkably little affected by iodine catalysis or refluxing (Figs. 1–3). A rather surprising spectroscopic feature is the absence of a definite *cis*-peak. This phenomenon is to be explored further and might be (in part) connected with the "retro" structure. The appearance of very high *cis*-peaks was not expected *a priori* in this set, since the central double bond (usually responsible for the highest peak of this type) is missing and, furthermore, the two double bonds located nearest the center are of the spatially "hindered" type.

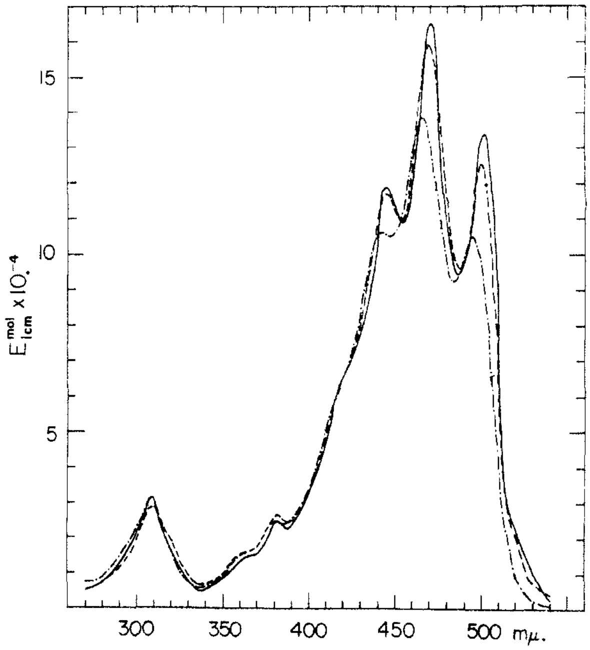


Fig. 1.—All-*trans*-dehydro-β-carotene in hexane: —, fresh solution; – – –, after refluxing 1 hr.; – · – ·, after iodine catalysis, in light.

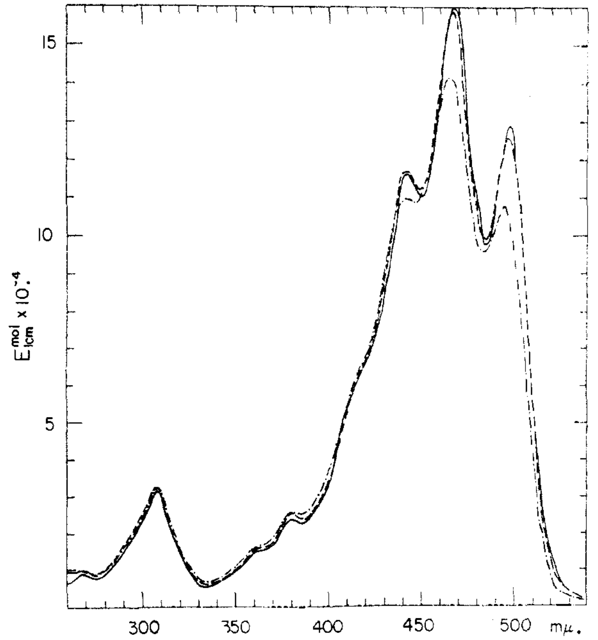


Fig. 2.—Neo-dehydro-β-carotene A in hexane: —, fresh solution; – – –, after refluxing 1 hr.; – · – ·, after iodine catalysis, in light.

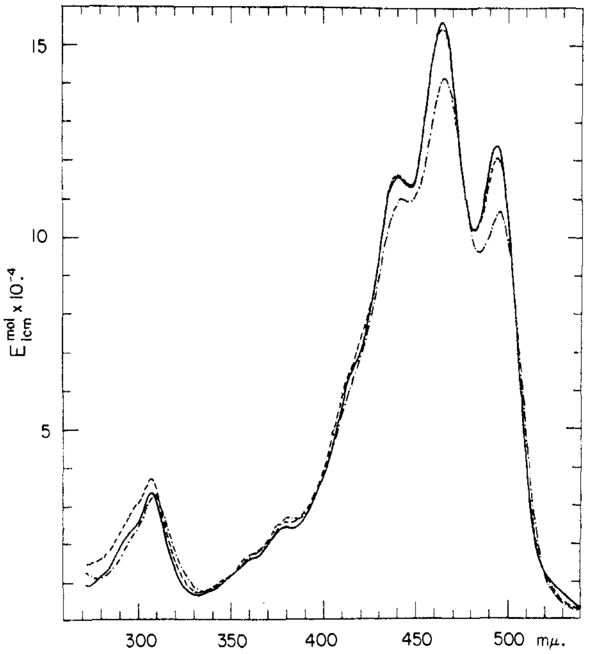


Fig. 3.—Neo-dehydro-β-carotene D in hexane: —, fresh solution; – – –, after refluxing 1 hr.; – · – ·, after iodine catalysis, in light.

A remarkable minor stereoisomer, termed neo-dehydro-β-carotene J, should be mentioned here. So far it has been obtained only by melting crystals of the all-*trans* or *cis* forms. It shows a degraded spectrum lacking fine structure. Fine structure does appear, however, upon iodine catalysis (Fig. 4) and at the same time the color of the solution,

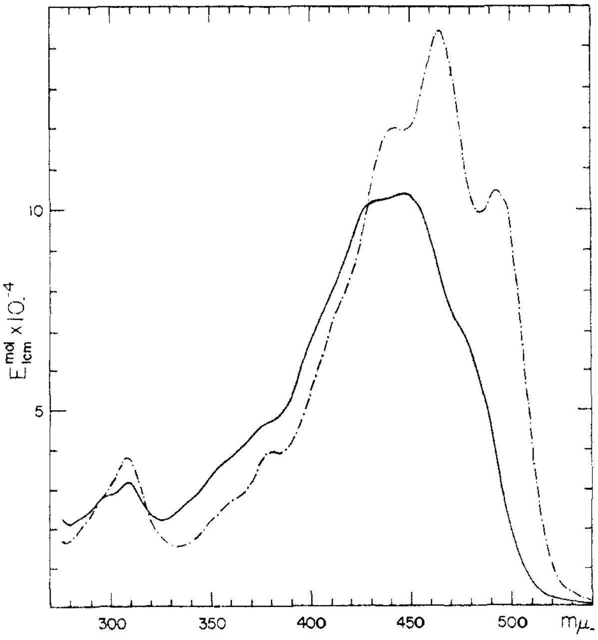


Fig. 4.—Neo-dehydro-β-carotene J in hexane (obtained by fusion of neo-A crystals): —, fresh solution; – · – ·, after iodine catalysis, in light.

initially yellow, turns intense orange-red. This phenomenon is analogous to that observed upon a similar treatment of prolycopene.[7] It can be interpreted by the assumption of a poly-*cis* structure and/or by the presence of at least one sterically hindered *cis* double bond.[3,8]

Experimental

Materials and Methods.—The term "lime–Celite" refers to a 2:1 mixture of Sierra Hydrated Lime, Superfine (U. S. Lime Products Corp., Los Angeles, Calif.) and Celite, No. 545 (Johns–Manville Co.). The alumina used was activated alumina, grade F, reground to 200 mesh (Aluminum Ore Co., E. St. Louis, Ill.). In general, elutions were carried out with acetone; however, in the case of all-*trans*-dehydro-β-carotene a 9:1 acetone–methanol mixture is preferable. Washing of solutions was carried out in a continuous apparatus.[9] Melting points (cor.) were taken in an electrically heated Berl block, in evacuated capillaries.

Preparation of Dehydro-β-carotene from β-Carotene.—A solution (cooled to -10°) of 800 mg. of the carotene in 1 l. of hexane was poured into a mechanically stirred solution (at -10°) of 800 mg. of iodine in 500 ml. of hexane, in the course of 1 min. After 2 more min. of stirring, the black precipitate was filtered, dissolved in 2 l. of acetone, kept at room temperature for 20 min., and then shaken vigorously with a solution of 5 g. of sodium thiosulfate in 250 ml. of water. After the addition of 400 ml. of hexane, the pigment mixture was transferred into the upper phase by the addition of water. After discarding the hypophase, the hexane solution was washed, dried, concentrated *in vacuo* to 200 ml., adsorbed on two 27×8 cm. lime–Celite columns, and developed with hexane + 6% acetone (the figures on the left represent the width of zones in mm.)

14 brownish and yellow minor zones
15 interzone
60 orange red: all-*trans*-dehydro-β-carotene
2 interzone
65 reddish-orange: neo-dehydro-β-carotene A
2 interzone
50 orange: neo-D
55 several minor zones and interzones
Filtrate: 300 mg. of unchanged β-carotene (and other substances)

Each of the three main zones was cut out, eluted, transferred to hexane and evaporated completely. Each crystalline residue was dissolved in about 2 ml. of warm chloroform, transferred to a centrifuge tube and evaporated again by means of a dry nitrogen stream (bath, 50°). The residue was dissolved in a minimum volume of benzene (bath, 50°) and the product crystallized out by the gradual addition of 4–5 vol. of methanol at 50°. After standing at room temperature for 1 hr., the crystals were centrifuged, washed with methanol (20°) and dried in an Abderhalden apparatus at 56° for 1 hr.; yields, 28 mg. of all-*trans*-dehydro-β-carotene, 48 mg. of neo-A, and 18 mg. of neo-D.

In analytical experiments similar in principle to that just described but including a crystallization of the crude product before chromatography, the following ratios were photometrically established in the mixture: all-*trans*-dehydro-β-carotene:neo-A:neo-D:a minor *cis* form:unreacted β-carotene:material unaccounted for = 16:15:10:0.5:46:13.

cis–trans **Isomerization by Refluxing.**—About 10 mg. of substance dissolved in 200 ml. of hexane was refluxed in an all-glass apparatus, in darkness, for 1 hr., then adsorbed on a 25×5.8 cm. column and developed with hexane + 6% acetone until all pigment zones were clearly separated by interzones. The individual stereoisomers were estimated photometrically. For this purpose each minor isomer whose molecular extinction coefficient was unknown was converted into the iodine equilibrium mixture and the molecular extinction value at $\lambda_{\max}$ of the latter (as established starting from crystalline stereoisomers) was used, *viz.*, 1.40×10^4 at 464–465 mμ, in hexane.

Table III demonstrates that no equilibrium was reached under the conditions applied.

TABLE III

COMPOSITION OF STEREOISOMERIC MIXTURES OBTAINED BY REFLUXING DEHYDRO-β-CAROTENES IN DARKNESS (% OF STARTING MATERIAL)

	Starting material		
	All-*trans*	Neo-A	Neo-D
All-*trans*	53	5	2
Neo-A	24	50	13
Neo-D	4	16	70
Neo-E	2	10	8
Minor isomers	1	6	1
Unaccounted for	17	13	7

cis–trans **Isomerization by Melting Crystals.**—About 15 mg. of all-*trans* crystals sealed *in vacuo* in a Pyrex tube (diam., 6–7 mm.) were kept in a bath at 200° for 90 sec., then cooled in ice-water. The material was dissolved in 2–3 ml. of benzene. After dilution with hexane to 50 ml. it was adsorbed on a 24×4.8 cm. column (developer, hexane + 6% acetone)

5 yellow and brownish
20 interzone
18 orange red: all-*trans*
2 interzone
15 reddish orange: neo-A
1 yellow: neo-B
3 interzone
12 yellow-orange: neo-C
4 orange-pink: neo-D
1 interzone
5 pale orange: neo-F
4 interzone
8 very pale yellow: neo-G
5 interzone
8 very pale buff: neo-J

The following ratios, measured photometrically, show that about half of the material was present in the form of stereoisomeric dehydro-β-carotenes. All-*trans:* neo-A:neo-B:neo-C:neo-D:neo-E:neo-F:neo-G:neo-H:neo-J: unaccounted for = 8:14:2:12:12:3:1:1:0:1:46. Very similar ratios were obtained by melting the neo-A and neo-D isomers. Although the neo-A form has a substantially lower melting point than has the all-*trans* compound, it was found that in this case also a bath temperature of 200° was required for the formation of the neo-J form. Fusion of neo-A at 170° did not produce any neo-J form.

Photochemical *cis-trans* **Isomerization.**—A solution of 2 mg. of pigment in 25 ml. of hexane contained in a Pyrex volumetric flask was exposed to light from two parallel 120-cm. fluorescent (3500°) Mazda lamps, white and yellow, from a distance of 30 cm. for 8 hr. The ratios, all-*trans:* neo-A:neo-D:unaccounted for, were as follows: (a) starting from the all-*trans* form: 84:8:2:6; (b) from neo-A: 5:86:3:6; and (c) from neo-D: 1:6:91:2.

cis-trans **Isomerization by Iodine Catalysis, in Light.**—A solution of about 12 mg. of substance in 100 ml. of hexane was catalyzed by the addition of 0.1 mg. of iodine in 1 ml. of the same solvent, illuminated for 30 min. as described above (distance, 60 cm.) and then chromatographed (column, 25×5.8 cm., developer, hexane + 8% acetone)

1 yellow-brown: unidentified
45 interzone
28 orange-red: all-*trans*
4 interzone
30 reddish-orange: neo-A
2 yellow: neo-B
2 interzone
20 yellow-orange: neo-C
10 pinkish orange: neo-D
4 interzone
8 yellow: neo-E
1 interzone
6 orange: neo-F
3 interzone
7 pale yellow: neo-G
3 interzone
6 very pale orange: neo-H

---

[7] L. Zechmeister, A. L. LeRosen, A. W. Schroeder, A. Polgár and L. Pauling, THIS JOURNAL, **65**, 1940 (1943).

[8] G. F. Garbers, C. H. Eugster and P. Karrer, *Helv. Chim. Acta*, **35**, 1850 (1952).

[9] A. L. LeRosen, *Ind. Eng. Chem., Anal. Ed.*, **14**, 165 (1942).

The neo zones C + D were cut out jointly, and their separation was achieved by developing with a 3:1 benzene–hexane mixture on alumina (the original sequence was then inverted).

The photochemically established ratios appear in Table IV.

TABLE IV

COMPOSITION OF STEREOISOMERIC MIXTURES OBTAINED FROM DEHYDRO-β-CAROTENES BY IODINE CATALYSIS, IN LIGHT (% OF STARTING MATERIAL)

	Starting material		
	All-*trans*	Neo-A	Neo-D
All-*trans*	17	18	17
Neo-A	28	30	30
Neo-B	4	5	3
Neo-C	10	11	10
Neo-D	12	15	15
Neo-E	4	6	5
Neo-F	6	6	3
Neo-G	3	2	1
Neo-H	1	1	1
Unaccounted for	15	7	16

Acknowledgment.—The authors wish to thank Dr. A. J. Haagen-Smit and Mr. G. Swinehart for microanalyses.

PASADENA, CALIFORNIA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, JOSEPH E. SEAGRAM AND SONS, INC.]

The Chromatographic Separation of Hardwood Extractive Components Giving Color Reactions with Phloroglucinol

BY R. A. BLACK, A. A. ROSEN AND S. L. ADAMS

RECEIVED MAY 11, 1953

By application of paper chromatography, four aromatic aldehydes giving phloroglucinol color reactions have been isolated in the ethanol extracts of oak hardwood. They have been identified as vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde. In general, both coniferaldehyde and sinapaldehyde were found to exist in other hardwoods examined, while only coniferaldehyde was found in soft woods. The lignin color reaction with phloroglucinol may thus be associated with sinapaldehyde as well as coniferaldehyde.

Introduction

Plant physiologists and botanists have long known that lignified membranes can be identified by the characteristic colors which are formed with certain organic reagents. As early as 1834, Runge[1] showed that a blue color is formed when sprucewood is treated with phenol in the presence of hydrochloric acid. Since that time, a number of organic and inorganic reagents have been shown to undergo color reactions with wood, and many investigators have sought an explanation for the color formation. In 1913, Czapek[2] extracted a fraction from sprucewood—hadromal—which he thought to be the color-forming substance in wood. The composition of this extract was not determined until Adler and Ellmer[3] isolated both vanillin and coniferaldehyde from a (spruce) hadromal preparation. It now seems evident that the characteristic color tests for lignin are dependent upon the presence of coniferaldehyde groups.[4,5] An important reaction of wood is the formation of a magenta color with phloroglucinol in the presence of hydrochloric acid. At the present time, coniferaldehyde and its methyl ether[6] are the only pure compounds which have been shown to give colors with phloroglucinol comparable to those obtained with wood and certain isolated lignin preparations.

Klason[7] and Freudenberg[8] found evidence of the presence of low molecular weight lignin in the extractives of wood; however, owing to the small quantity present an investigation of the identity of this lignin-like material was not carried out. By the application of paper chromatography it was possible to study the trace lignin-like substances in hardwood extractives. In addition to vanillin and syringaldehyde—the usual oxidation products of hardwood—two other aldehydes which give purple colorations with phloroglucinol were shown to be present. The magenta color formation of one spot suggested the presence of coniferaldehyde as one aldehyde. The presence of the sinapaldehyde grouping may be suspected also from the known chemistry of hardwood. In addition, a sinapaldehyde grouping is indicated from work of Pew.[9] In this work a part of the resorcinol color with spruce and aspen wood corresponds to the condensation product of resorcinol with coniferaldehyde substituted in the 5-position with a methoxy or propenyl group.

Experimental

Preparation of Wood Extracts.—(a) White oak chips were ground into a coarse meal. A 500-g. portion of the ground wood was covered with 95% ethyl alcohol in a glass-stoppered flask, and the mixture was allowed to stand for two days. The solvent was decanted and the process was repeated similarly three times. The ethyl alcohol extract was concentrated to 30 ml. by distillation at 30° under reduced pressure in a stream of nitrogen. The concentrate was extracted repeatedly with ethyl ether, and the ether extract was taken nearly to dryness *in vacuo*. The residue

---

[1] F. F. Runge, *J. prakt. Chem.*, **1**, 24 (1834).

[2] F. Czapek, "Biochemie der Pflanzen," Vol. I, Jena, 1913, p. 689.

[3] E. Adler and L. Ellmer, *Acta Chem. Scand.*, **2**, 839 (1948).

[4] For a discussion and bibliography of the relation of coniferaldehyde to the lignin color reaction, *cf.* F. E. Brauns, "The Chemistry of Lignin," Academic Press., Inc., New York, N. Y., 1952, chapter 4.

[5] Also *cf.* E. Hagglund, "Chemistry of Wood," Academic Press., Inc., New York, N. Y., 1951, chapter 4.

[6] E. Adler, K. J. Bjorkqvist and S. Häggroth, *Acta Chem. Scand.*, **2**, 93 (1948).

[7] P. Klason, "Beitrage zur Kenntnis der Chemischen Zusammensetzung des Fichtenholzes," Berlin, 1911, p. 34.

[8] K. Freudenberg, A. Janson, E. Knopf and A. Hagg, *Ber.*, **69**, 1415 (1936).

[9] J. C. Pew, THIS JOURNAL, **73**, 1678 (1951).