TABLE II

APPEARANCE UNDER THE POLARIZING MICROSCOPE

Derivs.	Crystal system	Habit and extinction	Optical character, biaxial	Elongation	Observed ns[a] Low	High
C_2	Triclinic or monoclinic	Flat needles, 40–41°	Negative	Positive	1.53[b]	1.595[c]
C_3	Triclinic	Fibrous, 2–13°	Negative(?)	Positive	1.441–1.442	1.545–1.546
C_4	Triclinic	Flat, elongated, 3–6°	Positive	Positive	1.40–1.45	1.52–1.525
C_5	Triclinic	Flat, some rectangular	.....	Positive in needles	1.46	1.52
C_6	Triclinic	Flat, elongated, 5–6°	Positive	Positive	1.46–1.475	1.515–1.520
C_7	Triclinic	Flat, elongated. Rectangular, 0–2°	Positive	Positive	1.48–1.485	1.52–1.530

[a] The ns given above represent those which are observed on the crystals as they lie on the slide in immersion liquids. Since the principal optical directions lie oblique to this surface, the actual minimum index alpha and maximum index gamma cannot be directly measured. [b] Beta. [c] Gamma.

Summary

1. The melting points of the normal fatty acid diamides of ethylenediamine are given, from acetyl to heptadecoyl, less C_9.

2. The solubilities and some crystallographic properties of the first six members of the series are recorded.

LEXINGTON, VIRGINIA RECEIVED JUNE 24, 1935

[CONTRIBUTION FROM SEVERANCE CHEMICAL LABORATORY, OBERLIN COLLEGE]

Preparation of a Potent Vitamin A Concentrate[1]

BY HARRY N. HOLMES, HAROLD CASSIDY, RICHARD S. MANLY AND EVA R. HARTZLER

Since Vitamin A has not yet been isolated, it is highly desirable that improved methods for the preparation of potent concentrates be developed.

Other workers in this field have obtained Vitamin A concentrates of very high values. Karrer, Morf and Schöpp[2] in 1931 succeeded in preparing a pale yellow oil of approximately 105,000 "blue value." (Values based upon the Carr–Price[3] color test and upon Karrer's own calculation that his standard cod-liver oil had a Vitamin A potency of 10 blue value). Heilbron, Heslop, Morton, Webster, Rea and Drummond[4] have published data on a concentrate of 65,000 blue value, while Carr and Jewell[5] have more recently obtained a concentrate of 78,000 blue value. The English workers, like Karrer, based their valuation upon the Carr–Price color test. Their concentrates were prepared by vacuum distillation.

The "blue value" signifies the number of blue units read with a Lovibond tintometer for 0.04 g. of solid dissolved in anhydrous chloroform immediately after reaction with the antimony trichloride reagent. It is obvious that "blue units per gram" could also be calculated by multiplying "blue value" by 25.

Concentration by Freezing.—The liver oil of halibut (Hippoglossus) was saponified in the Parke–Davis laboratories, the non-saponifiable portion removed by a solvent and most of the cholesterol frozen out. In our laboratory the first step was to transfer the concentrate from methyl alcohol to pentane by washing out the alcohol with water.

The pentane solution was next subjected to drastic freezing for a week or ten days in a bath of ethyl alcohol and carbon dioxide snow. To filter out the noticeable precipitate we employed in our earlier work an ultra-filter bomb of heavy metal which could be entirely surrounded by a bath of ethyl alcohol and carbon dioxide snow. Through this strong filter (Fig. 1) the filtrate could be forced by a pressure of 50–100 pounds of nitrogen. This method was very satisfactory when working with small quantities as it prevented any warming up of the mixture with annoying dissolving of the precipitate during filtration and also permitted the exclusion of air. With larger quantities, however, the filtration of such

(1) This research was made possible through the cordial coöperation of Parke, Davis & Company, of Detroit, and the Abbott Laboratories, of North Chicago. Paper read at the New York Meeting of the A. C. S., Division of Biochemistry, April 22, 1935.

(2) Karrer, Morf and Schöpp, *Helv. Chim. Acta*, **14**, 1036 (1931).

(3) Carr and Price, *Biochem. J.*, **20**, 497 (1926).

(4) Heilbron, Heslop, Morton, Webster, Rea and Drummond, *ibid.*, **26**, 1178 (1932).

(5) Carr and Jewell, *Nature*, **131**, 92 (1933).

gummy material was found to be too slow. In our more recent work we have adopted a suggestion by Dr. Karl Link, using suction filtration through a layer of carbon dioxide snow packed in a coarsely sintered glass filter. Such filtration is rapid, requiring only a few minutes for completion. Moreover, the carbon dioxide snow prevents warming up of the solution and provides an inert atmosphere for protection of the vitamin. The filtrate was then allowed to warm up to room temperature and some of the solvent was removed under suction to remove the dissolved carbon dioxide.

The solution (under nitrogen) was stored in carbon dioxide snow until ready for use. By this process the concentrate usually was improved from the 30,000–40,000 blue value as received in our laboratory to a value of 45,000–50,000. In one instance a blue value of 60,000 was obtained.

Concentration by Tswett Columns.—The column method of filtration through adsorbent powders as originated by the botanist, Tswett,[6] for the separation of plant pigments is peculiarly suited to the concentration of such a heat-sensitive substance as Vitamin A. The more highly adsorbed materials appear in bands near the top of the column, while the less strongly adsorbed materials appear farther down the column.

Karrer and Morf[7] used this method quite extensively in research with vitamin A and with the carotenoid pigments. They used calcium oxide or hydroxide, while Karrer, Morf and Schöpp[2] used alumina, and Karrer and Schöpp employed calcium carbonate.[8]

Although Karrer and his associates cut the adsorbent column into three sections and extracted from the rich middle section, we have found it to be advantageous to obtain fractions of different degrees of potency by washing the entire column with a suitable solvent. The less strongly adsorbed and more soluble materials are washed through first with the more strongly adsorbed and less soluble material following at a slower rate. Frequent changes of the receiver (maintaining an atmosphere of nitrogen) then enable us to obtain filtrate fractions of varying composition.

Although such a well-known adsorbent as carbon must have been tried, its use has not been reported, probably because of serious loss of the vitamin due to oxidation. We have used Norit carbon very extensively with excellent results, but only after taking great precautions in its activation to remove air and replace it with nitrogen.

Since carbon is a non-polar adsorbent while magnesia is somewhat polar it would be expected that the two should show somewhat different preferential adsorption from a complex mixture such as the crude vitamin A concentrate. With this idea in mind we have adopted the procedure of running the solution of concentrate first through carbon and then through a magnesia prepared from the precipitated hydroxide with the idea that the carbon would remove certain impurities and the magnesia others with a resulting product of greater purity than that obtained from either adsorbent alone.

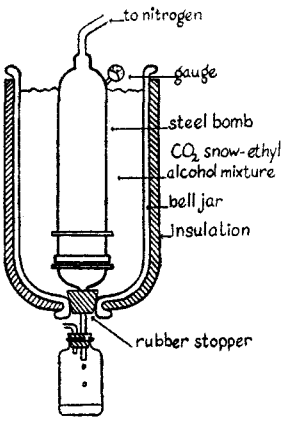

Fig. 1.—Low temperature filtration.

Results.—A large number of filtrate fractions have been obtained in this Laboratory with blue values of 100,000 or higher.

Using carbon alone we have obtained 41 fractions from several different oils with blue values of from 90,000 to 130,000.

TABLE I

No. of fractions	Blue value
23	90,000–100,000
12	100,000–110,000
5	110,000–120,000
2	120,000–130,000
1	130,000

Using magnesia alone two fractions were obtained with blue values between 90,000 and 100,000.

Using carbon followed by magnesia we have obtained 14 fractions with blue values between 90,000 and 140,000. They are distributed as shown in Table II.

Our first attempt using this procedure gave us the best results so far obtained. Circumstances, however, did not permit us to obtain a biological assay although we had about half a gram of the

(6) Tswett, *Ber. Botan. Ges.*, **24**, 384 (1906).
(7) Karrer and Morf, *Helv. Chim. Acta*, **16**, 625 (1933).
(8) Karrer and Schöpp, *ibid.*, **16**, 745 (1933).

TABLE II

No. of fractions	Blue value
7	90,000–100,000
2	100,000–110,000
1	110,000–120,000
1	120,000–130,000
2	130,000–140,000
1	140,000

richest material. We had removed the solvent from the oily residue under reduced pressure and had stored it under nitrogen in carbon dioxide snow for two days. At the end of that period the oily material had become crystalline, and its blue value had fallen to 1050. We learned too late that our valuable half gram should have been stored at once in cold methyl alcohol for protection.

It may be of interest to note that Karrer considered a standard cod liver as having a "blue value" of 10. Upon this basis our best concentrate was at least 14,000 times as potent in vitamin A as such standard cod-liver oil while Karrer's best concentrate was 10,500 times his standard.

Description of Color Test.—The values given in this paper were all measured by the Carr–Price reaction[3] using antimony trichloride in anhydrous chloroform according to the directions of the "Cod-liver Oil Colour Test Sub-Committee of the British Pharmacopoeia Commission" and reading blue values with a Lovibond tintometer.

Drummond and Morton[9] examined six oils biologically, colorimetrically and spectroscopically and found very good agreement between the three methods. More recently Lathbury[10] has stated that cod-liver oils and vitamin A concentrates show a constant ratio between the results of the biological, chemical and physical methods of measurement.

We read rather consistently, with our particular tintometer, several per cent. higher than Parke, Davis & Company, so all values in this paper have been corrected accordingly. Recently, we found our colorimetric assays of a group of four liver oils in close agreement with those by Parke, Davis & Company in Detroit, and in their English laboratory at Hounslow. Two of these oils had been cross checked with the spectro-absorption instrument in Morton's laboratory (England) and Brode's laboratory (Ohio State University) as well as in Detroit.

(9) Drummond and Morton, *Biochem. J.*, **23**, 785 (1929).
(10) Lathbury, *ibid.*, **28**, 2254 (1934).

Bio-assay of a Potent Concentrate.—Parke, Davis & Company made a bio-assay on one of our earlier potent concentrates which was obtained by use of a carbon column and which gave for us a color assay of 94,000 blue value. The material was dissolved in a commercial cottonseed oil to which had been added 1% of soy bean lecithin as antioxidant. The bio-assay value obtained corresponded to 91,200 blue value, the feeding level required being 0.24 gamma per day, a feeding level remarkably low in comparison with the others recorded in the literature.

The same sample was also analyzed in the Parke–Davis laboratory for vitamin D and found to contain 18,620 Steenbock D units per gram.

Effect of Moist Air on Color Test.—We have recently demonstrated in this Laboratory that an increase in humidity has an inhibiting effect on the formation of the blue color with the antimony trichloride reagent. By enclosing our tintometer, and all apparatus, in a glass case in which we were able to control the humidity to some extent we obtained readings showing an average of over 10% decrease in the blue values with a rise in relative humidity from 35 to about 60%. The best vitamin A values we have so far obtained were read in July, 1934, under humid conditions in our basement, which averaged about 20°F. (11°C.) cooler than the official outdoor temperature. It seems probable, therefore, that our reported values of that month really deserved a higher rating.

Materials

Pentane.—The pentane used was a low boiling petroleum fraction (b. p. 30–45°) obtained from the Viking Distributing Co., Charleston, W. Va.

Chloroform.—U. S. P. grade from the Dow Chemical Co., Midland, Michigan.

Antimony Trichloride.—Baker C. P. anhydrous antimony trichloride. The reagent was made up with especially purified dry chloroform to a concentration of 21–23% of antimony trichloride in chloroform.

Carbon.—Grade "A," pharmaceutical Norit, obtained from L. A. Salamon Co., 216 Pearl St., New York City.

Magnesium Oxide.—In the earlier work we prepared our own magnesium oxide from Parke-Davis milk of magnesia by filtering and drying. This process was quite tedious, however, and we have recently been using a similar magnesia, "Micron Brand," 2641, obtained from the California Chemical Corp., Newark, California. This was used by Strain[11] in the purification of carotenes.

Alumina.—The alumina used was purchased from the J. T. Baker Company, Phillipsburg, N. J., under the trade name of "Hydralo."

(11) Strain, *J. Biol. Chem.*, **105**, 523 (1934).

Silica.—The silica used was Holmes' chalky silica gel prepared in the Severance Laboratory.

"Hyflo Super Cel."—A siliceous earth with practically no adsorbent qualities, purchased from Johns-Manville, Cleveland.

Nitrogen.—Compressed gas, from the Ohio Chemical Co., Cleveland, containing less than 0.05% oxygen. This trace of oxygen was removed by passing the gas through alkaline pyrogallol.

Methods

Our columns were set up according to the diagram in Fig. 2. The tip of the column tube was tightly plugged with glass wool and the entire system was then filled with nitrogen. A thin mixture of activated carbon and pentane, well shaken, was then poured into the column and allowed to settle. While the level of the liquid fell to the upper level of the column of carbon the tube was tapped with a block of wood to dislodge gas bubbles. A pentane solution of the concentrate was then carefully added and allowed to filter down through the carbon with the aid of slight suction at B, while nitrogen entered the top of the column at A. After all the solution of concentrate had entered the carbon, pentane was carefully added through the separatory funnel and allowed to wash the concentrate down through the carbon. Fractions of the filtrate of from 50 to 150 cc. each were collected. Upon changing receivers, stopcocks B and C at the bottom were closed (cutting off suction), B was opened to admit nitrogen to the partially evacuated receiver before removal, and a clean nitrogen-filled bottle was connected.

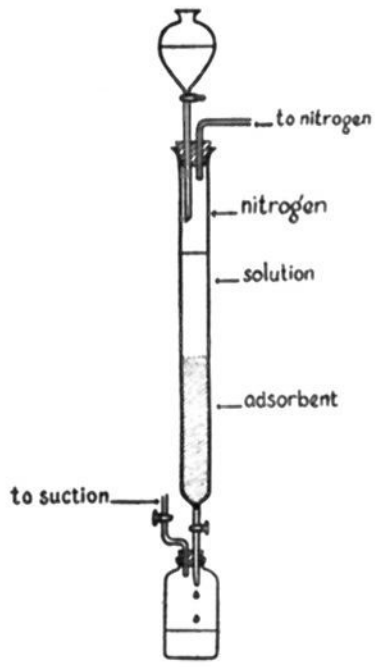

Fig. 2.—Column for selective adsorption.

Only slight suction can be used, otherwise the powdered adsorbent packs too tightly for effective filtration. To aid in hastening the filtration through magnesia columns we used a heat-treated siliceous earth, "Hyflo Super Cel," mixed in equal part by weight with the magnesia as suggested by Strain in his work on carotenes. This siliceous earth is a poor adsorbent but aids in more uniform packing of the magnesia and in more rapid filtration. Both powders were activated together (after thorough mixing) in order to remove any moisture or oxygen present.

Our usual practice was to filter about 6 g. of concentrate, contained in 50 cc. of pentane solution, through a column of carbon 2.5 cm. in diameter and 23 cm. high (about 40 g. of carbon). The filtrate from a column of this size contained from 1 to 40 mg. per cc.

Summary

1. Cholesterol and some other impurities were frozen out of a pentane solution of the non-saponifiable portion of halibut-liver oil; the resulting solution was then fractionated by Tswett adsorption columns with an improved technique. Using a column of a specially prepared oxygen-free carbon followed by treatment with a column of a new type magnesia a concentrate of vitamin A at least 40% more potent than any previously recorded was obtained.

2. Lovibond tintometer readings were checked biologically and spectrographically in the Parke–Davis laboratories and elsewhere.

3. The most potent concentrate had a blue value of at least 140,000.

OBERLIN, OHIO RECEIVED JULY 16, 1935

[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

The Mercuration of Ethylenes and Reactions of the Methoxy Mercurials

BY GEORGE F. WRIGHT[1]

Introduction

When an unsaturated compound is treated with mercuric acetate in alcohol solution, the elements of methoxymercuric acetate combine with the ethylene. The nature of the product has been the subject of controversy for some years and the evidence has been well summarized by Adams, Roman and Sperry.[2] These authors as well as others[3,4] have rejected Manchot's hypothesis[5] that such mercurials are molecular compounds of the type $\cdot RCH{=}CHR'\cdot R''OHgX$ (I) and they have preferred the second of two mechanisms of formation which designate the reaction as ordinary addition to a double bond. More recent work has substantiated the structure for the mercurials as III rather than I. Marvel and co-workers,[6] by separating disastereomers with different rotations from the mercuration of optically active cinnamic esters, have shown that

(1) National Research Fellow in Chemistry.

(2) Adams, Roman and Sperry, THIS JOURNAL, **44**, 1781 (1922).

(3) Mills and Adams, *ibid.*, **45**, 1842 (1923).

(4) Middleton, *ibid.*, **45**, 2763 (1923).

(5) Manchot, *Ann.*, **420**, 174 (1920); *ibid.*, **421**, 316, 331 (1921).

(6) Sandborn and Marvel, THIS JOURNAL, **48**, 1409 (1926); Griffith and Marvel, *ibid.*, **53**, 789 (1931).