COLORIMETRIC ESTIMATION OF VITAMIN A AND β -CAROTENE WITH ANTIMONY PENTACHLORIDE

TOSHIRO MURATA AND JUNKO NAGASHIMA

*Pharmaceutical Faculty, Kumamoto University, Kuhonji, Oemachi,
Tsuboi, Kumamoto*

(Received February 19, 1960)

The most prevalent chemical method for estimating vitamin A is based on the Carr-Price reaction, involving the sensitive coloration of both vitamin A and β -carotene with antimony trichloride. The mechanism of the color formation was studied by Meunier (1), and it was assumed to be due to the replacement of the lone pair of the electron belonging to the OH-group in the structure of vitamin A. At first the oxonium compound was formed, followed by the transformation of the double bonds in the structures, mesomerism, whereby a color was formed due to halochromy.

In practice, vitamin A is dissolved in chloroform, to which chloroform solution of antimony trichloride is added. A great amount of antimony trichloride, however, is required for developing the color sufficiently. In order to obtain the suitable color density for estimation, 10^4 — 10^5 times moles of antimony trichloride must be added to one mole of vitamin A. It is also the case with β -carotene.

Brüggemann *et al.* (2) reported that the color formation was not due to antimony trichloride, but to antimony pentachloride usually contained in the commercial sample of antimony trichloride.

In this study, the estimation of vitamin A and of β -carotene with antimony pentachloride was attempted, and the method was compared with that using antimony trichloride.

EXPERIMENTAL AND RESULTS

The method for estimating vitamin A with antimony trichloride was carried out according to that described in "Eisei Shikenho" (An Official Methods of Analysis authorized by Pharmaceutical Association of Japan).

1. Estimation of Vitamin A in Cod-Liver Oil by Both Methods

The cod-liver oil was saponified and extracted with ether. After distillation of the ether, the unsaponifiable residue was dissolved in chloroform. 1 ml each of the solution was added to 9 ml of antimony chloride solution. The color formed was estimated colorimetrically at 620 m μ .

Antimony trichloride solution used contained 250 mg of the chloride per ml, while the antimony pentachloride solution used contained various amounts

of the pentachloride. To each 100 ml antimony chloride solution, 1.0 ml of acetic anhydride was previously added.

By changing the amount of SbCl_5 added, various colorations were observed; 23.4 mg/ml gave reddish blue, 2.34 mg/ml blue just like the color given with SbCl_3 , 1.2 mg/ml faint but distinctive blue, and 0.15 mg/ml reduced the color markedly. All the color densities developed by adding SbCl_5 , however, were smaller than those developed by adding SbCl_3 as shown in Table I.

TABLE I
Colorimetric Determination of Vitamin A in Cod-Liver Oil with
 SbCl_3 and SbCl_5

Reagent added		Vitamin A content (i.u./g)					
Kind of chloride	Concentration	1	2	3	4	5	6
	mg/ml						
A. SbCl_3	250	2000	2013	2127	1401	1451	1414
B. SbCl_5	2.34	1860	1816	1893	1296	1237	1345
B/A (%)		93.1	90.2	88.9	92.5	85.3	95.1
A. SbCl_3	250	1145	1122	1148			
B. SbCl_5	1.2	1010	987	967			
B/A (%)		88.3	87.9	84.1			
A. SbCl_3	250	3033	3153	3033			
B. SbCl_5	0.15	129	129	125			
B/A (%)		4.25	4.09	4.12			

If the commercial sample of SbCl_3 contains about 0.06% of SbCl_5 as reported by Brüggeman *et al.*, the reagent containing 0.15 mg of SbCl_5 per ml may be sufficient to form the color. Impurity of the reagent and degradation of the SbCl_5 in the procedure might cause lower values.

2. Estimation of Vitamin A with Antimony Pentachloride under a Certain Condition

From the result of the above experiments, it is obvious that the concentration of SbCl_5 to be added vitamin A need not be so high as SbCl_3 . In

FIG. 1 Absorption Spectrum of SbCl_5 -Vitamin A

FIG. 2 Effect of Concentration of Reagent on Color Density

usual method for determining vitamin A with SbCl_3 , 1 ml of chloroform solution of the unsaponifiable matter was added to 9 ml of SbCl_3 -reagent. The smaller the volume of the SbCl_3 -reagent to be added, the more rapid and simpler the procedure might be. Thus 3.5–4.5 ml of chloroform solution of the unsaponifiable matter was added to 0.01 ml of SbCl_5 -reagent in the present studies.

Estimation of Absorption Maximum—After adding 0.01 ml of SbCl_5 -reagent to 3.5 ml of the sample, absorbance was estimated spectrophotometrically at each wavelength. The results are shown in Fig. 1. The maximum absorbance lied at about $620\text{ m}\mu$ in agreement with the case of SbCl_3 -vitamin A compound.

Effects of Changing Concentrations of SbCl_5 on Absorbance—In this experiment, the reagents containing 11.7, 58.5, 117, 585, 1170, and 1560 mg of SbCl_5 per ml respectively were used. Spectrophotometric estimation at $620\text{ m}\mu$ was carried out 15 seconds after adding the reagent to the sample. The results are shown in Fig. 2. At the concentrations higher than 585 mg per ml of SbCl_5 it was inconvenient to carry out the procedure because of the emission of smoky chlorine gas. Further addition above 1170 mg per ml resulted in the change of the color. So the concentration of SbCl_5 was most conveniently used at 468 mg per ml.

Decoloration of Colored Complex—Standard cod-liver oil (J. P. VI) was used for determining the content of vitamin A. Each absorbance was determined at the interval of 15, 20, 30, 40, 50, 60, 70, 80, and 90 seconds, respectively, after adding 0.01 ml of SbCl_5 reagent to 4.5 ml of the sample solutions. The estimation was unable to carry out more rapid than 15 seconds after adding the SbCl_5 -reagent. The results are shown in Fig. 3.

Standard Curve of Vitamin A Determined with SbCl_5 —Cod-liver oil samples containing 2.5, 5.0, 7.5, 10.0 i.u. of vitamin A, respectively, were colorimetrically determined under the conditions as described above. The results gave linear relationship as shown in Fig. 4.

Colorimetric Determination of Vitamin A in Cod-liver Oil—The cod-liver oil containing 2560 i.u. per g of vitamin A as estimated previously with

FIG. 3 Rate of Decoloration

FIG. 4 Standard Curve of Vitamin A Determined with SbCl_5

TABLE II
Estimation of Vitamin A Content in Cod-Liver Oil

No. of experiment	T	E	A content
	%		I.U./g
1	32.2	0.4908	2500
2	32.3	0.4922	2500
3	32.3	0.4922	2500
4	32.2	0.4908	2500

SbCl_3 , was used as a sample. The value of vitamin A in the oil was on an average 2500 I.U. as shown in Table II.

3. *Estimation of β -Carotene with Antimony Pentachloride*—Crystalline β -carotene was used as a sample. To each 3.5 ml of the chloroform solution containing various amounts of β -carotene, 0.01 ml of the reagent containing 468 mg of SbCl_5 per ml was added, and the color produced was colorimetrically estimated at 620 $\text{m}\mu$. Almost linear standard curve was obtained at the concentrations of 0–22 μg per ml. (Fig. 5).

FIG. 5 Standard Curve of β -Carotene Determined with SbCl_5

DISCUSSION

Brüggemann *et al.* reported that the Carr-Price reaction was due to the formation of a colored substance by the reaction of vitamin A with SbCl_5 which is ordinarily contained in the commercial sample of SbCl_3 , and their view was confirmed by the authors. Namely, the color developed with SbCl_5 had the same absorption maximum at 620 $\text{m}\mu$ as that with SbCl_3 , and both vitamin A and β -carotene can be determined colorimetrically using SbCl_5 . They reported further that the amount of SbCl_5 sufficient to develop the color corresponded to 0.06 per cent of that of SbCl_3 , a finding which could not be confirmed by the authors. The amount of SbCl_5 required for coloration was 1/3150 of that of SbCl_3 . The color formed with SbCl_5 faded as rapidly as that with SbCl_3 . The addition of a very small amount of the reagent made the procedure easier and more rapid, and the further color change was considerably reduced. Another convenience of using SbCl_5 was the prevention of staining the glass apparatus by the SbOCl formed.

β -Carotene could also be colorimetrically estimated using SbCl_5 . Comparing the standard curve of β -carotene with that of vitamin A, the mechanism of formation of color in β -carotene with SbCl_5 might be different from that of vitamin A. These experiment suggests that estimation of vitamin A with SbCl_5 in the presence of much β -carotene will give false values.

SUMMARY

Vitamin A and β -carotene form with SbCl_5 color substances having an absorption maximum at $620\text{ m}\mu$, at which wavelength the colorimetric estimation can be easily and rapidly carried out.

Sufficient color for estimation is obtained by adding 0.01 ml of SbCl_5 -chloroform solution containing 468 mg of SbCl_5 per ml to 3.5 ml of sample chloroform solution.

Staining the glass by the SbOCl formed in the procedure was insignificant with the use of SbCl_5 .

REFERENCES

1. Meunier, P., *Compt. Rend. Acad. Sci.* **221**, 64 (1945).
Meunier, P., *Bull. Soc. Chim. France Mém.* (5) **13**, (1946).
2. Brüggemann, J., Krous, W., and Tiews, J., *Chem. Ber.* **85**, 315 (1952).