An improved colorimetric procedure for the analysis of vitamin A

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Factors affecting the reaction between antimony trichloride and 2,3-dichloropropanol (2,3-DP) with retinol and related compounds were examined. It is shown that purified 2,3-DP with the addition of antimony trichloride produces a stable colour complex with retinol absorbing at 555 nm. A tentative scheme for the reaction mechanism and the possible structure of the complex are also presented.

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On a examiné les facteurs affectant la réaction du trichlorure d'antimoine et du dichloro-2,3 propanol (2,3-DP) avec le rétinol et d'autres composés qui lui sont reliés. On a montré que l'addition de chlorure d'antimoine à du 2,3-DP provoque la formation, par réaction avec le rétinol, d'un complexe de couleur stable absorbant à 555 nm. On propose un schéma réactionnel et une structure possible pour le complexe.

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Introduction

Vitamin A and compounds having vitamin A activity are most commonly assayed in food materials or samples of biological origin by; (*1*) bioassay, (*2*) ultraviolet absorption, (*3*) fluorometric analysis, and (*4*) colorimetric analysis.

A large number of related compounds have varying degrees of vitamin A activity. Mild physical or chemical treatment can convert a biologically active compound such as all-*trans* retinol into a compound of low potency like the eleven *cis* isomer. Most physico-chemical tests respond to the different isomers and even to many retinol analogues as if they were all-*trans* retinol. These procedures give an upper limit to the estimate of the amount of vitamin A in a food or biological material. The true vitamin A activity can only be determined by bioassay techniques. These are unfortunately so cumbersome and time-consuming that they have been replaced in most routine testing by the fast, relatively simple, cheap and reliable physico-chemical tests.

Another major difficulty in assaying foods is the presence of carotenoids, food additives, and other materials which interfere with the physico-chemical procedures employed in the analysis. In order to eliminate these interferences extensive chromatographic and extraction purification is necessary in the analysis of most foods. The higher level of vitamin present and the simpler character of most pharmaceutical preparations makes these systems inherently easier to analyze than foods.

The ultraviolet absorption method (measurement of the absorption at the maximum in the vitamin A curve) is an excellent procedure if few interfering compounds are present and if the vitamin is present in relatively high concentration. This method is one of those recommended by the Association of Official Analytical Chemists (AOAC) for the determination of vitamin A in margarine (2). For this purpose it is a difficult but acceptable procedure.

Vitamin A fluoresces strongly near 480 nm with excitation at 330–360 nm. This fluorescence has been little used for quantitative purposes until recently, because of extraneous fluorescence from phytofluene and other fluorescent materials present in many food materials and biological samples. Improved column chromatographic methods of removing the interfering fluorescing compounds have sparked a renewal of interest in the fluorometric procedure (3, 4). This is an excellent, very sensitive research method, especially where the concentration of vitamin A is low, if the interfering compounds can be satisfactorily removed.

Most vitamin A analysis is carried out by colorimetric techniques. Vitamin A reacts with many Lewis acids to produce a transient blue colour (5). In another of the official methods of

the AOAC (2), vitamin A in food materials is determined by reacting a vitamin A solution (extracted from the food with nonpolar anhydrous solvents) with a 20–25% solution of antimony trichloride in chloroform. The blue colour produced (λ_{max} = 620 nm) reaches maximum intensity in 5 to 10 s and then rapidly fades. This procedure, which is usually called the Carr–Price (6) method, has the advantage over the ultraviolet technique of being less liable to interference and somewhat more sensitive. Its major disadvantages are: (*1*) it is necessary to take readings of the absorbance within 5 to 10 s of addition of the reagent, (*2*) the poisonous and corrosive nature of the reagent, and (*3*) the development of the colour is affected by traces of water and alcohol. These problems make the method less precise than the ultraviolet technique.

Budowski and Bondi (7) have introduced a second colorimetric procedure. This involves the conversion of vitamin A to anhydrovitamin A with anhydrous ethanolic – HCl or *p*-toluenesulphonic acid. The reaction is very specific for vitamin A but low results are obtained if even small traces of water are present.

A third colorimetric procedure was described by Sobel and Werbin (8). They showed that the reaction of 'activated' 1,3-dichloro-2-propanol (1,3-DP) with vitamin A produces a pink coloured complex which has its absorption maximum at 555 nm and which is stable for 2 to 10 min. The method has never become popular because of the difficulty of reproducibly activating 1,3-DP and because the procedure is less sensitive than the Carr–Price. Sobel and Werbin activated the chemical by vacuum distilling 1,3-DP from a 2.0% solution of antimony trichloride in chloroform. Unfortunately, the 1,3-DP supplied by many commercial sources either cannot be activated by Sobel and Werbin's method or shows only a weak activity with vitamin A.

Penketh (9) has suggested that the activating principle is hydrogen ion. Sobel and Werbin (10) stated that their preparations contained varying amounts of antimony trichloride but that this was not the activating chemical, because direct addition of antimony trichloride did not activate 1,3-DP.

In the study reported here we have concentrated our efforts: (*1*) on improving and understanding the 'activation' of 1,3-DP and the isomer 2,3-dichloro-1-propanol (2,3-DP) and (*2*) on determining the mechanism of the reaction. Our purpose was (*a*) to develop procedures using dichloropropanol compounds which would be more convenient than the common physicochemical methods, and (*b*) to suggest, from a study of the reaction mechanism, other compounds which may give a more intense colour reaction with vitamin A.

Results and Discussion

(a) Preliminary Results with 1,3-DP

When 1 ml of a solution containing less than 150 μg/ml of vitamin A is added to 3 ml of 1,3-DP activated as described by Sobel and Werbin (10), a transient blue colour appears (λ_{max} = 620 nm), but it is replaced within approximately 1 min by a pink colour (λ_{max} = 555 nm). The Carr–Price procedure also produces a blue colour with maximum absorption at 620 nm.

Using the chloramine T method (11) it was found that commercially activated 1,3-DP (glycerol dichlorohydrin for vitamin A analysis from Eastman Kodak, Distillation Products Division) and our own 1,3-DP, activated as described by Sobel and Werbin (10), contained approximately 0.15% reducing material, undoubtedly antimony trichloride. Efforts were concentrated on attempting to activate 1,3-DP by direct addition of antimony trichloride. This was found to be impossible with most commercial samples of 1,3-DP. A pink colour could, however, be developed if the directly activated solution were heated immediately after addition of vitamin A. A sample from BDH described as dichlorohydrin had decomposed slightly and was, therefore, distilled and dried before addition of antimony trichloride. This solution developed a strong pink colour (λ_{max} = 555 nm) when mixed with 30 μg/ml of retinol acetate. Similar distillation of the other samples of 1,3-DP prior to direct addition of antimony trichloride did aid activation but the pink colour produced with retinol acetate was quite weak. An analysis of the dichlorohydrin solution showed that it consisted of an approximately 50:50 mixture of 1,3-DP and 2,3-DP. A sample of 2,3-DP which had been obtained previously, activated readily after distillation. Even crude, undistilled 2,3-DP, prepared as described in the Experimental section,

TABLE 1. Effect of antimony trichloride concentration on absorption and chromophore stability at 555 and 660 nm for retinol–acetate–2,3-DP and retinal–2,3-DP respectively*

Concentration of $SbCl_3$ (gm/100 ml)	Absorbance at 555 nm (retinol acetate)	Time to reach maximum absorption (min)	Absorbance at 660 nm (retinal)	Time to reach maximum absorption (s)
0.1	0.135	6	0.00	—
0.2	0.155	4	0.027	<30
0.5	0.160	2	0.013	<30
1.0	0.190	1	0.104	<30
3.0	0.210	0.6	0.137	<30
10.0	Not measured		0.260	60

*Three millilitres of a solution composed of 90 ml of 1,2-dichloroethane, 10 ml of 2,3-DP, and x g of antimony trichloride were mixed with 1 ml of retinol acetate or retinal in 1,2-dichloroethane. The retinol acetate concentration was 13.2 μg/ml and the retinal 4.64 μg/ml. The temperature was 20 °C.

activated easily, but the colour produced with vitamin A decayed rapidly, probably because of the presence of HCl. Both practical grade 1,3-DP and the 2,3-DP produced from allyl alcohol contain 3–15% impurity before distillation. These impurities, especially in the case of 1,3-DP, appear to inhibit the colour development. Our distillation procedure and Sobel and Werbin's method remove sufficient of these impurities from 1,3-DP to enable it to be activated by antimony trichloride. In Sobel and Werbin's method, the extent of this activation depended partly on the degree of purification achieved and partly on the amount of antimony trichloride carried over in the distillate. According to their own determinations this varied between 0 and 0.67%. Our analysis of several different samples of practical grade 1,3-DP showed that they contained varying amounts of several impurities and their ability to be activated was found to depend inversely on the amount of an impurity which eluted at a retention time of 0.12 relative to 1,3-DP.

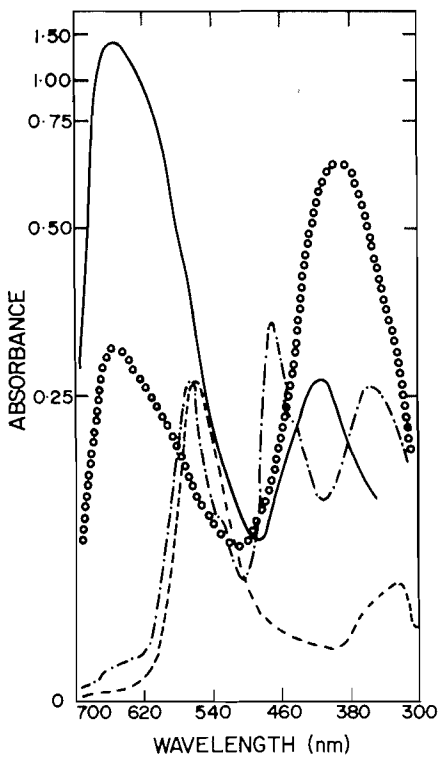

FIG. 1. Spectra of the 2,3-DP – $SbCl_3$ – vitamin A coloured complexes at 32 °C in chloroform: ○, retinal – 2,3-DP – 1% $SbCl_3$; —, retinal – 2,3-DP – 10% $SbCl_3$; - - -, retinol acetate – 2,3-DP – 1% $SbCl_3$; ·····, retinoic acid – 2,3-DP – 1% $SbCl_3$.

Our method of activation is capable of greater reproducibility because it is possible to control much more accurately the removal of impurities and the addition of antimony trichloride. Fortunately, 2,3-DP prepared from allyl alcohol (see Experimental section (*b*) for details) appears to contain few if any inhibiting impurities and it can be activated reproducibly by direct addition of antimony trichloride. In the remainder of this report, we describe the use of 2,3-DP, thus activated, in the analysis of vitamin A.

(b) Studies with 2,3-DP

(i) Effect of Antimony Trichloride Concentration

The dependence of the absorbance at 555 nm of the retinol acetate–2,3-DP chromophore and at 660 nm for the retinal–2,3-DP chromophore is presented in Table 1. The spectra of the vitamin A – 2,3-DP coloured complexes for retinol acetate, retinal, and retinoic acid are presented in Fig. 1. The rise and decay of the

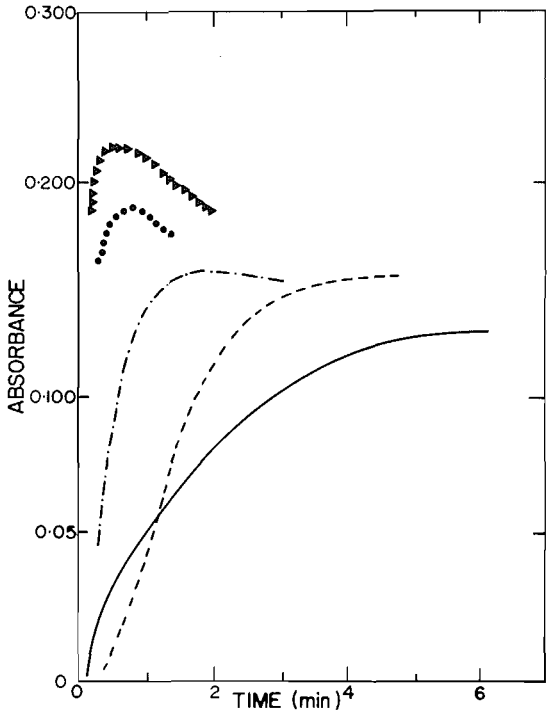

FIG. 2. Rate of change of the absorption at 555 nm as a function of $SbCl_3$ concentration. One millilitre of 13.2 μg/ml retinol acetate in 1,2-dichloroethane was mixed with 3 ml of 90:10:x dichloroethane – 2,3-DP – $SbCl_3$ at 20 °C: —, 0.1% $SbCl_3$; - - -, 0.2% $SbCl_3$; -·-·-, 0.5% $SbCl_3$; ···, 1% $SbCl_3$; ▲, 10% $SbCl_3$.

absorption intensity at 555 nm for retinol acetate are shown in Fig. 2. In earlier work it was found that, in the case of retinol acetate, 1% antimony trichloride was a good compromise between the intensity of colour developed and the rate of decay of absorbance. If maximum sensitivity is not required, possibly 0.5 or even 0.2% is a better choice. Retinol does not react as readily with 2,3-DP so at least a 1% solution is necessary to obtain a satisfactory absorbance value. A 10% solution gives a useful two and one-half fold increase in sensitivity.

(ii) Colour Complexes of Vitamin A Active Compounds

The absorbances relative to retinol acetate of several vitamin A active compounds are presented in Table 2. No absorbance was detected for retinoic acid when a 1% $SbCl_3$ solution was used. If a high concentration of retinoic acid was mixed with a 5% $SbCl_3$ solution, the complex spectrum shown in Fig. 1 was obtained.

TABLE 2. Absorbance of colour complexes of vitamin A compounds*

Compound	Absorbance relative to retinol acetate	Wavelength
Retinol acetate	1.0	555 nm
Retinol palmitate	1.0	555 nm
Retinal	1.6 (3.7)†	660 nm
Retinoic acid	0	

*Three millilitres of a solution composed of 90 ml of 1,2-dichloroethane or chloroform, 10 ml of 2,3-DP, and 1 g of antimony trichloride were mixed with 1 ml of the sample solution. The solvent for the sample was either 1,2-dichloroethane or chloroform and the temperature was 20 °C.
†10% $SbCl_3$ solution.

TABLE 3. Effect of solvent on the 555 nm chromophore*

Solvent	Absorbance at 555 nm / Absorbance at 555 nm in $CHCl_3$
$CHCl_3$†	1.0
Reagent grade $CHCl_3$	0.5
Nitromethane	0
Absolute alcohol	0
Methanol	0
2-Propanol	0
1,2-Dichloroethane	0.93
Carbon tetrachloride	—

*Three millilitres of a solution composed of 90 ml of solvent, 10 ml of 2,3-DP, and 1 g of antimony trichloride were mixed with 1 ml of retinol acetate, in the same solvent. The temperature was 32 °C and the experiments were conducted using a Spectronic Model 20.
†Chloroform was washed and distilled as described by Strohecker and Henning (1).

(iii) Effect of Solvent on the Absorbance at 555 nm

The effects of solvent were quite complex. Results are summarized in Table 3. Addition of 0.5 ml of alcohol per 4 ml of vitamin A – 2,3-DP solution completely prevents colour development. Even the 0.75% alcohol present in reagent grade chloroform severely inhibits colour development. Nitromethane also appears to be an inhibitor.

Carbon tetrachloride is immiscible with 2,3-DP in the proportions employed to construct Table 3. If the concentration of carbon tetrachloride is reduced, *i.e.* by adding 1 ml of retinol acetate in carbon tetrachloride to 3 ml of our standard chloroform–2,3-DP–$SbCl_3$ mixture, the absorbance obtained is identical with that obtained when only chloroform is present. The presence of carbon tetrachloride also appears to suppress the blue absorbance at 620 nm and to slow the reaction rate slightly. The effect of solvent will be

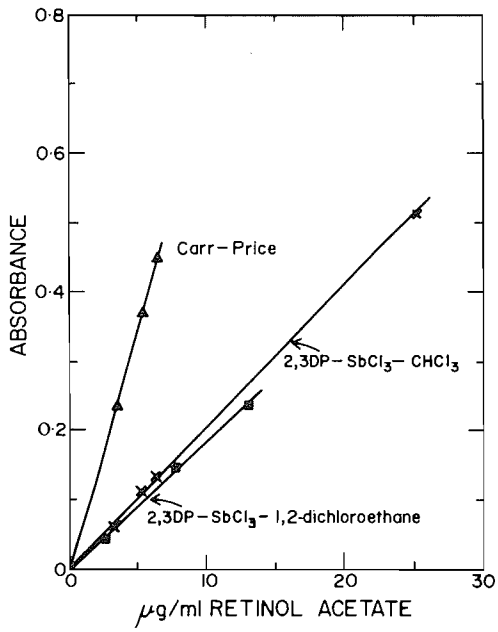

FIG. 3. Comparative Beer–Lambert plots for the Carr–Price and the 2,3-DP procedures ▲, ■. Curves were obtained at 32 °C in a Spectronic 20 and are corrected to a pathlength of 1 cm. In the solution containing 2,3-DP the ratios of solvent 2,3-DP–$SbCl_3$ were 90:10:1. The Carr–Price reagent contained 25 gm/100 ml $SbCl_3$.

further discussed in section (*vii*). Clearly, purified chloroform or 1,2-dichloroethane are the solvents of choice and alcohols should be either avoided or removed from all solvents.

(iv) Beer–Lambert Law Dependence

The Beer-Lambert law plots of retinol acetate in purified chloroform and 1,2-dichloroethane are shown in Fig. 3. The Carr–Price curve is also given. The absorbance of the coloured complex is linear in the concentration range 0–26 μg/ml when the 2,3-DP is diluted 9:1 with solvent. If 2,3-DP is used without dilution, the Beer–Lambert plot is not linear and the absorbance is about one-third less intense. On the basis of the above, the recommended procedure is to add 1 to 3 ml of a vitamin A solution in 1,2-dichloroethane to 3 ml of a 90:10:1 1,2-dichloroethane – 2,3-DP – antimony trichloride solution contained in a spectrophotometer cuvet and to record the maximum absorbance obtained at 555 nm (for retinol acetate).

One of our major goals has not been realized as yet, in that the absorbance is still about 3.5 times less intense than the corresponding Carr–Price absorbance when retinol acetate is assayed. As shown in Table 2, the sensitivity of the reagent for retinal is approximately the same factor higher, so that retinal can be detected by 2,3-DP with approximately the same sensitivity as retinol acetate by the Carr–Price method.

The lower sensitivity of this method for retinol acetate is not actually important in routine analysis. Most pharmaceutical preparations containing vitamin A have such a high level that dilution is necessary before analysis can be employed. Sebrell and Harris (12) show that most food sources of vitamin A contain considerably more than the 3 μg/g of vitamin A for which we found an absorbance of 0.076.

(v) Dependence of the Absorbance at 555 nm on Temperature

The absorbance at 26.5 °C was found to be 13% higher than at 20 °C. To prevent errors due to temperature effects, the procedure is best carried out in a controlled temperature room or a correction table can be prepared if this is not possible. If an instrument like the Spectronic 20 is used, the sample tube should not be inserted into the spectrophotometer until the pink colour appears, as the lamp of the Spectronic 20 noticeably heats the tube.

(vi) Stability of the Reagent

Two samples of reagent prepared by different workers from different samples of allyl alcohol and distilled at different maximum temperatures gave absorbances at 555 nm which differed by only 5% even though one reagent sample had been prepared 3 months before the other.

(vii) Mechanism of the Reaction

The first step in the reaction is certainly the Carr–Price reaction. By omitting the 2,3-DP we found that a strong transient absorption occurs at 620 nm even though the concentration of antimony trichloride is much smaller in our experiments than in the normal Carr–Price procedure. Solvents and the concentration of antimony trichloride markedly affect the absorbance at 620 nm. At antimony trichloride concentrations of less than 0.5% or in the presence of carbon tetrachloride or nitromethane even at 1% antimony trichloride concentration, the 620 nm absorbance is very low and does not vary appreciably with time.

In the presence (or absence) of 2,3-DP two peaks appear immediately at 397 nm with the

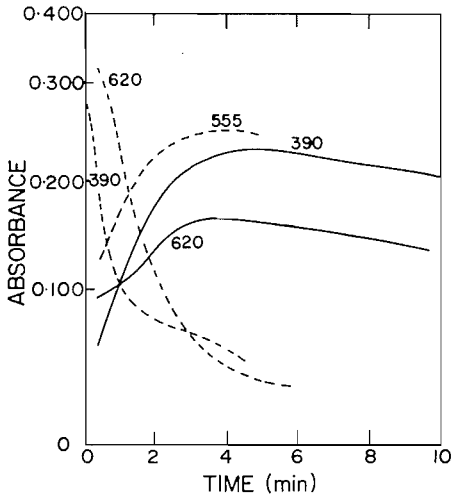

FIG. 4. Effect of 2,3-DP on the rates of change of the absorptions at 390, 620, and 555 nm: —, 3 ml of 1% $SbCl_3$ in $CHCl_3$ + 1 ml of 16.1 mg/ml retinol acetate; - - -, 3 ml of 90:10:1 $CHCl_3$/2,3-DP/$SbCl_3$ + 1 ml of 16.1 μg/ml of retinol acetate. Temperature 8 °C. Rates measured in a Beckman DB spectrophotometer.

hint of a third peak at 355 nm. The antimony trichloride solution absorbs strongly below approximately 370 nm with complete absorption occurring below 350 nm. Thus we could not determine the rate of change of the vitamin A concentration. Edisbury *et al.* (13) and Shantz *et al.* (14) showed that anhydrovitamin A is a product of the Carr–Price reaction. Anhydrovitamin A absorbs at 392, 371, and 351 nm in ethanol (12). The absorptions we obtained at 397 and 375 nm are undoubtedly due to anhydrovitamin A. At 10 °C in the presence of 2,3-DP, absorption at 390 nm was found to fall rapidly whereas in the absence of 2,3-DP it fell very slowly (Fig. 4). On the same time scale the pink colour rose to a maximum when 2,3-DP was present.

The relative values of the 620 and 390 nm absorptions depend on the concentration of antimony trichloride. At antimony trichloride concentrations in excess of 1% the 620 nm absorbance usually exceeds the 390 absorption whereas the reverse is true at lower concentrations of antimony trichloride.

Several different experiments have shown that the absorbance at 390 nm reaches a peak value before the 620 nm absorbance. For instance, 1 ml of a solution containing 9 μg/ml of retinol acetate in carbon tetrachloride was rapidly mixed at 30 °C with 3 ml of a solution containing purified chloroform, 2,3-DP, and antimony trichloride in the ratio 90:10:1. The 390 nm absorbance reached its maximum value in 6 s whereas the 620 nm absorbance took 14 s.

The rate and relative rate of decrease of the absorption at 620 and 390 nm depend on the concentration of antimony trichloride. As already stated, at low concentrations of antimony trichloride the 620 nm absorbance changes very little whereas the 390 nm absorbance decreases gradually from a fairly high value. At higher concentrations with purified chloroform or 1,2-dichloroethane as solvent, both absorptions decrease at approximately the same rate. The disappearance of the 620 and 390 absorptions and the rise of the absorption at 555 nm are best fitted by kinetic equations based on the assumption of reversible first-order kinetics. In the presence of 1% antimony trichloride all forward rate constants measured (k_{620}, k_{390}, k_{555}) agree within 20–30%.

If 0.5 ml of absolute ethanol is added to the reaction mixture after the 620 and 390 nm absorptions have reached their maximum values and the pink complex is present, the absorptions at 620 and 555 nm drop to zero within the mixing time of the chemicals, even at 0 °C. The 390 nm absorption does not change.

The present findings also indicate that a relatively stable complex absorbing at 555 nm is formed in the presence of both antimony trichloride and 2,3-DP. The structure of this complex has not been fully established but can be reasonably assumed to be that given in Fig. 5 by analogy with the known reaction of retinol with ethanol and Lewis acids. Earlier workers have shown that on treatment with anhydrous ethanol – HCl, vitamin A is first converted to anhydrovitamin A (13, 14) and on prolonged treatment, to isoanhydrovitamin A which bears an ethoxy group at 4, 14, or 15. This compound is reported to show absorption peaks at 332, 348, and 366 nm (15), while the Carr–Price complex absorbs at 620 nm (13). Whether or not the complex displays a weaker absorption peak at shorter wavelengths is not reported. Unfortunately, the strong absorption of antimony trichloride in the ultraviolet precludes the possibility of determining unequivocally whether a similar compound is formed between anhydrovitamin A and 2,3-DP in the reaction under study.

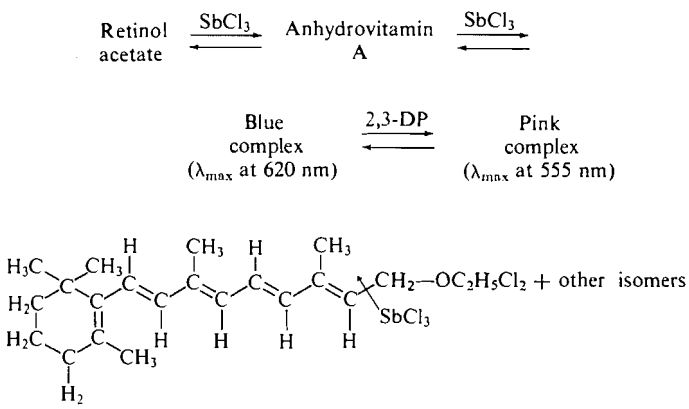

FIG. 5. Proposed reaction mechanism and the postulated structure of the pink complex.

The proposed mechanism for the reaction scheme is shown in Fig. 5. The first step is the conversion of retinol to anhydrovitamin A by antimony trichloride.

Lewis acids interact further with the anhydrovitamin A to form a π-complex with the conjugated system. The strong inductive effect of the chlorine atoms produces a fractional charge separation over the π-complex. The most strongly affected molecular orbital will be the highest occupied orbital (highest energy). Since the long wavelength transition is due to the promotion of an electron from this to the lowest unoccupied molecular orbital, the λ_{max} of the transition will be altered. The effect of the formation of the π-complex will be to destabilize (increase the energy of) the ground state (highest occupied molecular orbital) and to stabilize the excited state (lowest unoccupied molecular orbital). This is brought about by the stabilization of the necessary dipole produced in the optical transition. The decrease in the energy difference between the two states leads to the observed strong bathochromic shift from the ultraviolet to 620 nm.

The slower subsequent addition of 2,3-DP produces the complex shown in Fig. 5 and in effect reduces the conjugation path by a further formal double bond. Since the energy difference between the highest occupied and lowest unoccupied molecular orbital increases with decreasing conjugation path length, interaction with antimony trichloride now gives rise to the transition at 555 nm. Justification for this assumed scheme can be gained from the fact that the Carr–Price complex of vitamin A_2 (with six double bonds in the unperturbed state) absorbs at 693 nm (a shift of 73 nm compared to the retinol complex). If this 73 nm shift is of the right order for addition or subtraction of one double bond then the calculated long wavelength absorption maximum of the Lewis acid complex should be 547 nm. As has already been stated the measured absorption maximum occurs at 555 nm, only 8 nm removed from the calculated value.

Blatz *et al.* (16, 17) postulated extensive *cis–trans* isomerism of retinol in the reaction with Lewis acids leading to the formation of cyclic structures. In view of the known hindrance to rotation due to steric interference between the methyl groups at positions 9 and 13 and the proximal H atoms this is felt to be unlikely though selective *cis–trans* isomerism is obviously possible.

Further Work

Work is proceeding on determining if 2,2′,3,3′-tetrachloro-1-propanol, 2,3-dibromo-1-propanol, and 2,3-diodo-1-propanol have any advantage over 2,3-DP as colorimetric reagents for vitamin A analysis. Isolation of the isoanhydrovitamin A analogue (Fig. 5) is also being attempted.

Chemicals and Experimental Detail

(a) Chemicals

Retinol acetate, 1,3-DP for vitamin A determination, 2,3-dichloro-1-propanol (2,3-DP), allyl alcohol, and antimony trichloride were obtained from Fisher Scientific. Most of the 1,3-DP was manufactured by Eastman Kodak, except for 100 ml from the Baker Chemical Company. Retinol acetate, retinal, retinol, retinoic acid, and retinol palmitate were obtained from the Sigma

Chemical Company. Dichlorohydrin was supplied by British Drug Houses (BDH), Chloroamine-T by Eastman Kodak, and chlorine gas by Matheson.

Carbon tetrachloride, nitromethane, 1,2-dichloroethane, absolute ethanol, and chloroform were obtained from Fisher Scientific. Alcohol was removed from the chloroform by the method described by Strohecker and Henning (1).

Chemicals were analyzed on a 6 ft by 0.25 in. 15% EGSS-X on Gas Chrom Q 100–120 mesh column mounted in an F and M 810 gas–liquid chromatograph. A similar DEGS column was also satisfactory.

Absorbance measurements were made in 1 cm quartz cells in a Beckman DB spectrophotometer. In our mechanism studies, the temperature of the cells was controlled to ±1 °C by a Tamson TEZ9 circulator and a Neslab Instrument PBC-4 bath cooler.

(b) Preparation of 2,3-Dichloro-1-propanol (2,3-DP)

Chlorine gas was bubbled through cooled pure allyl alcohol contained in 500 ml gas washing bottles until glc analysis showed that the reaction was complete. Solvents were not used as they were found to be difficult to remove from the 2,3-DP. A temperature programmed run from 110 to 200 °C at a rate of 4 °C/min adequately separated the reaction products on the glc column. The 2,3-DP eluted in approximately 12 min. The chromatogram showed that there were three major impurities and several minor components. Only a trace of 1,3-DP was present. If the reaction was not taken to completion a considerable amount of allyl chloride was detected. If, however, the reaction was continued until the solution turned yellow due to excess chlorine no allyl chloride was found. Presumably one of the other impurities is the completely chlorinated product of allyl chloride.

The solution also contained a considerable amount of HCl either from the cylinder gas or from a side reaction. To purify the 2,3-DP, water was added and the acid was neutralized to p*H* 6–7 with sodium bicarbonate or carbonate. The neutralized solution was separated from the sodium chloride, washed with water, and then dried over anhydrous sodium sulphate. The impurities were distilled from the 2,3-DP at a pressure of a few torr and a maximum temperature of 92 °C. The 2,3-DP darkens above this temperature and rapidly decomposes above 120 °C. The 2,3-DP left in the reaction flask had a slight reddish-brown tinge and was quite viscous.

(c) Carr–Price Procedure

A 25% $SbCl_3$ solution in $CHCl_3$ (3.5 ml) was rapidly added from a wide-mouthed pipet to 1 ml of vitamin A solution contained in a 1.13 cm pathlength Spectronic 20 cell mounted in the spectrometer. Readings were taken at the 'pause' point, 5 s after mixing (temperature 32 °C).

(d) Rate Study Procedure

All chemicals and glassware were cooled to the spectrophotometer's cell temperature in the main tank of the circulator. Nitrogen gas was circulated through the cell compartment of the spectrophotometer to prevent fogging of the cells. Activated 2,3-DP (3 ml) or a total of 3 ml of activated 2,3-DP plus solvent containing the requisite amount of antimony trichloride were placed in a test-tube in the tank of the circulator. A solution of vitamin A in the appropriate solvent (1 ml) was added, the solution agitated for 5–10 s on a vortex mixer and then poured into the precooled sample cell of the double beam spectrophotometer. Mixing was satisfactory at 10 °C but inclusion of air bubbles made results at 0 and −10 °C difficult to obtain.

The blank consisted of all components of the solution except vitamin A. The signal from the spectrophotometer was recorded on a 10 in. Beckman potentiometric recorder.

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