[CONTRIBUTION FROM THE DEPARTAMENTO DE QUIMICA ORGANICA DEL INSTITUTO "ALONSO BARBA" DE QUIMICA (C. S. I. C.)]

ADDUCTS OF VITAMINS A_1 AND D_2 WITH *p*-BENZOQUINONE

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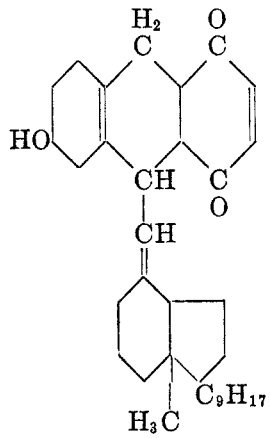
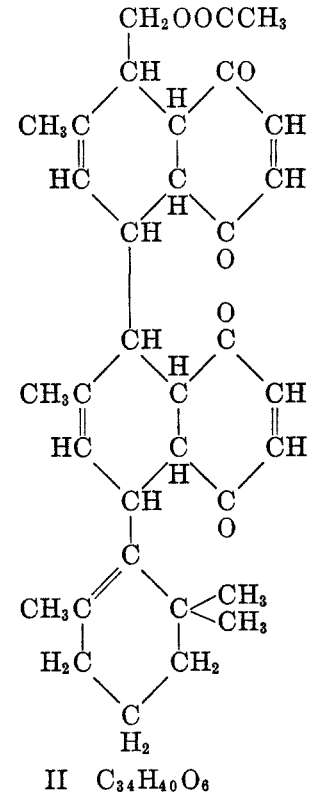
The adduct of Vitamin D_2 acetate and maleic anhydride was prepared by Windaus and Thiele (1). The formation of the adduct is said to be quantitative, although no numerical results in support of this are cited in their paper. Robeson and Baxter (2) described a method to estimate the percentage of neovitamin A in mixtures of this vitamin with Vitamin A; the method is based on the difference in reactivity of both vitamins with maleic anhydride. Kawakami and Hamano (3) had previously isolated the adducts of Vitamin A acetate and palmitate with maleic anhydride. These adducts are in the ratio of 1 mole of Vitamin A per 2 moles of philodiene. These are the only references found pertaining to the application of diene synthesis to vitamins for analytical purposes.



I $C_{34}H_{48}O_3$



II $C_{34}H_{40}O_6$

The application of our dienometric method using *p*-benzoquinone (4), to both vitamins A and D_2 offered special interest in connection with their estimation in preparations containing both vitamins. In the case of vitamin D_2 the formation of the acetate is not necessary since *p*-benzoquinone does not react with the alcoholic group.

As we did in cases previously studied (4), we first proved the addition of *p*-benzoquinone to the vitamins by the isolation and characterization of the corresponding adducts, which have not been described before. Vitamin D_2 reacts with *p*-benzoquinone in boiling benzene giving a 1:1 adduct $C_{34}H_{48}O_3$ (I). This was isolated as a yellow, non-crystalline powder melting at 95–100° with softening.

Vitamin A_1 acetate, under similar conditions, yields an adduct of 1 mole of vitamin with 2 moles of *p*-benzoquinone $C_{34}H_{40}O_6$ (II) formed by independent addition of the quinone to each of the two conjugations of the vitamin as in the case of vitamin A acetate and palmitate with maleic anhydride reported by Kawakami and Hamano (3).

In order to establish the optimum conditions of dienometry, we have studied in each case the influence of temperature, time of reaction, and relative concentrations of vitamin and philodiene. The estimation of *p*-benzoquinone in excess and, when necessary, of the hydroquinone formed, were carried out respectively with $N/100$ solutions of sodium thiosulphate and iodine, according to the technique already described (4).

The best results for Vitamin D_2 were obtained by heating the vitamin for three hours at 100° using an amount of quinone fifteen times in excess of theory for normal reaction with the vitamin. For the same period and using a five-fold excess of quinone, vitamin A gave quantitative yields. Correct results were obtained in the estimation of both vitamins together, using a strong excess of quinone and working at the same temperature and time as before.

EXPERIMENTAL

Adduct of vitamin D_2 with p-benzoquinone. Vitamin D_2 (5 g.) and *p*-benzoquinone (1.5 g.) in benzene (50 ml.) were refluxed for four hours. The solution took on a red color. During the reaction quinhydrone was formed; it crystallized on cooling and was removed. The benzene was distilled off, and the red-brown viscous residue was dissolved in alcohol and reprecipitated by the addition of water. By repeating this treatment several times a viscous oil was obtained which was dried 2–3 hours *in vacuo* on the steam-bath. The oil changed to a yellow powder melting at 95–100° with softening.

Anal. Calc'd for $C_{34}H_{48}O_3$: C, 80.95; H, 9.52.
Found: C, 81.05; H, 9.66.

Oxime. Hydroxylamine hydrochloride (5 g.) and sodium hydroxide (2 g.) in water (50 ml.) were added to a solution of the adduct (2 g.) in sufficient alcohol to get a clear solution; the mixture was refluxed for two hours. After cooling, water in excess was added and the white product obtained was filtered and washed with water. It was purified by dissolving in alcohol and precipitating with water. After repeating this treatment two or three times a white product was obtained melting at 140° (decomp.).

Anal. Calc'd for $C_{34}H_{49}NO_3$: N, 2.69. Found: N, 2.44.

Adduct of vitamin A_1 acetate with p-benzoquinone. A solution of vitamin A_1 acetate (0.3 g.) in alcohol (25 ml.) was mixed with another solution of *p*-benzoquinone (0.3 g.) in alcohol (25 ml.) The red solution formed was refluxed for five hours without formation of quinhydrone. The alcohol was distilled off and the dark brown viscous product obtained was dissolved in alcohol and precipitated with water. This treatment was repeated three times to ensure the elimination of excess quinone. The dry product, a vitreous solid, melted at 148–150° with softening.

Anal. Calc'd for $C_{34}H_{40}O_6$: C, 75.00; H, 7.35.
Found: C, 75.11; H, 7.62.

Oxime. Hydroxylamine hydrochloride (0.5 g.) in sodium hydroxide (10 ml. of a 10% solu-

tion) was added to the adduct (0.2 g.) in alcohol (25 ml.); the mixture was refluxed for one hour. On cooling no crystallization occurred; on addition of water a reddish powder was precipitated. This was filtered and washed with water, dissolved in alcohol, and reprecipitated by adding water. The dry product melted at 135–140°.

TABLE I

DIENOMETRIC ESTIMATION OF VITAMIN D_2

T, °C.	TIME (HOURS)	MOLE QUINONE / MOLE VITAMIN	AMOUNT OF VITAMIN		ERROR, %
			Taken (mg.)	Found (mg.)	
75	10	11	4.04	3.43	15
100	10	11	4.04	3.61	10
100	3	11	3.98	3.83	4
100	3	14	3.98	3.90	2
100	3	18	4.00	3.92	2
100	3	18	2.00	1.97	1.5

TABLE II

DIENOMETRIC ESTIMATION OF VITAMIN A_1 ACETATE

T, °C.	TIME (HOURS)	MOLE QUINONE / MOLE VITAMIN	AMOUNT OF VITAMIN		ERROR, %
			Taken (mg.)	Found (mg.)	
100	3	1.5	48.00	34.00	29
100	3	5	44.00	44.00	0
100	6	5	44.00	14.80	6
100	3	7	37.00	37.00	0
100	3	7	20.00	19.80	2

TABLE III

DIENOMETRIC ESTIMATION OF VITAMIN D_2 AND VITAMIN A_1 ACETATE

T, °C.	TIME (HOURS)	MOLE QUINONE / MOLE VITAMIN	VITAMIN TAKEN		QUINONE, G.		ERROR, %
			D_2 (mg.)	A_1 (mg.)	Calc'd	Found	
100	3	5	3.28	39.6	0.0269	0.0134	50
100	3	20	4.01	44.8	0.0305	0.0299	2

Anal. Calc'd for $C_{34}H_{41}NO_6$: N, 2.36. Found: N, 2.50.

Dienometric estimation of vitamins D_2 and A_1. The results are given in Tables I, II and III.

In view of the above results the following titration method is proposed.

Dissolve the sample of Vitamin D_2 or A in absolute alcohol and dilute to a known volume. Place a 2-ml. aliquot of this alcoholic solution in a 15 × 1.5 cm. glass tube. Then add a portion of an alcoholic solution of *p*-benzoquinone (1.08 g. per 100 ml. of absolute alcohol distilled over sodium) sufficient to give a 15–20 fold molar ratio of *p*-benzoquinone to Vitamin D_2 or a 6–8 fold molar ratio of *p*-benzoquinone to Vitamin A_1.

At the same time a blank assay is prepared by adding an equal portion of the *p*-benzoquinone solution to another glass tube containing 2 ml. of absolute alcohol.

Both tubes are then sealed and heated in an oven at 100° for three hours. At the end of

this time each tube is cooled, opened, and the contents transferred quantitatively, by washing with absolute alcohol, to 50-ml. volumetric flasks and diluted to volume.

For the titration of the hydroquinone, a 2-ml. aliquot of the solution from the volumetric flask containing the Vitamin D_2 or A_1 is placed in a 300-ml. Erlenmeyer flask, diluted with 100 ml. of distilled water, and made alkaline by adding 20 ml. of a saturated sodium bicarbonate solution. Several drops of starch solution are added and the contents are titrated with $N/100$ iodine solution. The iodine solution is added dropwise until a faint blue color persists. The hydroquinone thus titrated is converted to *p*-benzoquinone by the following expression:

$$X = \frac{\text{ml. } N/100 \text{ iodine soln.} \times 0.54}{1000}$$

For the titration of the excess *p*-benzoquinone another 2-ml. aliquot from the same volumetric flask containing the vitamin is placed in a 300-ml. Erlenmeyer flask and diluted with 100 ml. of distilled water. Then 1 ml. of 30% potassium iodide solution and 1 ml. of concentrated hydrochloric acid are added. The mixture is stirred for one minute and the free iodine is determined with $N/100$ sodium thiosulphate solution. The quinone thus titrated is given by the following expression:

$$X' = \frac{\text{ml. } N/100 \ Na_2S_2O_3 \text{ soln.} \times 0.54}{1000}$$

The blank assay is processed in the same manner and the values resulting from the iodine titration and the thiosulphate titration are expressed as Y and Y'. The amount of *p*-benzoquinone that is condensed by reaction with the amount of vitamin D_2 or A_1 present is determined from the following expression:

$$\text{grams of } p\text{-benzoquinone condensed} = (Y + Y') - (X + X')$$

Finally:

$$\text{grams of } p\text{-benzoquinone condensed} \times 3.666 = \text{g. Vitamin } D_2$$

$$\text{grams of } p\text{-benzoquinone condensed} \times 1.518 = \text{g. Vitamin } A_1 \text{ acetate}$$

SUMMARY

The adducts of vitamins A_1 acetate and D_2 with *p*-benzoquinone and their monoöximes were prepared.

A dienometric method, using solutions of *p*-benzoquinone as the philodiene, has been described and was applied to the quantitative estimation of each vitamin and of mixtures of both. The most suitable conditions of temperature and molar ratios for the estimation were also determined.

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