LXXXIII. NOTE ON THE ULTRAVIOLET ABSORPTION OF CRYSTALLINE PRE- PARATIONS OF VITAMIN B₁.

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SINCE Peters and Philpot [1933] stated that two samples of crystalline vitamin B₁ prepared at Oxford showed absorption maxima at 247 $m\mu$, whereas other workers [Heyroth and Loufbarrow, 1931] had correlated vitamin B₁ activity with absorption about 260 $m\mu$ it seemed doubtful whether any relation existed between absorption in the ultraviolet region of the spectrum of vitamin B₁ preparations and their biological activity.

Subsequently the opportunity occurred of examining several crystalline samples of vitamin B₁ prepared independently in three different laboratories, namely two samples from Prof. Peters, two from Prof. Windaus, and two from Dr Van Veen to whom grateful acknowledgment is made for the opportunity of making the comparison.

The object of the investigation was to discover what differences, if any, in the ultraviolet absorption existed between the samples of vitamin B₁ at hand when examined under similar conditions. The observation of Peters and Philpot [1933] that on rendering an acid solution of vitamin B₁ alkaline there was a shift of the maximum absorption to shorter wave-lengths and the appearance of a new and labile maximum (330–340 $m\mu$) suggested a basis of comparison. Since comparison of the changes of absorption in samples of unknown constitution obtained by varying p_H is likely to be more sensitive to differences in composition than a single determination at a given p_H , absorption measurements were made on all six samples in $N/200$ HCl at successive time intervals after bringing to a p_H between 11.0 and 12.0, and then subsequently after reacidification.

The effect on the absorption of varying the solvent was also tried.

EXPERIMENTAL.

The absorption measurements were made with a Hilger quartz E 316 spectrograph with Spekker photometer [Twyman, 1931; Twyman and Allsopp, 1934], using a condensed spark between tungsten steel electrodes as light source.

A weighed amount of each sample was originally dissolved in 75 % alcohol containing $N/200$ HCl and examined in a suitable layer thickness (0.5–2.0 cm.). The solution was diluted with 75 % acid alcohol to give a suitable density value in a layer of 5 cm. thickness and measured again. It was found that over this small range of dilution (22 to 10 times) Beer's law is followed within the limits of determination (± 2 %).

The 5 cm. cell was of standard Hilger type with centre filling funnel. For the determination of the alkali change a suitable volume (obtained by trial) of *N* NaOH was added through the funnel and the tube shaken for a few seconds, the thumb occluding the funnel opening, the enclosed bubble acting as mixer. The absorption was measured at once and then at intervals of 5 mins.

The time taken to expose a plate with the method here used is about 3 mins., and from the results of succeeding measurements it was evident that during this time some change in absorption had taken place. The absorption curves of the alkaline solutions are therefore mixed curves and cannot be taken as having accurate quantitative significance. Nevertheless, comparison of the curves with those obtained by Peters and Philpot [1933], who used a method requiring only 5–15 secs. exposure [Philpot and Schuster, 1933], shows quite good agreement.

RESULTS.

The results of the determinations are grouped in Table I, which shows the values obtained for the six samples in *N*/200 HCl in 75 % alcohol. The extinction coefficient has its usual meaning and *c* is in g. per litre. In order that the

Table I.

Source and designation	Salt	ϵ/c for salt	% free base in salt	ϵ/c for free base	Wave-length of maximum, $m\mu$
1. 26 (59)	Sulphate	40.2	65	61.9	247
2. 26 (52)	"	31.8	60	53	246
3. 61 A (15)	Hydrochloride	46.4*	78.5	59.1	247
4. 64 (19)	"	45.0*	—	—	247
5. Windaus I	"	38.8	—	—	245
6. Windaus II	"	45.6	78.5	60.6	247
		45.3†			
7. Van Veen I	"	39.3	—	—	245
8. Van Veen II	"	40.4	—	—	245

1–4 Prepared by Kinnersley *et al.* [1935].

* Quoted from Peters and Philpot [1933].

† Determination by Prof. Pohl, private communication by Prof. Windaus.

extinction coefficients of the sulphates and hydrochlorides should be comparable, they have been reduced to correspond to concentration of free base. The close correspondence between 61 A (15) and 64 (19) and Windaus II is very remarkable, and after reducing to free base value this is extended to 26 (59). The discrepancy in 26 (52) is discussed by Kinnersley *et al.* [1935]. The samples Van Veen I and II and Windaus I are also very similar and differ from the former group in having a markedly lower extinction coefficient and a maximum at λ 245 instead of λ 247 $m\mu$.

Fig. 1 shows the absorption curves of 26 (59), Windaus II and Van Veen II in *N*/200 HCl in 75 % alcohol.

All six examples showed the alkali change of Peters and Philpot. As stated above, the method employed is really too slow for the making of measurements of rates of change occupying only about 15 mins. It is not surprising therefore that no difference in this respect was noticeable among the six samples.

The solvent was found to have great influence upon the absorption. Fig. 2 shows different absorption curves obtained with the same sample of vitamin. If the vitamin is dissolved in neutral 80 % alcohol and then acidified, it does not show the same absorption as when dissolved originally in acid alcohol. The same applies to aqueous solutions.

It will be noted that in neutral alcoholic solution the vitamin shows two well-defined bands at 234 and 268 $m\mu$. These correspond very closely with the bands found by Heyroth and Loufbarrow [1934] in some specimens of vitamin B₁ [see also Smakula, 1935]. In simple distilled water the absorption curves

Fig. 1.

Fig. 1. Vitamin B₁. 1 ..... 26 (59); 2 ——— Windaus; 3 ——— Van Veen.

Fig. 2.

Fig. 2. Vitamin B₁ hydrochloride. 1 ..... Dissolved originally in acid alcohol; 2 ----- 80 % alcohol acidified; 3 ——— 80 % alcohol.

obtained with the vitamin are poorly reproducible. The reason for this was traced to the fact that between p_H 5.5 and 7.0 the vitamin is extremely sensitive to change in hydron concentration, and the slight variations in the p_H of the distilled water used were sufficient to cause marked changes in the absorption curve. All curves obtained under these conditions are mixtures of curves 2 and 3, Fig. 2. The importance of measuring the absorption of the vitamin under standard conditions of hydron concentration is therefore obvious.

DISCUSSION.

It appears that the purest samples of crystalline vitamin B₁ in acid alcohol all show selective absorption with a maximum at 245–247 $m\mu$. That others should have correlated vitamin B₁ activity with absorption at 260 $m\mu$ is difficult to explain. In this regard Prof. Windaus is now in agreement that his specimens show a maximum at 247 $m\mu$ (private communication to Prof. Peters) as against a previously published maximum at 260 $m\mu$.

The marked influence of hydron concentration on the absorption of vitamin B₁ has no doubt been a source of confusion. There is the additional possibility that in less pure samples the impurities not only may have maxima about 260 $m\mu$, but may also alter the absorption of the vitamin.

It also appears that vitamin B₁ preparations of highest activity give very similar absorption curves. The extinction coefficients, the behaviour of the absorption on changing the hydron concentration and the maxima of absorption are almost identical.

It is therefore probable that the absorption measured is that of the vitamin B₁ molecule, and that in the three laboratories from which the above samples are derived one and the same entity is being used.

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