Polarographic Studies on Fat-soluble Vitamins in Nonaqueous Media

Part IV.* Polarography of Vitamin D in Acetonitrile as Solvent

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Vitamin D₂ and vitamin D₃ gave a single reduction-wave, respectively, in polarography in acetonitrile containing 0.05 M-tetrabutylammonium iodide. The reduction-wave of vitamin D₃ was indistinguishable from that of vitamin D₂, whose half-wave potentials were evaluated identically at -2.1_0 V vs. Hg. Proportionalities between wave-height and concentration were held in the concentration range from 4×10^{-4} M (ca. 6000 IU·ml⁻¹) to 1.2×10^{-3} M (ca. 18000 IU·ml⁻¹).

In both cases, the conjugated three double-bond system may be reduced to two double-bond system, which corresponds to the third reduction-step of vitamin A.

Ergosterol which has a conjugated two double-bond system and an isolated double-bond gave no reduction-wave under the present conditions.

INTRODUCTION

Vitamin D₂ and D₃, whose structures have been established as derivatives of sterins, are especially famous in vitamin D-substances and their vitamin D-effects are almost equal to each other. Each of them has a conjugated three double-bond system on which their color reactions are generally based¹⁾.

It has been reported that vitamin D, as well as vitamin A, gives no reduction-wave under ordinary conditions in polarography²⁾. However, conjugated double-bond system, unless it is so short, may give reduction-wave in suitable media as described in Part II³⁾ of this series of works. It has been also polaro-

graphically known that isolated double-bond and conjugated two double-bond system can scarcely cause reduction-waves in any solvent with ordinary supporting electrolyte. In the case of vitamin D, the reduction potential must be very negative because the number of conjugated double-bonds is only three, and adoption of aprotic solvent such as acetonitrile must be needed.

EXPERIMENTAL

Crystallines of vitamin D₂ (ergocalciferol), D₃ (cholecalciferol) and ergosterol (provitamin D₂) were reagents of commercial extra pure grade. Tetrabutylammonium iodide obtained as described in Part I⁴⁾ of this series of works also served as supporting electrolyte* in the present study. Acetonitrile as solvent

* Part III: This Journal, **26**, 777 (1962).

1) e.g. H. Schaltegger, *Helv. Chim. Acta*, **29**, 285 (1942).

2) Y. Asahi, *Rev. Polarography (Japan)*, **8**, 1 (1960).

3) R. Takahashi and I. Tachi, This Journal, **26**, 771 (1962).

4) I. Tachi and R. Takahashi, This Journal, **26**, 238 (1962).

* Aged reagent of tetrabutylammonium iodide contains decomposition-products which badly injure the polarogram. Then, it can be easily refreshed by two or three times recrystallizations with pure benzene.

and nitrogen gas for de-aeration were purified in the same manner as previously described⁹.

Stock solutions were 2×10^{-3} M in acetonitrile for vitamin D and 0.5 M in acetonitrile for tetrabutylammonium iodide. The final concentration of supporting electrolyte was 0.05 M, so that the composition of basal electrolytic solution was the same as that in the study on vitamin K reported in Part I.

Conditions and procedures of all experiments, as well as instruments, were the same as adopted in the previous investigations.

RESULTS AND DISCUSSION

(1) Reduction-wave of Vitamin D₂.

As shown in Fig. 1, vitamin D₂ showed a single reduction-wave in the given medium at a potential close to the end-rise caused by depolarization of the supporting electrolyte.

As residual current was considerable, wave-height was defined as the difference between the height at the inflection point of current-potential curve and the corresponding height of the blank curve. Wave-heights measured in this manner were plotted in Fig. 2 against the corresponding concentrations of vitamin D₂. As a whole, proportionality between

wave-height and concentration can be established in the concentration range from 4×10^{-4} M (ca. 6000 IUD·ml⁻¹) to 1.2×10^{-3} M (ca. 18000 IUD·ml⁻¹), though the plotted points are somewhat scattered. Appearances of the dependency of polarogram on the concentration are also illustrated in Fig. 1.

The half-wave potential in acetonitrile containing 0.05 M-tetrabutylammonium iodide was -2.1_0 V vs. Hg (mercury-pool electrode in the same electrolytic solution), which can be converted into -2.9_8 V vs. NCE_{aq} (aqueous normal calomel electrode) by the method of pilot ion as employed in Part I⁴). The half-wave potential seemed to be independent of the concentration of depolarizer.

(2) Results on Vitamin D₃ and Ergosterol.

Vitamin D₃ and ergosterol were also treated in the same medium as above adopted. Vitamin D₃ gave such a single reduction-wave which could not be distinguished from that of vitamin D₂. The half-wave potential and the behavior of the limiting current were the same as those of vitamin D₂.

FIG. 1. D.C. Polarograms of Vitamin D₂ in Acetonitrile Containing 0.05 M-Bu₄NI.

The concentrations of vitamin D₂ are (a) 1.2×10^{-3} M (Definition of wave-height; broken line is the blank-current.), (b) 8×10^{-4} M, (c) 4×10^{-4} M, (d) 0 M (blank).

Ergosterol gave no reduction-wave in the

FIG. 2. The Relationship between Wave-height and Concentration of Vitamin D₂.

potential range which could be realized before the end-rise in acetonitrile containing tetrabutylammonium iodide as supporting electrolyte.

(3) Reduction Mechanism.

The reduction mechanism of vitamin D was studied with a.c. polarography in 60 vol% benzene-acetonitrile mixture containing 0.1 M-tetrabutylammonium iodide which is the same medium as adopted in the studies on vitamin A in Parts II & III. In Fig. 3, shown are a.c. polarograms of vitamin D₃ and reduction-products of vitamin A-palmitate obtained by electrolyses at controlled potentials as describ-

FIG. 3. A. C. Polarograms in 60 vol% Benzene-Acetonitrile Mixture Containing 0.1 M-Bu₄NI.

(a,b,c) 7.5×10^{-4} M-Vitamin A-palmitate, (a) before electrolysis, (b) after electrolysis at constant potential of -1.45 V vs. Hg, (c) after electrolysis at constant potential of -1.90 V vs. Hg; (d) 4×10^{-4} M-Vitamin D₃.

ed in Part III.

When vitamin A-palmitate is reduced stepwise by electrolyses at fixed potentials of -1.45 V and -1.90 V vs. Hg, the third reduction-wave becomes distinct. The a.c. polarogram of vitamin D_3 shows the reduction-wave corresponding to the third wave of vitamin A-palmitate which was defined as reduction of conjugated three double-bond system in Part III. Thus it may be concluded that the reduction-wave of vitamin D_3 is caused by reduction of this conjugated three double-bond system.

It was also confirmed in the present experiments that polarographic behaviors of vitamin D_2 and D_3 are the same. This suggests the polarographic reduction of vitamin D_2 is also based on the conjugated three double-bond system and not influenced by the isolated double-bond in its side-chain. Therefore, isolated double-bond and, as shown by the fact that ergosterol gives no reduction-wave, conjugated two double-bond system are unable to cause any reduction-wave in the present medium.

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