ON THE NATURAL OCCURRENCE OF UBICHROMENOL

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The presence of ubichromenol in the unsaponifiable fraction of animal lipids was first reported by Lowe et al.[1] in 1953, and quantitative data have since been presented for its concentration in liver[2], kidney[3] and other organs. Laidman and coworkers[4] recently have established that this compound is a hydroxychromene isomer of ubiquinone.

In the course of an investigation of the substances in animal tissues which interfere in the determination of vitamin E using the Emmerie-Engel reagent[5], it was observed that a strongly reactive compound was formed upon refluxing ubiquinone in ethanolic KOH. This compound was isolated on alumina (Bio-Rad Laboratories, Brockmann III) by developing the column with increasing proportions of peroxide-free diethyl ether in distilled petroleum ether (30° - 60°), the reactive principle being eluted with 10% diethyl ether. Upon spectroscopic examination this compound was found to have absorption characteristics identical with those for ubichromenol[2] (λ_{max} in cyclohexane at 275 and 330 mμ, with marked inflections at 233 and 283 mμ).

After crystallization four times from ethanol, the identity of the compound was confirmed by synthesis of the acetate ester (acetic anhydride in toluene containing a small amount of anhydrous pyridine) which exhibited the loss of selective absorption at 330 mμ characteristic of ubichromenol acetate[2]. Catalytic hydrogenation using palladium on $BaSO_4$ gave a λ_{max} at 290 mμ for perhydroubichromenol and oxidation of this product with $FeCl_3$ in ethanol resulted in a shift in absorption to λ_{max} 279 mμ.

The observation that ubichromenol can be formed from ubiquinone under these circumstances raised the question of its natural occurrence in animal tissues. As all analytical data present in the literature were obtained by procedures involving saponification, it was decided to investigate the occurrence of ubichromenol in animal lipids by a method which avoided the use of alkali.

In studies on the isolation of vitamin E and its metabolites in this laboratory, it has been found possible to separate these compounds efficiently from the bulk of animal lipids, without saponification, by fractional crystallization from ethanol at -70° C. As ubichromenol is readily crystallized from ethanol, other solvents were investigated and acetone was found to be suitable. Preliminary tests using lipid that had been obtained by ethanol extraction of visceral adipose tissue from pigs, showed that this material could be effectively crystallized from acetone at a dilution of 1:30 (w/v) by immersion in a dry ice-acetone bath for 1 hour. Separation of the crystallate was achieved by filtration under suction through Whatman No. 42 paper using a pyrex funnel fitted with a dry ice-acetone jacket. At a concentration of 0.2 mg per ml no crystallization of ubichromenol from acetone was observed at this temperature. The recovery of ubichromenol from a solution of visceral lipid in acetone (1:30, w/v) was determined after crystallizing the lipid and washing 6 times with precooled acetone. At a concentration comparable to that reported to be present in kidney lipid[3], 84% was recovered in the filtrate.

A sample of 3.6 kg of fresh ground pig kidneys was divided into 3 equal parts. Ubichromenol was isolated from the first portion by the procedure outlined by Mervyn and Morton[3]. The same procedure was applied to the second portion after the addition of 50 mg of ubiquinone prior to saponification. The third fraction was dried with anhydrous Na_2SO_4 and extracted in darkness for 16 hours by shaking with 4 liters of absolute ethanol. This procedure had previously been found to be an efficient means of extracting other lipids from animal tissues. After filtering and washing with ethanol, the filtrate was dried over Na_2SO_4 and the solvent was removed <u>in vacuo</u> at 70° C. under nitrogen. The residue, weighing 32 gm, was dissolved in 100 ml of diethyl ether, 500 ml of

acetone was added and the solution was stored overnight at -20°C. The phospholipids were removed by filtration and washed with 500 ml of cold acetone. Evaporation of the filtrate left a residue weighing 11.5 grams. This material was dissolved in 350 ml of acetone, placed in a -70° C. bath for 1 hour and filtered as previously described. The crystallate was washed 6 times with 30 ml of acetone precooled to -70° C. and the residue obtained by evaporation of the solvent was dissolved in petroleum ether. The material then was chromatographed on alumina by the same method used for the unsaponifiable fractions obtained from the other two portions of the original kidney sample.

The first portion of the sample, which was subjected to the procedure of Mervyn and Morton[3], yielded 11.0 mg of ubichromenol, in part as the acetate ester. This figure represents a concentration of 9.2 μg per gm of fresh tissue, which is in good agreement with the values reported by these investigators. The second fraction yielded 28.2 mg of ubichromenol (equivalent to 23.5 μg per gm of tissue), indicating that about 35% of the 50 mg of ubiquinone added prior to saponification had been converted to ubichromenol in the course of the procedure. No evidence was obtained for the presence of ubichromenol in that portion of the kidney sample which had not been subjected to saponification.

These results indicate that ubichromenol is not a natural constituent of animal lipids but is formed by cyclization of ubiquinone in the presence of alkali with a corresponding reduction from 10 to 9 in the number of isoprene units in the side chain. The amount of ubichromenol obtained is influenced by refluxing time, maximum yields being obtained after about 30 minutes. When ubiquinone is refluxed in ethanolic KOH for 2 hours the main product is a dark yellow compound which is fluorescent in ultraviolet light and is eluted from alumina only with highly polar solvents.

Recently, Links[6] has reported that ubiquinone is partially converted to ubichromenol on alumina, the rate of conversion being decreased by the addition of water. In the present studies 6% water was added to the alumina used for chromatography and the conversion on the column, if any, was minor in comparison with that effected by alkali.

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