UBIQUINONES AND SOLANESOL IN HUMAN AND RAT TISSUES

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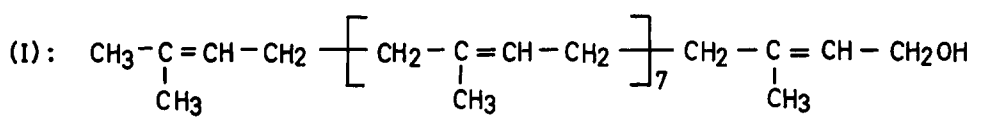
Received February 15, 1960

From the results available up to now it seems clear that among the different forms of ubiquinones, ubiquinone(50) is preferred in the animal tissue (Lester and Crane, 1959). An exception is the rat liver. In vitamin A deficient rats about 90% of the total amount of ubiquinone was found to be ubiquinone(45) and only 10% were identified as ubiquinone(50) (Rüegg et al., 1959)(Gloor and Wiss, 1959a). Using normal rat livers, about the same ratio of these two isoprenologues could now be detected.* Furthermore it seemed interesting to examine whether the occurrence of ubiquinone(45) in the higher animal is typical for liver tissue. Therefore, ubiquinone from human liver was isolated and identified. Only one form, the ubiquinone(50), was present.

In the course of our isolation work an unsaturated alcohol with isoprene structure could be obtained in crystalline form. It could be shown that this substance, isolated from human heart and liver, was identical with solanesol (I):



Rowland et al. (1956), who first isolated solanesol from different types of tobacco, favoured a chain length of 10 isoprene units. Later on, however, it could be definitely proved that the molecule contains only 45 carbon atoms, e.g. nine isoprene units (Kofler et al., 1959; Erickson et al., 1959). The amount of solanesol estimated by the intensity of the colour produced by exposition to iodine vapour after separation by thin layer chromatography, was 4 to 6 µg per gram heart, and 20 to 50 µg per gram liver tissue. Thus a human heart contains approximately 2 to 5 mg a human liver approximately 30 to 80 mg of solanesol.

In previous investigations (Gloor and Wiss, 1959b) it could be shown that

* This agrees with recent findings of R.E.Olson, G.H.Dialameh and R.Bentley (personal communication).

ubiquinone(50) is synthesized by the rat liver, using mevalonic acid as a precursor. We have subjected a biosynthetic ubiquinone mixture, highly purified, to paper chromatography, which allows a clear separation of different isoprenologues, and found that both, ubiquinone(45) and ubiquinone(50), were labelled. 84% of the radioactivity corresponds to the first, and 16% to the latter homologue.

The question arose whether solanesol is involved in the ubiquinone metabolism, as a precursor or as a degradation product. Using the same isolation procedure as for human tissue, it could be demonstrated that in the rat liver solanesol is present, but only in very minor amounts. Solanesol isolated from rat liver, after administration of ^{14}C-labelled mevalonic acid, turned out to be non-radioactive.

Preliminary investigations have shown that solanesol, besides its occurrence in tobacco, is also present in varying amounts in other plant materials. It seems therefore probable that the solanesol found in animal tissues is supplied by nutritional ingredients.

Experimental

(a) Isolation of ubiquinones

From 502 g of livers from rats, kept on a normal stock diet, ubiquinones were isolated as described previously (Rüegg et al., 1959). After saponification and chromatography on alumina (Brockman activity I + 7% H_2O) 220 mg of a crude mixture of ubiquinones were obtained. By partion chromatography on a column of 23 g polyethylene powder (Hostalen W) ubiquinone(45) and (50) were separated, yielding the same ratio of these two isoprenologues as previously found in vitamin A deficient liver, e.g. about 90% ubiquinone(45) and 10% ubiquinone(50).

Three human livers (1328 g, 1144 g, 990 g) were treated in the same manner. After chromatography on alumina the three samples were pooled. 990 mg of crude ubiquinone containing about 220 mg pure substance, estimated by UV.-spectra, were distributed in a counter current apparatus (Craig) with isooctane and methanol as solvents. After 25 partition steps the fractions 18 to 21 were pooled. Their residue (138 mg) was chromatographed on a column containing 15 g of polyethylene powder (Hostalen W) using mixtures of increasing amounts of acetone in water (210 ml 75%, 450 ml 80%, 500 ml 85%, and 300 ml pure acetone).

(b) Isolation of solanesol from human liver and heart

Human liver (3462 g) and heart (774 g) was worked up as described above. The fractions obtained by column chromatography were subjected to thin layer chromatography. This method, as described by Stahl (1958, 1959) turned out to be useful for detecting solanesol. Silica gel G (Merck, Darmstadt) was used as adsorbent,

Table I

No. of fractions	Solvent in ml	Residue in mg	Melting Points
1	1-610	20,7	Oil, no ubiquinone spectrum
2	610-624	1,0	48-49°
3	624-638	2,0	49-51°
4	638-652	2,2	50°
5	652-666	2,0	49-50°
6	666-680	1,2	49°
7	680-708	1,9	50°
8	708-757	2,3	49°
9	757-1470	84,4	Oil, no ubiquinone spectrum

Paper chromatography of the different fractions (Table I) revealed only spots corresponding to ubiquinone(50).

2% methanol in benzene as solvent. By iodine vapour as developing agent, the solanesol spots could be made visible. The fractions containing solanesol were pooled and rechromatographed on alumina (activity I + 7% water). The residue of the solanesol fractions (225 mg) was acetylated in 5 ml acetic anhydride and 2 ml pyridine, and worked up according to standard procedures. After repeated chromatography on alumina, the solanesyl-acetate was saponified and the resulting alcohol chromatographed on alumina. 50 mg of pure crystalline solanesol was obtained (mp. 38°). It was identified by comparison with solanesol obtained from tobacco on the paper chromatogram , and by mixed melting point. An isoprenologue of solanesol prepared synthetically by addition of one isoprene unit to solanesol (Rüegg et al 1959) could be clearly distinguished from it on the paper chromatogram . For further identification solanesol, isolated from human livers, was acetylated with ^{14}C-acetic anhydride and pyridine. Thus 31.8 mg of ^{14}C-solanesyl acetate were obtained, added to 470 mg of pure unlabelled solanesyl acetate, and crystallised 5 times from the 10-fold amount of pure acetone at -10°C. The specific activities of samples of consecutive crystallisations were: 357, 314, 315, 309 and 333 cpm/m 200 mg of mother liquor from the first two crystallisations were subjected to a 25-fold Craig counter current distribution between isooctane and methanol. The three fractions containing the largest amount of material (119 mg) were redistributed once again in the same solvent system. After a third distribution we isolate 30 mg in the main fraction. This was crystallized once from acetone and the specific activity determined as 343 cpm/mg (all counting was performed in a liquid scintillation counter).

(c) Solanesol from rat liver

320 mg of unsaponifiable material from 60 g of rat liver was chromatographed twice on alumina as described above. The fractions containing solanesol according to thin layer chromatography were pooled and purified further by the same technique on a preparative scale. For this purpose 20 mg of substance were put as a streak on a starting line of 15 cm. Material thus obtained was identified by paper chromatography before and after acetylation, using solanesol from tobacco as reference substance.

Livers of 55 rats, each having been dosed with 500 µg of ^{14}C-mevalonic acid (Gloor and Wiss, 1959b) intraperitoneally, were saponified and repeatedly chromatographed on alumina. Fractions eluted after ubiquinone but before cholesterol contained the solanesol and trace amounts of these other two substances. They were combined, evaporated and the residue (less than 10 mg) mixed with 500 mg of pure unlabelled solanesol, isolated from tobacco. The total radioactivity of this mixture was about 200,000 cpm. By five subsequent crystallisations from the 30-fold amount of methanol at 0°C the activity dropped from about 400 to 171, 49, 3 and 0.6 cpm/mg, indicating that the solanesol of rat liver was not labelled.

Summary

Ubiquinone(45) and ubiquinone(50) are present in normal rat livers in the same ratio as previously found in livers of vitamin A deficient rats. After administration of ^{14}C-mevalonic acid both, ubiquinone(45) and ubiquinone(50), were radioactive. Human livers contain the ubiquinone(50) only. From human livers and hearts solanesol was isolated in crystalline form, and proved to be identical with a sample obtained from tobacco. After administration of ^{14}C-mevalonic acid, it was not possible to detect radioactivity in the rat liver solanesol.

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