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Substance SC (Ubichromenol): A Naturally-Occurring Cyclic Isomeride of Ubiquinone-50

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(Received 6 July 1959)

Lowe, Morton & Harrison (1953) reported that the unsaponifiable-lipid fraction from livers of vitamin A-deficient rats showed selective absorption in the ultraviolet region at 275 and 330 m μ . Two materials were concentrated by chromatography on alumina, a fraction with an absorption peak (in cyclohexane) at 275 m μ and an inflexion near 330 m μ , and a second fraction showing peaks at 275 and 332 m μ

with marked inflexions at 233 and 283 m μ . The latter has hitherto been designated 'substance SC' and it is now proposed to call it 'ubichromenol'. The former, known provisionally as 'substance SA' is now called 'ubiquinone' (Morton, Wilson, Lowe, & Leat, 1957; Morton, 1958; Morton *et al.* 1958) or coenzyme Q₁₀ (Q₂₇₅) (Crane, Hatefi, Lester & Widmer, 1957; Wolf *et al.* 1958).

Substances SA and SC were found to be normal minor constituents of rat liver, kidney and intestine, and it was shown (Heaton, Lowe & Morton, 1955, 1957) that in this species vitamin A depletion brought about a pronounced rise in substances SA and SC in the liver and a slight rise in substance SC in the intestine. Substance SA was reported (Cunningham, Lowe, Mervyn, Morton & Vernon, 1955; Lowe, Morton & Vernon, 1957; Cunningham & Morton, 1959) in the liver and kidneys of many species. Substance SC was detected in the liver of many species and in the kidneys of most; it occurred in rather larger amounts in 'normal' human kidneys (Mervyn & Morton, 1958, 1959) but was not detected in nephritic kidneys.

Lowe (1953) and Heaton (1955) isolated, from the liver of vitamin A-deficient rats, small amounts of substance SC as a yellow oil showing absorption maxima at 233, 275 ($E_{1\text{cm}}^{1\%}$ varying from 67.0 to 94.5), 283 and 332 $m\mu$. Heaton found that substance SC could be acetylated, the acetate having an absorption peak at 317 $m\mu$ instead of 332 $m\mu$. It contained no nitrogen, and SC acetate on analysis gave an empirical formula $C_{11}H_{17}O$. The Rast method indicated a molecular weight of 385 but this observation has been misleading (see below). The infrared-absorption spectrum of substance SC showed a hydroxyl band which disappeared on acetylation. The present paper describes the isolation and characterization of substance SC from human kidney.

MATERIALS AND METHODS

Materials. Human kidneys were made available by the help of Professor H. L. Sheehan, Department of Pathology, University of Liverpool, and were obtained from post-mortem examinations by Dr J. C. Brundret (Sefton General Hospital), Col. B. Blewitt (Newsham General Hospital), Dr C. A. St Hill (Royal Southern Hospital), Dr S. Whitwell (Broadgreen Hospital) and Dr H. E. Vickers (Walton Hospital), all in the Liverpool area. The kidneys were stored either at 0° or -30° until collection, which was generally within 2 days. After removal of the perirenal fat and membrane, and most of the pyramid fat, the kidneys were cut into pieces (about 2.5 cm.³).

Preparation of unsaponifiable extract. The kidney tissue was 'saponified' in 1 kg. portions. The tissue was added to a boiling mixture of 1 l. of 0.25% (w/v) pyrogallol in ethanol and 0.5 l. of aqueous 60% (w/v) KOH, and the mixture was refluxed for 45 min. After cooling, water (3 vol.) was added and the solution was extracted with 4 × 0.5 l. of light petroleum (b.p. 40–60°)–ether (4:1, v/v). The combined extracts were washed with water until free from alkali, dried over anhydrous Na_2SO_4 and filtered through a sintered-glass funnel; the filtrate was evaporated (finally under nitrogen) and the residue was weighed.

Crystallization of sterol. The unsaponifiable lipids were dissolved in the minimal volume of hot light petroleum (b.p. 40–60°) and the solution was left overnight at -30°.

The sterol which had crystallized out was filtered off and washed with cooled light petroleum, the washings being added to the filtrate. The total filtrate was reduced in volume, cooled and a further crop of crystalline sterol was later removed.

Evaporation of solvents. Solvent was removed from each fraction by distillation on a boiling-water bath to a relatively small volume. This was transferred to a small conical flask and the remaining solvent was evaporated in a stream of nitrogen on a boiling-water bath, and the fraction carefully desiccated *in vacuo* and weighed.

Chromatography. Grade 0 alumina (P. Spence and Co., Widnes, Lancs.) was treated with *n*-HCl and washed with water until free from chloride. The alumina was dried by heating to 500° for $\frac{1}{2}$ hr. and immediately before use was partially deactivated with water to Brockmann grade 3. The unsaponifiable material which remained after two crops of sterol crystals had been removed was chromatographed in 2 g. portions on alumina columns (100 g.). The solvents used to elute different fractions from the column were: light petroleum (b.p. 40–60°, dried over sodium wire and redistilled before use), diethyl ether (dried over sodium wire and redistilled from reduced iron) and methylal (redistilled). Usually five fractions were collected from the column:

- (1) 1 l. of 3% ether in light petroleum (pre-ubiquinone fraction).
- (2) 1 l. of 5% ether in light petroleum (ubiquinone fraction).
- (3) 1 l. of 10% ether in light petroleum (SC fraction).
- (4) 1.5 l. of 30% ether in light petroleum or 1 l. of ether (fraction containing sterol and vitamin A).
- (5) 250 ml. of methylal (in some experiments only) (fraction containing sterol and vitamin A).

Substance SC was usually all in fraction (3) but some occasionally occurred in fractions (2) or (4), whereupon the particular fractions were rechromatographed on small columns.

Corresponding SC fractions from many runs were bulked and rechromatographed on alumina. The fractions rich in substance SC were then chromatographed on MgO (British Drug Houses Ltd.: 'for chromatographic analysis'), usually 0.5 g. of SC fraction on a 50 g. column. Solvents used to elute fractions from each column were: (1) 1 l. of 4% ether in light petroleum; (2) 1 l. of ether; (3) 250 ml. of methylal (SC fraction). If MgO from a freshly opened bottle is used a little substance SC may be eluted by ether but most of it is removed only by the more polar methylal.

Acetylation. Substance SC (100 mg.) was dissolved in pyridine (1 ml.) and the solution was cooled before the addition of excess (10 ml.) of acetic anhydride. The mixture was left overnight in the dark at room temperature, and was then poured into cold 5*N*-HCl (10 ml.). The mixture was extracted with light petroleum (3 × 10 ml.). The bulked extracts were washed with water until free from chloride and the solvent was removed.

Hydrogenation. Hydrogen uptake was determined in a microhydrogenation apparatus (J. W. Towers Ltd.). The solvent was acetic acid–ethanol–cyclohexane (1:1:1, by vol.). Adams catalyst (PtO) was used and the hydrogen was at atmospheric pressure.

Spectrophotometry. Ultraviolet-absorption spectra were measured by a Unicam SP. 500 photoelectric spectrophotometer.

meter. Unless otherwise stated, the figures reported refer to cyclohexane solutions. Infrared-absorption spectra were measured with solvent-free films. The instrument was a Perkin-Elmer double-beam spectrometer, model 21 (Department of Organic Chemistry, University of Liverpool).

Nuclear magnetic resonance. The spectra were prepared by Dr L. H. Sutcliffe and Mr J. Feeney of the Department of Inorganic and Physical Chemistry, University of Liverpool. The materials were dissolved in CHCl_3 , which was also used to provide the reference peak. The instrument was a Varian 4300-B nuclear magnetic resonance spectrometer run at 40 Mc/sec. All band measurements are positive shifts on the high-field side of the reference peak.

Analyses. These were carried out in the microanalysis laboratory of the Department of Organic Chemistry, University of Liverpool.

RESULTS

Yield of substance SC from human kidney. Human kidney (46.5 kg.) was saponified as described and yielded crude unsaponifiable material (140.75 g.). After two crystallizations, which yielded 92.9 g. of crude cholesterol, the residual unsaponifiable material, 41.2 g., was chromatographed in portions on alumina. The 3% of ether in light petroleum eluates contained 4.04 g. of material, λ_{max} 268 m μ . The ubiquinone-containing fractions eluted by 5% of ether in light petroleum yielded 5.12 g. of material, λ_{max} 272 m μ , $E_{1\text{cm}}^{1\%}$ 100. The SC-containing fractions (eluted by 10% of ether in light petroleum) contained 9.32 g. of material with an inflexion at 233, λ_{max} 275 ($E_{1\text{cm}}^{1\%}$ 11.1), inflexion 282 and λ_{max} 331 m μ . The subsequent fraction (15.87 g. eluted by ether) contained chiefly sterol with vitamin A as the principal absorbing entity, λ_{max} 327 m μ .

The SC-containing material (9.32 g.) was re-chromatographed in portions on alumina, and yielded a ubiquinone-containing fraction (5.01 g.; λ_{max} 272 m μ , $E_{1\text{cm}}^{1\%}$ 6.9) and a sterol fraction (1.87 g.). The SC fraction (1.91 g.) eluted by 10% of ether in light petroleum showed inflexion 233, λ_{max} 275 ($E_{1\text{cm}}^{1\%}$ 29.3), inflexion 283 and λ_{max} 332 m μ . This last fraction was chromatographed on MgO. Light petroleum containing 4% of ether eluted 1.40 g. of material with negligible selective absorption. The material (0.441 g.) eluted by ether and methylal showed absorption peaks at 233, 275 ($E_{1\text{cm}}^{1\%}$ 92.6), 283 and 332 m μ . Extraction of the adsorbent with hot methylal yielded 0.03 g. of material with no characteristic selective absorption.

The SC fraction (0.441 g.) was crystallized, first at -30° and then at 0° , from ethanol until the $E_{1\text{cm}}^{1\%}$ values at 275 m μ of the crystals and mother liquors (separated by filtration at low temperature) were equal and constant. After seven crystallizations the yield of substance SC was 0.153 g. with $E_{1\text{cm}}^{1\%}$ (275 m μ) 96.1 (see Fig. 1). These pale-yellow

Fig. 1. Absorption curve of substance SC (ubichromenol), 0.00419% in cyclohexane.

Fig. 2. Absorption curve of SC acetate (ubichromenyl acetate), 0.00459% in cyclohexane.

crystals melted at about 18° but a precise melting point was not recorded. Further quantities of substance SC were obtained from the final mother liquors.

SC acetate. This was prepared as described and purified by chromatography on alumina. Substance SC (100 mg., nearly pure) gave acetate quantitatively and after crystallizations from ethanol yielded 60 mg. of white solid, m.p. about 18°, showing absorption peaks at 230, 275 ($E_{1\text{cm.}}^{1\%}$ 95.2), 285 and 314 $m\mu$ (see Fig. 2).

Infrared spectra. The infrared spectrum of substance SC is shown in Fig. 3a and clearly indicates the presence of an aromatic ring. An aromatic structure generally gives rise to bands near 3030 cm.^{-1} ($=\text{C}-\text{H}$ stretching vibration) and near 1600 and 1500 cm.^{-1} ($\text{C}=\text{C}$ vibrations), cf. Bellamy (1954, p. 54). Substance SC as a film shows bands at 3049, 3021 and 2994 cm.^{-1} , and also at 1605 and 1492 cm.^{-1} . Normally, aromatic structures show a weak band at 1580 cm.^{-1} , which is seen as a shoulder on the main 1600 cm.^{-1} peak. In substance SC there is a distinct peak at 1575 cm.^{-1} and this may indicate the presence of a double bond conjugated with the aromatic ring (cf. Bellamy, 1954, pp. 60-61). No clear indication of the degree of substitution of the aromatic ring of substance SC is given in the infrared spectrum.

Substance SC contains one or more hydroxyl groups, as is shown by the band at 3546 cm.^{-1} . Clearly there is no carbonyl group in substance SC, from the absence of the strong $\text{C}=\text{O}$ band in the region 1650-1750 cm.^{-1} . The presence of one or more methoxyl groups is indicated by peaks at 1272 and 1098 cm.^{-1} . The bands at 1179, 1359 and 1389 cm.^{-1} are in the region associated with

skeletal vibration and CH deformation frequencies of isopropyl groups.

The infrared spectrum of SC acetate is shown in Fig. 3b. In this spectrum the bands associated with hydroxyl groups are no longer present and the new bands at 1754 and 1190 cm.^{-1} are indicative of a phenolic acetate.

Analyses. Substance SC: Found, C, 81.93; H, 10.67; O (direct), 8.05; OMe, 7.34%. SC acetate: Found, C, 80.81; H, 10.59; OMe, 7.05%. From the analyses it is apparent that substance SC contains four oxygen atoms, two being in methoxyl groups and one being in an acetylatable hydroxyl group. The fourth oxygen atom cannot belong to a non-acetylatable hydroxyl group (see infrared spectrum of SC acetate) nor to a carbonyl group (see infrared spectrum of substance SC). The C:H ratio of substance SC shows that the molecule must be considerably unsaturated. Its molecular weight, calculated from the analyses, is about 860 for four oxygen atoms, and therefore that of SC acetate would be 902. This makes substance SC very like ubiquinone-50 ($\text{C}_{59}\text{H}_{90}\text{O}_4$) which requires C, 82.08; H, 10.51; OMe, 7.2%. A monoacetate related to ubiquinone (I) would require C, 80.96; H, 10.24; OMe, 6.85%.

Hydrogenated substance SC and SC acetate. The hydrogen uptake of substance SC was 1.242 moles of hydrogen/100 g., which for a molecular weight of about 860 indicates 10.7 double bonds. For SC acetate the value was 1.307 moles/100 g., which for a molecular weight of 902 indicates 11.8 double bonds. These hydrogen uptakes for substances SC and SC acetate were reproduced, and there seems therefore no doubt that SC acetate takes up 1 mol.prop. of hydrogen more than does substance SC.

The ultraviolet-absorption spectra of hydrogenated substance SC (perhydro-SC) and hydrogenated SC acetate (perhydro-SC acetate) are given in Fig. 4, and their infrared spectra in Figs. 5b and 5c respectively. The maxima at 233, 275, 283 and 332 $m\mu$ of substance SC disappear on hydrogenation and in perhydro-SC are replaced by one maximum at 291 $m\mu$, $E_{1\text{cm.}}^{1\%}$ 42.6. A double peak at 275 and 283 $m\mu$ of the intensity found in substance SC is consistent with it being an aromatic substance with one double bond conjugated with the ring (styrene type) (Laitinen, Miller & Parks, 1947). The disappearance of this double peak on hydrogenation would therefore be expected. The absorption maximum at 291 $m\mu$ of perhydro-SC and its intensity (molecular-extinction coefficient) are similar to those of aromatic substances with two oxygen functions in the *para* positions. Phenols, catechols and resorcinols all show peaks near 280 $m\mu$ (Burawoy & Chamberlain, 1952; Coggeshall & Lang, 1948; Hart & Haglund, 1950; Friedel &

Fig. 3. Infrared-absorption curves: (a) substance SC (ubichromenol), film, minimum thickness; (b) SC acetate (ubichromenyl acetate), film, 0.075 mm.

Orchin, 1951), whereas quinols and tocots give rise to peaks near 290 m μ .

Supporting evidence for one phenolic hydroxyl group in substance SC is given by the effect of

alkali on the ultraviolet spectrum. Perhydro-SC in ethanol shows $\lambda_{\max}$ 291 m μ , $E_{1\text{cm}}^{1\%}$ 42 and on addition of sodium ethoxide there is a reversible shift to $\lambda_{\max}$ 308 m μ with a similar intensity (Fig. 6). This shift is consistent with salt formation by a phenol. The isosbestic points indicate a two-component system, i.e. one hydroxyl group. (We have observed that with two free hydroxyl groups in an aromatic ring there is a bathochromic shift in alkali but no isosbestic points are seen on the curves obtained with different amounts of alkali, e.g. 2-methylresorcinol in ethanol shows $\lambda_{\max}$ 274 and 280 m μ , $\epsilon_{\max}$ 1330, whereas in excess of alkali there is a single peak at 286 m μ , ϵ 2245.) With substance SC, alkali similarly causes reversible changes in the spectra, the peaks at 233 and 332 m μ moving to 246 and 358 m μ respectively (Fig. 7). We have already indicated that the double peak of substance SC at 275 and 283 m μ at the intensity observed is due to a styrene type of chromophore. It would seem that substance SC possesses an aromatic ring with a double bond conjugated to it, a free hydroxyl group in that ring and probably an oxygen function in the ring in the

Fig. 4. Absorption curves: —, perhydro-SC (perhydro-ubichromenol); ---, perhydro-SC acetate (perhydro-ubichromenyl acetate). Both 0.0133% in cyclohexane.

Fig. 5. Infrared-absorption curves: (a) cyclized derivative of ubiquinol, film, minimum thickness; (b) perhydro-SC (perhydro-ubichromenol), film, 0.025 mm.; (c) perhydro-SC acetate (perhydro-ubichromenyl acetate), film, minimum thickness.

Fig. 6. Effect of sodium ethoxide on ultraviolet absorption of perhydro-SC (perhydro-ubichromenol). —, Neutral solution, 0.0138% in ethanol; ---, 0.00217M sodium ethoxide; - · - ·, 0.0087M sodium ethoxide; · · ·, 0.0217M sodium ethoxide.

para position to the hydroxyl group. The ultra-violet-absorption spectrum of perhydro-SC acetate (two peaks 276 m μ , $E_{1\text{cm}}^{1\%}$ 21.3; 282 m μ , $E_{1\text{cm}}^{1\%}$ 20.9) resembles that of α -tocopheryl acetate and is consistent with substance SC having a similar type of structure.

In order to confirm the uptake of an additional mol.prop. of hydrogen by SC acetate, perhydro-SC was acetylated and further hydrogenation attempted. There was, however, no uptake of hydrogen. The material from this experiment showed λ_{max} 283 m μ , $E_{1\text{cm}}^{1\%}$ 20.5 with a shoulder at 276 m μ , $E_{1\text{cm}}^{1\%}$ 18.7. Thus acetylated perhydro-SC is similar to but not identical with perhydro-SC acetate.

The infrared spectrum of perhydro-SC acetate shows a weak hydroxyl band at 3546 cm.⁻¹

Fig. 7. Effect of sodium ethoxide on ultraviolet absorption of ethanolic SC (ubichromenol). —, Neutral solution, 0.00113% in ethanol; — — —, 0.00143 M-sodium ethoxide; - - - - -, 0.00429 M-sodium ethoxide; - · - · -, 0.00858 M-sodium ethoxide.

(Fig. 5c), which is absent from the otherwise similar infrared spectrum of the material recovered from the attempted hydrogenation of acetylated perhydro-SC. Since partial saponification in the hydrogenation procedure can be discounted, it must be assumed that a new hydroxyl group is formed on hydrogenation of SC acetate, and this must be associated with the uptake of an additional mol.prop. of hydrogen. It must further be concluded that the unidentified ether oxygen present in substance SC may be in a ring and that this ring splits on hydrogenation of SC acetate but not on hydrogenation of substance SC.

A cyclized derivative of ubiquinone. So far substance SC appears to contain an aromatic ring with *para*-oxygen substitution, two methoxyl groups and a large unsaturated side chain, and these are also present in ubiquinol (Morton *et al.* 1957). Ubiquinol can be converted into a chroman-type of molecule by a cyclization involving the double bond of the first isoprene unit of the side chain and the hydroxyl group in the *ortho* position to this (Bouman, Slater, Rudney & Links, 1958). A structure of this type (II) is more in accord with the desiderata for the structure of substance SC, with the exception that there is no double bond conjugated to the ring, whereas there is evidence for this in substance SC. An attempted cyclization of ubiquinone by the method described for tocopheryl quinone by Bouman & Slater (1957) gave a very small yield. J. Green (personal communication) suggested that SnCl₄, concentrated HCl and acetic acid might prove a better reductive cyclizing reagent. In accordance with his procedure 0.2 g. of ubiquinone-50, 20 g. of SnCl₄, 200 ml. of HCl and 800 ml. of acetic acid were refluxed for 2–3 hr. on a boiling-water bath. This mixture was extracted with ether to yield a product which was crystallized from ethanol at –30° after chromatography on alumina. The cyclized product, 114 mg., showed λ_{max} 290 m μ , $E_{1\text{cm}}^{1\%}$ 40.0. In ethanol, this cyclized product showed λ_{max} 290 m μ , $E_{1\text{cm}}^{1\%}$ 40, and on addition of sodium ethoxide the peak shifted to 308 m μ , $E_{1\text{cm}}^{1\%}$ 40. This material thus showed the same ultraviolet-absorption spectrum and the same behaviour on addition of alkali as perhydro-SC.

(I)

(II)

The infrared-absorption spectrum of this cyclized product (Fig. 5a) is (apart from an unexplained peak at 1748 cm^{-1}) virtually identical with that of perhydro-SC. Both of these spectra differ from that of substance SC in a few details, and these differences are largely in accord with the expected structures. Substance SC has a band at 1575 cm^{-1} not present in the cyclized product or in perhydro-SC, and, as already pointed out, this may be due to a conjugated double bond. Substance SC has a strong band at 1302 cm^{-1} , which is much reduced in intensity in perhydro-SC and the cyclized product. This band is due to C—H stretching and deformation vibration of a *cis* double bond. If it is assumed that substance SC has an all-*trans* side chain, as in ubiquinone (Morton *et al.* 1958), the 1302 cm^{-1} frequency would be due to the double bond conjugated to the aromatic ring. The band at 1466 cm^{-1} in substance SC is less strong than that in the cyclized product and also in perhydro-SC. This band is in the region associated with the C—H deformation frequency of methylene groups.

There is no band at 1333 cm^{-1} in substance SC, whereas in the cyclized product there is a weak band and in perhydro-SC the band is stronger and quite distinct. This frequency is associated with the C—H deformation of a tri-substituted carbon atom ($\geq\text{C—H}$), which would arise in perhydro-SC on saturation of a polyisoprenoid side chain. The bands at 1416 and 833 cm^{-1} of substance SC are less intense in perhydro-SC. These frequencies in the region associated with C—H stretching and deformation vibrations of polyisoprenoid structures, $\text{R}_1\text{R}_2\text{C=CHR}_3$. However, in the cyclized product these bands are also weaker than in substance SC, and this is surprising since the cyclized product should have the same side chain as substance SC.

Oxidation of perhydro-SC. A ready confirmation of the supposed structure of substance SC would be the oxidation of perhydro-SC, to open the oxygen ring and give rise to perhydroubiquinone. Perhydro-SC (4 mg.) in 10 ml. of ethanolic solution of FeCl_3 (0.2 %, w/v) was kept overnight. The solution then was extracted with light petroleum and the extract showed $\lambda_{\text{max.}}$ $279\text{ m}\mu$, $E_{1\text{cm.}}^{1\%}$ approx.

160 , $\lambda_{\text{max.}}$ $407\text{ m}\mu$, $E_{1\text{cm.}}^{1\%}$ 4.3 in ethanol. Authentic perhydroubiquinone shows $\lambda_{\text{max.}}$ $278\text{ m}\mu$, $E_{1\text{cm.}}^{1\%}$ 163, $\lambda_{\text{max.}}$ $407\text{ m}\mu$, $E_{1\text{cm.}}^{1\%}$ 4.3 (Morton *et al.* 1958). On addition of sodium borohydride to an ethanolic solution of the oxidation product, the spectrum showed $\lambda_{\text{max.}}$ $290\text{ m}\mu$, $E_{1\text{cm.}}^{1\%}$ approx. 50. Perhydroubiquinol has $\lambda_{\text{max.}}$ $290\text{ m}\mu$, $E_{1\text{cm.}}^{1\%}$ 49.5. Addition of further FeCl_3 reversed the changes. This is good evidence for the production of perhydroubiquinone by oxidation of perhydro-SC.

Nuclear magnetic resonance. The nuclear magnetic resonance spectra of ubiquinone-50 (human kidney), ubiquinone-30 (baker's yeast), substance SC and the cyclized product were examined. The conclusion drawn from the spectra by Dr Sutcliffe and Mr Feeny was that the nuclear magnetic resonance spectrum of substance SC fully supported the idea of ring closure.

(1) The allyl hydrogens manifested in the spectra of ubiquinone-50 and ubiquinone-30, by peaks at 157 and 163 cyc./sec. and 161 and 167.5 cyc./sec. respectively, have disappeared in the spectrum of substance SC, as expected.

(2) In the spectra of the two ubiquinones the peak for the methoxyl group is single and in substance SC two methoxyl group peaks are shown: ubiquinone-50, 129 cyc./sec.; ubiquinone-30, 132 cyc./sec.; substance SC 132 and 133.8 cyc./sec. The two methoxyl groups in ubiquinone are equivalent whereas in substance SC they are non-equivalent.

(3) In substance SC, ring hydrogens are registered: 24.1 and 33.7 cyc./sec., and 61.8 and 72.0 cyc./sec., and the splitting of the two bands shows the two hydrogens in the ring to be on *ortho*-carbon atoms.

(4) In substance SC, a phenol hydrogen — 3.9 cyc./sec. is recorded. The high-field bands in substance SC, namely 208, 223.5 and 232 cyc./sec., are similar to those of ubiquinone-50 and -30 and it is reasonable to assume an isoprenoid side chain. Intensity measurements indicate that substance SC has nine isoprenoid units in the side chain (not counting the first isoprene unit which is involved in the ring).

All the evidence so far stated would agree with substance SC having structure (III).

The nuclear magnetic resonance spectrum of the cyclized product derived from ubiquinone showed that ring closure had taken place (the methoxyl groups were non-equivalent, allyl hydrogens had disappeared, phenol hydrogen had appeared) and also indicated that the heterocyclic ring hydrogens did not belong to an ethylenic linkage. Further, the spectrum showed that most of the unconjugated double bonds in the side chain had disappeared. The high field peaks, 190, 206, 226, 240 and 254 cyc./sec. are no longer similar to those obtained from the three previous compounds with the isoprenoid type of side chain. The peaks might indicate a linear saturated side chain but intensity measurements show that perhaps two double bonds remain.

The treatment of ubiquinone with SnCl_2 in a strongly acid medium appears to have effected the expected cyclization and in addition to have reduced some of the double bonds of the side chain, possibly with further cyclization.

DISCUSSION

The substance hitherto designated SC is somewhat widely distributed in animal tissues in quite small amounts. It first became accessible to study because its concentration in rat liver increased several-fold in advanced vitamin A-deficiency (Heaton *et al.* 1957). The evident structural relationship to ubiquinone which has now emerged might suggest that under these circumstances it was derived from ubiquinone by aberrant metabolism and that its accumulation reflects a metabolic block.

On the other hand, the best source of substance SC so far known is 'normal' human kidney, and its disappearance from the kidneys of patients who

died from one form or another of nephritis (Mervyn & Morton, 1959) suggests that the metabolic abnormality lies in the absence of substance SC. This might result from failure to achieve synthesis or it might be the result of exhaustive hyperactivity in some process mediated by substance SC. It is too early to discuss how these two approaches to the biochemistry of substance SC are inter-related.

The structural investigation makes it appropriate to suggest the name *ubichromenol* for substance SC.

Kofler (1946) described a quinone from alfalfa and other plants which appeared to be a tetrasubstituted benzoquinone (m.p. 48–49°) of molecular weight about 800. It showed peaks at 254 and 262 $m\mu$ and a much weaker maximum near 320 $m\mu$. Since the isolation of ubiquinone (co-enzyme Q_{10}) Kofler's quinone has been studied afresh and it is now regarded as possessing the structure (IV), where $n = 9$. (Trenner *et al.* 1959; Kofler *et al.* 1959).

It thus differs from ubiquinone in lacking a methyl group at position 6 and in having methyl groups instead of methoxyl group at positions 2 and 3.

The occurrence in plants and animals of a polyisoprenoid side chain $\text{C}_{50}\text{H}_{81}$ has increased interest in the isolation by Rowland, Latimer & Giles (1956) of an allylic alcohol solanesol ($\text{C}_{45}\text{H}_{73}\cdot\text{OH}$) from tobacco leaves. The properties of this alcohol strongly suggest, however, that it is made up of nine isoprene residues joined together as in Kofler's quinone and ubiquinone 45 (Erickson *et al.* 1959; Kofler *et al.* 1959).

Rowland (1958) has since isolated from flue-cured tobacco a compound, solanachromene (V). The compound exhibits in the ultraviolet region absorption peaks at 233, 267 (inflection 275) and 332 $m\mu$ ($\log \epsilon$, 4.37, 3.76, 3.74 and 3.63 respectively). This absorption spectrum is consistent with that of ubichromenol, where the double peak in the middle ultraviolet region is displaced towards longer wavelengths and increased a little in intensity.

The chromene ring has also been found in ageratochromene (VI) from the essential oils of some *Ageratum* species (Alertsen, 1955). Crane (1959) has discovered that Kofler's quinone, which he designates Q_{254} , is concentrated in chloroplasts,

(IV)

(V)

(VI)

whereas mitochondria from the same leaves contain coenzyme Q_{10} (ubiquinone-50). The association of the former with photosynthetic electron transport and of the latter with mitochondrial electron transport provides some guide for future work on solanachromene and ubichromenol.

Interesting possibilities arise from this work, namely that tocopherol analogues with polyisoprenoid side chains may well be found in nature with the chromene structure. The naturally occurring vitamins K with polyisoprenoid side chains may well be accompanied by or be in metabolic relationship with cyclized derivatives.

SUMMARY

1. A normal minor unsaponifiable lipid, hitherto designated substance SC, and present in kidney and other tissues of many species, has been isolated from normal human kidney, its concentration in this tissue being about 10 mg./kg.

2. Substance SC is a yellow crystalline compound, m.p. about 18°. It is isomeric with ubiquinone, $C_{59}H_{90}O_4$, but is not itself a quinone.

3. Substance SC contains a chromene ring system and a large polyisoprenoid side chain. Perhydro-SC can be oxidized by ferric chloride to perhydroubiquinone. Substance SC is therefore a cyclic isomeride of ubiquinone, the cyclization involving the first isoprenoid unit of the side chain and the adjacent oxygen function.

4. The name ubichromenol is suggested for substance SC.

In addition to expressing gratitude to those mentioned above, we wish to thank Dr F. M. Dean, Dr W. B. Whalley, Dr O. Isler, Dr A. L. Morrison and Professor O. Wiss for most useful discussions. We also thank Mr D. E. M. Lawson for assistance in the collection and saponification of tissue. Two of us (D.L.L. and J.F.P.) are holders of Agricultural Research Council Research Studentships.

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