of low dielectric constant and ion-solvent interaction would be more and consequently Φ^0 will be larger.
The plot of o^0 vs $1/\varepsilon$ (ε = dielectric constant) is found to be linear, indicating the dependence of Φ^0 on dielectric constant and the slope is of the order $NO_3 > Br^- > Cl^-$.
Conductance. The equivalent conductance of all the 6 salts are found to be linear with $C^{1/2}$, which indicates that the Debye-Huckel-Onsager theory of electrolytic conductivity is applicable to these ions. The Walden product $\Lambda^0\zeta$, which can be employed to study ion-solvent interaction (table 3) is almost constant. The constancy of $\Lambda^0\zeta$ is most presumably due to the compensating contribution of positive temperature coefficient of the conductance to the negative temperature coefficient of the viscosity of the solvent. Hence it appears that the temperature dependence of $\Lambda^0\zeta$ is not very helpful in the study of ion-solvent interaction in dioxane-water mixtures.

Table 3. $\Lambda^0\zeta \cdot 10^2/\Omega^{-1} \cdot cm^2 \cdot$ poise

Mass fraction of fraction temperature in °C	10%	20%	30%	10%	20%	30%
	NaCl			KCl		
30	124.88	123.82	123.26	136.23	134.50	134.06
35	124.00	123.85	123.35	134.77	134.89	134.05
40	124.87	124.55	123.23	136.24	135.49	134.19
45	123.35	123.36	123.56	135.66	135.59	134.74
	NaBr			KBr		
30	118.67	118.58	118.40	130.02	129.27	129.21
35	118.83	118.74	118.77	129.60	129.78	129.47
40	118.85	119.72	118.41	130.31	130.07	129.30
45	118.17	117.73	118.41	129.65	129.85	129.58
	$NaNO_3$			KNO_3		
30	114.24	113.76	113.54	125.59	124.45	124.33
35	113.66	115.68	113.71	124.43	124.65	124.41
40	113.90	112.15	113.60	125.36	124.94	124.49
45	113.67	112.08	113.36	125.30	125.15	124.43

The size of the anions studied are almost of the same order[22] and the $\Lambda^0\zeta$ values are of the order $Cl^- > Br^- > NO_3^-$. The lesser the value of $\Lambda^0\zeta$, the greater is the ion-solvent interaction. During migration, these common ions which are fairly small, are covered with a sheath of solvent molecules resulting in a larger size of the solvedynamic unit, so that the size of the dissolved ions and ion-solvent interaction in dioxane-water mixtures is of the order $NO_3^- > Br^- > Cl^-$.

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Biochemical studies of pigments from a pathogenic fungus *Microsporum cookei*.V. Evidence for the transmembrane permeability of xanthomegnin across phospholipid bilayer membranes

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Summary. Direct evidence is provided for the transmembrane permeation of xanthomegnin across phospholipid bilayer mebranes using ascorbate-loaded liposomes. This process may be associated with an uncoupling effect on the oxidative phosphorylation of mitochondria.

Xanthomegnin, a binaphthoquinone pigment isolated from a pathogenic fungus *Microsporum cookei,* has been shown to uncouple the oxidative phosphorylation of rat liver mitochondria[1-3] and to lose its uncoupling effect by the O-methylation of the phenolic hydroxyl groups of the pigment[3]. In general, it has been well documented by many experiments using liposomes and reconstituted membrane vesicles that compounds which uncouple mitochondrial respiration discharge the electrochemical potential by acting as proton conductors on mitochondrial inner membranes[4-12]. We have investigated the transmembrane permeability and proton conductivity of xanthomegnin across phospholipid bilayer membranes to study the molecular mechanism for the uncoupling effect on mitochondrial respiration. This communication deals with spectroscopic evidence for the transmembrane permeability of xanthomegnin.
Materials and methods. Xanthomegnin was isolated from dried mycelium of *M.cookei* by a procedure previously described[2]. Cytochrome c (bovine heart muscle) and Tris were purchased from Sigma Chemical Co. Bio-Gel P-150 was a product of Bio-Rad Laboratories. Egg lecithin (the product of Sigma Chemical Co.) was used after further purification by means of silicic column chromatography. Other reagents were of the purest form commercially available. The ascorbate-loaded liposomes were prepared by ultrasonic oscillation of egg lecithin suspension (20 mg egg lecithin in 2 ml of 0.1 M ascorbate). The ascorbate in

the outside of the liposomes was completely removed by using gel filtration chromatography (Bio-Gel P-150). Sucrose (0.25 M), which contained 20 mM Tris-HCl (pH 7.4), was used as a development solvent in order to prevent hypotonic disruption. The permeation of xanthomegnin into the liposomes was reduced spectrophotometrically by tracing the absorbance at 412 nm which resulted from the reduction of xanthomegnin by the ascorbate in the liposomes. Reduction of xanthomegnin caused a spectral alteration showing a marked increase of the absorbance at 412 nm[2]. The reduction of cytochrome c was observed by the reduced minus oxidized difference spectrum of cytochrome c. Spectroscopical observations were carried out with Hitachi Recording Spectrophotometer EPS-3T.

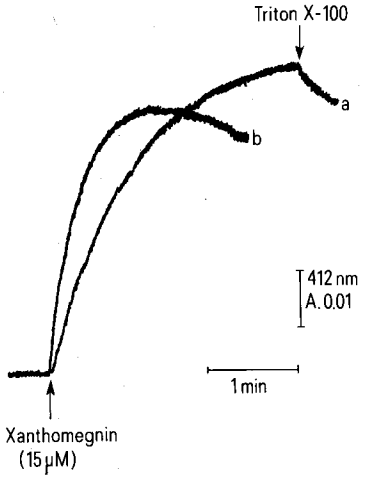

Fig. 1. Reduction of xanthomegnin by intra-liposomal ascorbate. Reaction medium contained 0.25 M sucrose, 50 mM Tris-HCl (pH 7.4) and 2.0 mg/ml of liposomal phospholipids in the final volume of 2 ml. Incubations were carried out at 25 °C. Curve a and b were incubated with and without 0.5% Triton X-100, respectively.

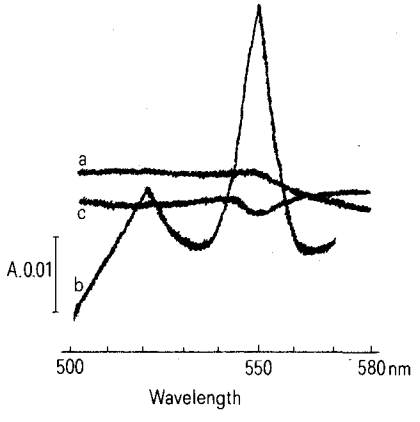

Fig. 2. Reduction of external cytochrome c by internal ascorbate. The basal medium contained 0.25 M sucrose, 50 mM Tris-HCl (pH 7.4), 0.2 mg/ml of cytochrome c, and 1.74 mg/ml of liposomal phospholipids. Curve a shows the control line. Curve b: 12 μM xanthomegnin was added to the sample cuvette. Curve c: Additionally Triton X-100 was mixed to both of the cuvettes in a final concentrations of 0.5%.

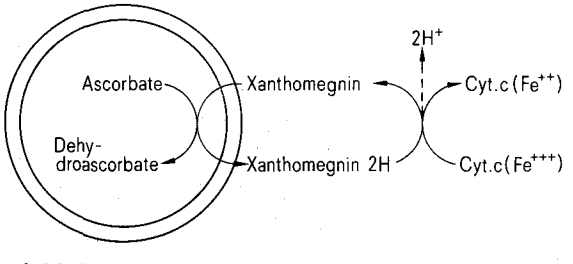

Fig. 3. Model for the reduction of external cytochrome c by internal ascorbate through xanthomegnin.

Results and discussion. Figure 1 shows the reduction of xanthomegnin by ascorbate in liposomes. As seen in curve a, addition of xanthomegnin caused a marked increase of the absorbance at 412 nm, showing a reduction of xanthomegnin by ascorbate in the liposomes. Addition of Triton X-100 after the complete consumption of ascorbate caused no further increase in absorbance but instead caused a temporal decrease which resulted from an auto-oxidation of the reduced pigment by aeration. On the other hand, disruption of the liposomes by Triton X-100 caused a high rate of absorbance increase as shown in curve b. These results suggest that xanthomegnin permeates into liposomes across the phospholipid bilayer membranes and was reduced by the intra-liposomal ascorbate. Figure 2 shows the reduced minus oxidized difference spectrum of cytochrome c. Curve a demonstrates the resultant spectrum of externally added cytochrome c to the liposomal suspension. The addition of xanthomegnin caused a reduction of cytochrome c, showing a typical reduced minus oxidized difference spectrum of cytochrome c (curve b). Subsequent addition of Triton X-100 to both of the cuvettes led to the destruction of the liposomes, which was accompanied by the reduction of cytochrome c without xanthomegnin in the reference cuvette. As a result, the spectrum became straight line as shown in the curve c.

These results demonstrate that xanthomegnin, reduced by ascorbate, diffused out of the liposomes to reduce cytochrome c on the outside of the liposomes. This is shown schematically in figure 3. The transmembrane permeation of xanthomegnin across phospholipid bilayer membranes was indicated spectroscopically using ascorbate-loaded liposomes. On the other hand, in our previous articles[2,3], the possibility of proton conductivity of xanthomegnin was suggested by comparing the uncoupling effects of this compound with those of luteosporin and O-methylxanthomegnin. Therefore, these results suggest a molecular mechanism for the uncoupling effect of xanthomegnin on the oxidative phosphorylation of mitochondria. Perhaps xanthomegnin moves across the mitochondrial inner membranes in the opposite direction in charged or uncharged forms to discharge the electrochemical potential, which may lead to uncoupling of phosphorylation from the electron transport system of mitochondria.

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