acceptor. A similar complex between acridine and its cation has been already observed by Hammond (1964).

Acknowledgments

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Metabolism of Ubiquinone-7*

Isuke Imada, Masazumi Watanabe, Norichika Matsumoto, and Hiroshi Morimoto

ABSTRACT: Several new metabolites of ubiquinone-7 and one of their conjugates were obtained from the excrements and the tissues of rats and rabbits to which ubiquinone-7 had been administered. The three metabolites and one conjugate obtained from the excrements were identified as 2,3-dimethoxy-5-methyl-6-(3′-methyl-4′-oxopentyl)-1,4-benzoquinone (IV), *d*-2,3-dimethoxy-5-methyl-6-(3′-carboxy-3′-methylpropyl)-1,4-benzoquinone (V), *trans*-2,3-dimethoxy--5-methyl-6-(5′-carboxy-3′-methyl-2′-pentenyl)-1,4-benzoquinone (VII), and the disulfate XIII of the hydroquinone form of V, respectively, by comparison of their spectral data with those of synthetic samples. The earlier assumption that 2,3-dimethoxy-5-methyl-6-(5′-carboxy-3′-hydroxy-3′-methylpentyl)-1,4-benzoquinone lactone (III) is a metabolic end product was corrected and the lactone was proved to be an artifact formed from the conjugate of VII during the hydrolysis step.

The metabolite XIV from the adrenals was found to be an *ω-cis*-carboxylic acid which was formed by oxidation of the *cis*-methyl group in the terminal isoprene unit, while the metabolite XVIII from the ovaries was found to be the 26,27-dihydro compound of XIV, and they were obtained as the cholesteryl esters. The metabolic pathway of ubiquinone is discussed.

On the assumption that ubiquinone is excreted in the urine as a conjugate of 2,3-dimethoxy-5-methyl-6-(5′-carboxy-3′-hydroxy-3′-methylpentyl)-1,4-benzoquinone lactone (III), presumably as a glucuronide similarly to the excretion of α-tocopherol (Simon *et al.*, 1956a,b), Gloor *et al.* (1966) and Wiss and Gloor (1966) investigated III in the urine of rats to which labeled Q-9[1] had been administered, by the isotope dilution method and confirmed the presence of III in the urine. The authors investigated the metabolites of Q-7 (I), using the facts that a metabolite retaining the partial

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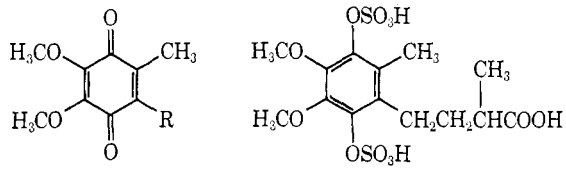
[1] Abbreviation used is: Q-*n*, ubiquinone-*n*.

structure of 2,3-dimethoxy-5-methyl-1,4-benzoquinone or its hydroquinone can be detected with leucomethylene blue, $K_3Fe(CN)_6$–$FeCl_3$ (1:1) spray reagents and by ultraviolet spectroscopy. In the present paper, the isolation of several metabolites is described.

Metabolites in Excrements. The urine of rats fed with I was hydrolyzed with a mixture of methanol and hydrochloric acid, and then purified by chromatography into nine quinones (I, III, IV, VI, VIII, IX, X, XI, and XII) and Q-7 hydroquinone (II) (Table I) after treating with diazomethane. IV, VI, and VIII were confirmed to be 2,3-dimethoxy-5-methyl-6-(3′-methyl-4′-oxopentyl)-1,4-benzoquinone, *d*-2,3-dimethoxy-5-methyl-6-(3′-methyl-3′-methoxycarbonylpropyl)-1,4-benzoquinone, and *trans*-2,3-dimethoxy-5-methyl-6-(3′-methyl-5′-methoxycarbonyl-2′-pentenyl)-1,4-benzoquinone, respectively, by comparison with the synthetic compounds (Watanabe *et al.*, 1970). The optical rotatory dispersion of VI showed a decreased amplitude ($[\alpha]_{398}^{25}$ −92°). VI and VIII were assumed to be artifacts which were formed by the acid treatment from *d*-2,3-dimethoxy-5-methyl-6-(3′-carboxy-3′-methylpropyl)-1,4-benzoquinone (V) and *trans*-2,3-dimethoxy-5-methyl-6-(5′-carboxy-3′-methyl-2′-pentenyl)-1,4-benzoquinone (VII), respectively. In order to confirm the presence of these acids, the urine was treated with diluted hydrochloric acid at 75° (Gloor *et al.*, 1966). From the reaction mixture, III and V, the structures of which were confirmed by synthesis (Morimoto *et al.*, 1969; Watanabe *et al.*, 1970), were newly obtained and the esters VI and VIII were not detected as expected. This result provides strong evidence that III is an artifact formed by lactonization during the process of extraction. The lactonization of γ,δ-unsaturated fatty acids by the attack of a proton is well known (Linstead and Rydon, 1933). The amounts of V and VII excreted in the urine of rats dosed with various amounts of I, increased with the amount of I administered, demonstrating that V and VII are metabolites of I (Table II).



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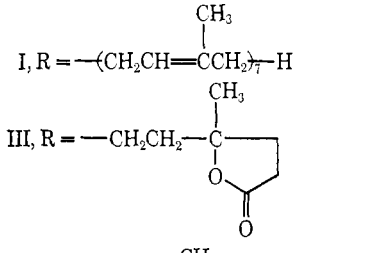
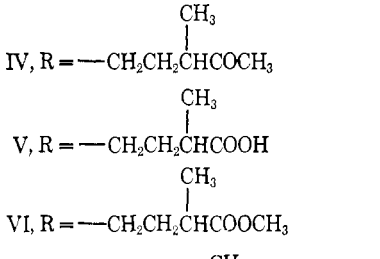
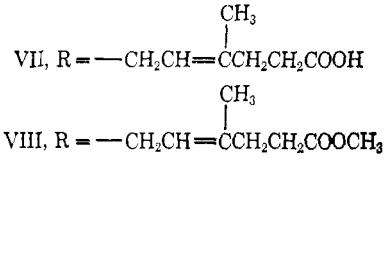
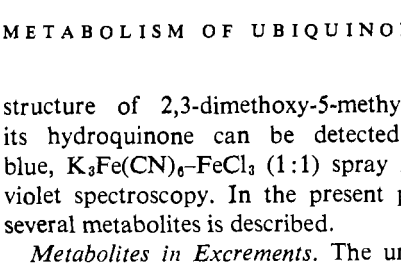
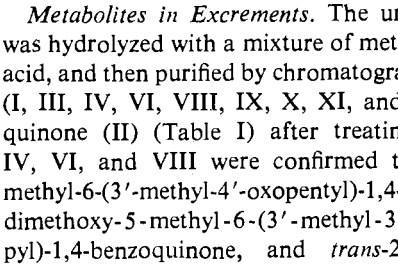
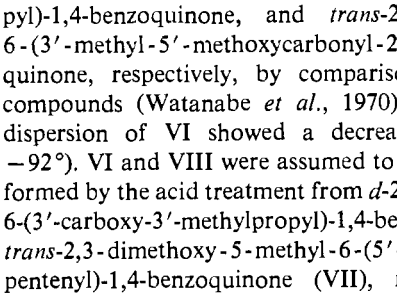












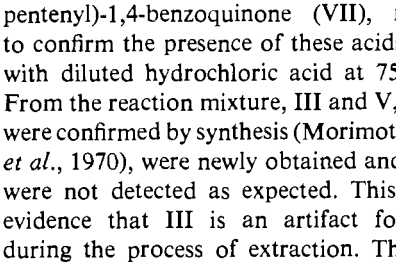


TABLE I: R_F Values of Quinones and a Hydroquinone Obtained from the Methylated Hydrolysate of Lipid-Free Urine.

Compd	System: Benzene–Ethyl Acetate (3:1)	System: Hexane–Ether (1:4)
III	0.30	0.20
IX		0.34
IV	0.55	0.57
VI	0.65	0.71
VIII	0.65	0.73
X	0.79	0.75
XI		0.79
XII		0.82
I	0.91	0.95
II	0.90	0.96

The compounds IX to XII were quinone because of the positive reaction with leucomethylene spray reagent, and they were considered to be metabolites of I because they were detected only in the acid hydrolysate of the urine of rats fed with I, but investigation was discontinued since they were minor components.

Since these quinones were not detected directly in the urine, they must be excreted as conjugates which can be easily converted into quinone or hydroquinone compounds by the acid treatment. In order to isolate the sufficient amount of the conjugates, the urine of rabbits to which I had been administered intravenously was collected. The conjugate XIII was obtained from the methanol extracts of the lyophilized urine as a white powder which gives V by the acid treatment. XIII did not show the color reaction for phenol, but showed it after being treated with sulfuric acid. XIII also exhibited a positive reaction for sulfur with the nitroprusside reagent (McGookin, 1959) and an ultraviolet spectrum indistinguishable from that of the disulfate of II. Therefore, XIII was concluded to be the disulfate of V-hydroquinone and the identification was accomplished by comparison with a synthetic sample (Watanabe *et al.*, 1970). VII was also found to be excreted as the disulfate of its hydroquinone since VII was not detected directly in the methanol extracts of the lyophilized urine powder but it was detected after the acid treatment. The conjugates of V and VII were present in the ratio of one to three, showing a difference from the rats urine (Table II).

In the ether extracts of the feces of rats fed with I, I and II were found in equal amounts. In the methanol extracts of the lipid-free feces, conjugates of V and VII were found in the ratio 7:3 from the contents of VI and VIII.

Metabolites in Liver. In the extracts of the liver of rats fed with I, three unknown quinones and conjugates of V and VII in the ratio 7:3 were found accompanied by endogenous Q-9, large amounts of I and II (Aramaki *et al.*, 1970).

TABLE II: The Amounts of Metabolites on Dosing with Various Amounts of Ubiquinone-7 (I).

	Intake of I				Metabolites (mg)			
	mg/kg per day	Total (mg)	No.	Days	V	VII	V + VII	%[a]
	0	0	6	5[c]	0.35 (56)[b]	0.28 (44)	0.63	
Male	15	230	6	5	5.45 (66)	2.74 (34)	8.19	3.6
rats	50	832	6	5	18.3 (63)	10.6 (37)	28.9	3.3
	150	2588	6	5	16.5 (70)	7.0 (30)	23.5	0.9
	0	0	10	6[c]	0.14 (59)	0.1 (41)	0.24	
Female	15	292	10	6	13.5 (63)	7.96 (37)	21.5	7.4
rats	50	832	10	6	21.7 (74)	7.9 (26)	29.6	3.6
	150	2196	9	6	31.4 (76)	9.7 (24)	41.1	1.9
Adult	0	0	1	2	0.27 (56)	0.21 (44)	0.48	
man	1.6	100	1	2	1.0 (52)	0.94 (48)	1.94	1.9

[a] Metabolites (V + VII)/I intaken. [b] % of a metabolite to the total metabolites (V + VII). [c] The days from the 162th day (male) and 168th day (female) after the beginning of administration.

TABLE III: R_F Values of the Metabolites Obtained from Adrenals and Ovaries, and Related Compounds.

	System				
Compd	Ether–Hexane–Acetic Acid (60:40:1)	Hexane–Ether (3:1)	Dimethylformamide–Water (9:1)[a]	Acetone–Water (9:1)[a]	Acetone[a]
XIV	0.42	0.03			
XV	0.93	0.65	0.31	0.0	0.21
XVI	0.72	0.44			
XVIII	0.42	0.03			
XIX	0.93	0.65	0.30	0.0	0.19
XX	0.72	0.44			
VIII	0.35	0.21			
V	0.23				
Cholesterol	0 48				
I	0.84	0.63	0.77	0.50	0.34
Q-9	0.82	0.62		0.20	0.31

[a] On paraffin-impregnated thin-layer plates (Morimoto and Imada, 1965).

Structures of unknown quinones have been not confirmed because of a small amount.

Metabolites in Adrenals and Ovaries. Aramaki *et al.* (1970) have shown by the analyses of endogenous Q-9 and I that extremely large amounts of I per gram of tissue are incorporated into the adrenals and ovaries in addition to the liver. In addition to these results, the authors have observed the presence of unknown quinones, XV and XIX, in the adrenals and ovaries, respectively. XV and XIX were separated from Q-9 and I by reversed-phase-layer chromatography, their R_F values being lower than Q homologs (Table III). XV and XIX were found to be similar to I by their positive reaction with the leucomethylene blue reagent and by their ultraviolet spectra. The infrared absorption spectrum of XV was very similar to that of I, but possessed an additional absorption characteristic of an α,β-unsaturated ester group at 1710 cm^{-1} (Figure 1). Though the molecularion peak (M) in the mass spectrum was not detected, probably due to its low volatility, it showed prominent peaks, M + 2 − R′ + 1, M − R′ + 1, M − ($CH_2CH{=}C(CH_3)COOR'$ + 1) and characteristic peaks of polyisoprenoid compounds, which were formed by successive fragmentation of the $CH_2CH{=}C(CH_3)CH_2$-unit, 522, 454, 386, and 318 *m/e* (Morimoto *et al.*, 1967). In the nuclear magnetic resonance spectrum (Figure 2) of XV, the signal at τ 8.35 due to the *ω-cis*-methyl of I disappeared and the signal at τ 8.40 due to the *ω-trans*-methyl of I shifted to a lower magnetic field (τ 8.21). From these data, XV was considered to be the ester of 2,3-dimethoxy-

TABLE IV: Quinones in Adrenals and Ovaries of Ubiquinone-7 (I) Feeding Rat.[e]

Organ	I Feeding (mg/kg per day)	Sex[a]	Compound[b] XV[c]	XIX[c]	I[d]	Q-9[d]
Adrenal	Control	M	282		45	27
	15	M	1290		116	50
	50	M	2120		432	126
	150	M	2310		707	145
	Control	F	254		26	99
	15	F	437			
	50	F	5060		1410	198
	150	F	3940		1007	225
Ovary	Control			792	212	117
	15			592	169	112
	50			1640	432	194
	150			3200	1250	226

[a] M, male; F, female. [b] μg/g tissue. [c] XV and XIX were quantified by using $E_{1cm}^{1\%}$ at 275 mμ = 142. [d] I and Q-9 were quantified by the procedure of Redfearn (1967). [e] Rats kept for 6 months.

5-methyl-6-(27′-carboxy-3′,7′,11′,15′,19′,23′,27′-heptamethyl-2′,6′,10′,14′,18′,22′,26′-heptacosaheptaenyl)-1,4-benzoquinone (XIV), in which the ω-*cis*-methyl of I was oxidized to a carboxyl. The hydrolysis of XV gave the acid XIV and the alcohol XVII. XIV was confirmed further by the ultraviolet, infrared, and mass spectra of XVI obtained by methylation of XIV. XVII was identified as cholesterol by comparison of the color reaction, the thin-layer chromatographic R_F value, and gas–liquid partition chromatography. These facts gave evidence that XV is the cholesteryl ester of XIV. The ovarial quinone XIX was very similar to XV except for the infrared absorption due to the saturated ester group (Figure 1). The hydrolysis of XIX gave cholesterol and the acid XVIII. XVIII was confirmed to be a dihydro compound of XIV, 2,3-dimethoxy-5-methyl-6-(27′-carboxy-3′,7′,11′,15′,19′,23′,27′-heptamethyl-2′,6′,10′,14′,18′,22′-heptacosahexaenyl)-1,4-benzoquinone by its ultraviolet and infrared spectra and by the ultraviolet, infrared, and mass spectra of its methyl ester XX. The amounts of XV accumulated were greater in the rats fed with I than in the control group, especially in the females and reached several times as much as I in some cases. The amounts of XIX also increased in dosing groups (Table IV). These data show that XV and XIX are metabolites of I. A compound which shows the same chromatographic R_F value and the same ultraviolet spectrum as those of XV or XIX was found in the tissues of rats kept without I. Therefore, endogenous Q-9 of rats was also metabolized to the ester of the Q-9 carboxylic acid derivatives in the adrenals and ovaries, and XIV and XVIII seem to be metabolized from I on the normal metabolic pathway.

Assay of Metabolites. It was reported by the authors that one of the metabolites can be measured by being converted into III (Morimoto and Imada, 1970). In this study, the assay procedure in which the conjugates are quantified

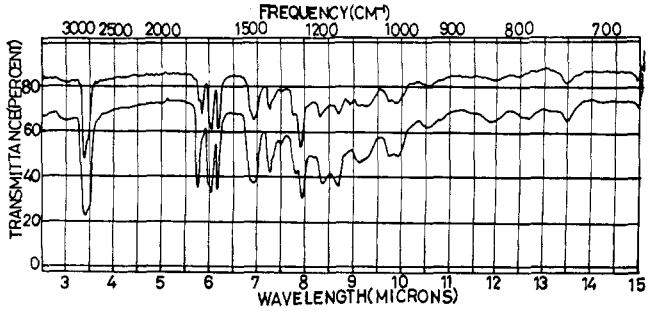


FIGURE 1: Infrared spectra of XV (upper curve) and XIX (lower curve).

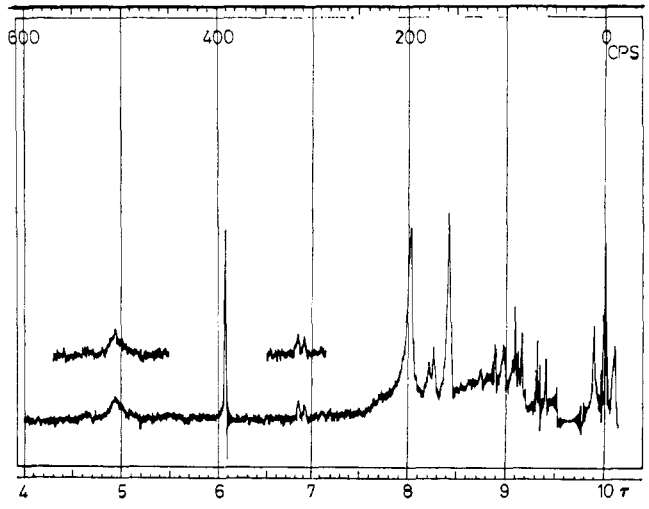
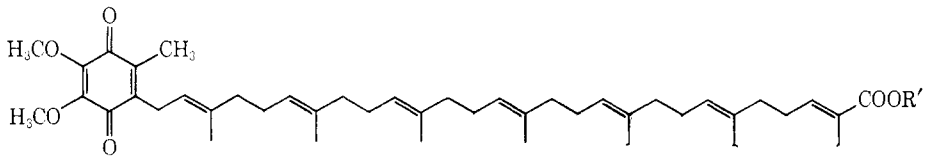


FIGURE 2: Nuclear magnetic resonance spectrum of XV.

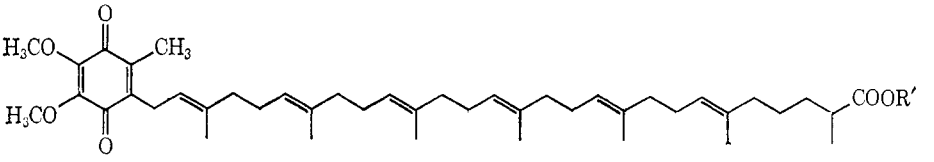
TABLE V: Condition of Acid Hydrolysis and the Resulting Quinones.

Acid	Solvent	Temp (°C)	Time (hr)	Quinones III	V	VI	VII	VIII
1.5 N HCl		75	2	++[a]	++	−[b]	−	−
	Water	75	1	++	++	−	+	−
		75	0.5	++	++	−	+	−
		75	0.5	+	+	++	−	++
	Methanol	21	13	−	−	+++	−	+++
		4	13	−	−	+	−	+
3 N HCl	Methanol	21	13	−	−	++	−	++

[a] +, ++, +++: Positive to the leucomethylene blue reaction in an ascending order of strength. [b] −: Negative to the reaction.



XIV, R′ = H
XV, R′ = cholesteryl
XVI, R′ = CH_3



XVIII, R′ = H
XIX, R′ = cholesteryl
XX, R′ = CH_3

as VI and VIII was investigated. As it is known that sulfate conjugates are hydrolyzed under relatively mild acid conditions (Lieberman and Dobriner, 1948; Burstein and Lieberman, 1958), the desired methyl esters, VI and VIII were found to be formed almost quantitatively when the urine powder was treated at room temperature in a mixture of methanol and hydrochloric acid, followed by oxidation with ferric chloride (Table V), and the VI and VIII thus formed were quantified by gas–liquid partition chromatography. This procedure can be applied to the assay of the ubiquinone metabolites in biological samples. Since VI and VIII can be separated by reversed-phase-layer chromatography, they can be quantified also by the absorbance at their absorption maxima at 277 and 275 mμ, respectively.

Experimental Section

General Comments. I was isolated from the cells of *Candida utilis* (Imada *et al.*, 1964). II, and its disulfate and diacetate, III, IV, V, VI, VII, VIII, and XIII, and 2,3-dimethoxy-5-methyl-6-carboxymethyl-1,4-benzoquinone (B) were synthesized in the author's laboratories (Morimoto *et al.*, 1965; Morimoto *et al.*, 1969; Watanabe *et al.*, 1970). The following chemicals were purchased from commercial sources: silica gel G and silica gel (less than 0.08 mm) from Merck A.G.; Florisil from Floridin Co.; APN from Apiezon Products Ltd.; QF-1, gas chrom Q (60–80 mesh) and gas chrom Z (80–100 mesh) from Applied Science Laboratories; DEAE-cellulose from Serva Co. Thin-layer chromatographic plates were prepared with silica gel G (Morimoto and Imada, 1965). Phenols were detected by spraying with a mixture of 1% $FeCl_3$–1% $K_3Fe(CN)_6$ (1:1) (Barton *et al.*, 1952) and quinones with the leucomethylene blue reagent (Linn *et al.*, 1959). Ultraviolet spectra were recorded by a Hitachi EPS-3T spectrophotometer in ethanol solution. Infrared absorption spectra were determined by Hitachi EPI-S2 and Hitachi EPI-2 spectrophotometers in liquid films. Nuclear magnetic resonance spectra were recorded by a Varian Model HA-100 spectrometer in carbon tetrachloride solution. Chemical shifts are expressed in τ values relative to tetramethylsilane as an internal standard. Mass spectra were recorded by a Hitachi RMU-6D double-focusing mass spectrometer at 70 eV, samples being introduced directly into the ion source at 200°. Optical rotatory dispersion was recorded by a Jasco ORD/UV-5 instrument in chloroform solution.

Animals. Sprague–Dawley rats (initial body weight: male 175 g, female 140 g) were kept on a standard food

powder supplemented with I. The daily intake of I per kilogram of body weight was adjusted to 15, 50, 150, and 2000 mg by controlling monthly the concentration of I in the food. Adult buck albino rabbits (initial body weight: 2.44 to 2.86 kg) were kept for 9 days on a standard diet of rabbit pellets. An aqueous solution of I was intravenously given to the rabbits in doses of 150 mg/kg body weight per day. Excrements were collected in a metabolic cage and urine was collected in a vessel containing toluene.

Quantitative Analysis of Q-7 (I) and Q-7 Hydroquinone (II). A sample (10 mg) was dissolved in ethanol (10 ml) and the solution was divided into two. I in the first part was measured directly by the usual procedure (Redfearn, 1967) and that in the second part after oxidation of II to I with $FeCl_3$ (Hoffmann *et al.*, 1964). II was calculated from the difference between the oxidized and direct values.

OXIDATION. To a solution (5 ml) of the sample in ethanol was added 10% ethanolic $FeCl_3$ (1 ml) and the mixture was shaken for 10 min. The reaction mixture was extracted with three 5-ml portions of petroleum ether. The extracts were washed with water and evaporated to dryness. The residue was dissolved in ethanol and made up to a volume of 5 ml.

ASSAY OF I. I was measured by comparison of the difference (ΔE) between the absorbance at 275 mμ of the sample solution and that of a solution treated with sodium borohydride (5 mg), $\Delta E_{1\,cm}^{1\%}$ of I = 186.

Quantitative Analysis of the Conjugates of V and VII. ACID TREATMENT AND PARTIAL PURIFICATION. A dried sample (1 g) was mixed with 1.5 N HCl in 90% methanol (10 ml) and stirred at room temperature overnight. To the reaction mixture was added 50% $FeCl_3$ (1 ml) and the mixture was shaken for 10 min. To the reaction mixture was added water (10 ml) and the mixture was extracted with three 15-ml portions of ethyl acetate. The extracts were evaporated to dryness. The residue was purified by thin-layer chromatography using benzene–ethyl acetate (3:1). The portion of silica gel corresponding to synthetic VI or VIII was extracted with ether. The extracts were evaporated to dryness. The residue was dissolved in carbon tetrachloride and made up to an aliquot volume for gas–liquid partition chromatography.

APPARATUS AND CONDITIONS. A helium gas chromatograph apparatus equipped with a hydrogen ionization detector (Ohkura gas chromatograph, Model 2100) and a Pyrex glass column (0.35 × 105 cm) packed with 5% APN on gas chrom Z were used. The gas flow rates of helium, hydrogen, and air were adjusted to 90, 60, and 600 ml per min, respectively, and temperatures to 210° (column) and 270° (evaporator); t_R values were 3.3 min (VI) and 8.2 min (VIII).

Isolation of Metabolites in Rats Urine. Urine (818 ml), collected during the 6 days from the 168th day after the beginning of administration from female rats which received I orally in doses of 150 mg/kg per day, was extracted with three 400-ml portions of ether. The extracts were washed with two 600-ml portions of water. The lipid-free urine and the washings were combined and concentrated to 220 ml in a freeze-drying apparatus. To the concentrate were added 35% HCl (75 ml) and methanol (300 ml), and the mixture was left standing at room temperature for 1 hr. The resulting mixture was extracted with three 200-ml portions of ethyl acetate. After being washed with water and dried over Na_2SO_4, the extracts were evaporated to dryness to give a brown oil (1.450 g). To a solution of the extracts in methanol (20 ml) was added an ethereal solution of diazomethane and the mixture was evaporated to dryness to give a brown oil. A solution of the oil in chloroform (3 ml) was applied on four silica gel plates and developed with benzene–ethyl acetate (3:1) to give several colored bands. The parts of R_F 0.0–0.52, 0.53–0.64, 0.65–0.79, and 0.80–1.0 were each extracted with ether to obtain orange-colored oils. The quinones contained in the oil from R_F 0.0–0.52 were purified by five successive preparative-layer chromatographic steps using hexane–ether (1:4). III (0.1 mg) and an unknown quinone IX (0.1 mg) were obtained as orange oils. A quinone contained in the oil from R_F 0.53–0.64 was purified similarly by thin-layer chromatography to give IV (1 mg) as an orange oil. The quinones contained in the oil from R_F 0.8–1.0 were purified by thin-layer chromatography using hexane–ether (1:4), benzene–ethyl acetate (3:1), and chloroform. A more polar unknown quinone XII (0.5 mg) and I (0.5 mg) were obtained as orange oils. One of the quinones contained in the oil from R_F 0.65–0.79 was purified by thin-layer chromatography using benzene–ethyl acetate (3:1). From the portion of silica gel corresponding to R_F 0.79 was obtained an unknown quinone X (0.5 mg) as an orange oil. A mixture (130 mg) of three quinones contained in the oil from R_F 0.65–0.75 of this thin-layer chromatography was purified by reversed-phase column chromatography as follows: a solution of the mixture in acetone (1 ml) was mixed with silica gel (2 g) containing 12.5% paraffin. The resulting mixture was air dried to remove acetone, and then it was overlapped on a column (2 × 18 cm) packed with silica gel (30 g) containing 12.5% paraffin. The column was eluted with acetone–water (1:1). The eluate was checked by thin-layer chromatography and fractionated into four fractions. Fractions 1 and 3 were purified by thin-layer chromatography using chloroform to give VI (20 mg) and VIII (10 mg) as orange oils, respectively. Fraction 4 was developed with acetone–water (3:2) on a silica gel plate treated with paraffin. The portion of silica gel corresponding to R_F 0.3 was extracted with ether. The extracts (2 mg) were purified by thin-layer chromatography using chloroform to give an unknown quinone, XI (0.5 mg) as an orange oil: VI, $[\alpha]^{25}D$ +0.57° (*c* 1.0); IX, λ_{max} 280 mμ, infrared spectrum 3400 (OH), 1714 (CO), 1665, 1640, 1615 cm^{-1} (quinone); X, λ_{max} 278 mμ, infrared spectrum 1740, 1270 (ester), 1665, 1650, 1610 cm^{-1} (quinone).

Isolation of Conjugates in Rabbits Urine. Urine (9.6 l.), collected during the 9 days after the beginning of administration from buck rabbits which received I intravenously in doses of 150 mg/kg per day, was lyophilized. The resulting powder (425 g) was extracted with three 400-ml portions of methanol. The extracts were concentrated to 200 ml. The concentrate was mixed with silica gel (150 g) and air-dried with occasional stirring at room temperature. The treated silica gel was overlapped on a column (5 × 42 cm) packed with silica gel (350 g) and eluted with ethyl acetate (0.5 l.), ethyl acetate–methanol (9:1) (1 l.), ethyl acetate–methanol (3:1) (2 l.), and ethyl acetate–methanol (1:1) (2 l.). The residue (16.6 g) of the ethyl acetate–methanol (1:1) fraction was dissolved in water, poured on a column (6 × 70 cm), packed with DEAE-cellulose (300 g), and then eluted with water (1.3 l.), 0.1 M NaCl (1.4 l.), 0.2 M NaCl (0.68 l., residue

3.8 g), 0.2 M NaCl (0.25 l.), and 0.2 M NaCl (0.47 l.), residue 5.8 g). The residue (3.8 g) was extracted with three 10-ml portions of methanol. The combined extracts were concentrated to 1 ml. The concentrate was purified by thin-layer chromatography using 1-propanol–NH_4OH (9:1). The main parts were masked with a glass plate and the uncovered edges were sprayed with $K_3Fe(CN)_6$–$FeCl_3$ reagent, then concentrated H_2SO_4 to detect the R_F value of the desired conjugate. The masked part corresponding to the blue-colored conjugate was taken off and extracted with three 10-ml portions of methanol. The extracts were evaporated to dryness to leave a white powder XIII (10 mg). The residue (5.8 g) was similarly worked up to obtain a white powder which was a mixture of the conjugates of V and VII.

Detection of Metabolites in the Conjugate. A sample was spotted on a silica gel plate. Methanolic HCl (3 N) was applied to the spot. After being kept at 75° for 30 min, the plate was developed with ethanol–chloroform (1:9). The metabolites which were contained in the conjugate were recognized as VI and VIII by spraying with the leuco-methylene blue reagent.

Metabolites in Feces. Q-7 HYDROQUINONE. Feces (22.4 g), collected during the 5 days from the 24th day after the beginning of administration from male rats which received I orally in doses of 150 mg/kg per day, were extracted with four 50-ml portions of ether. The extracts were evaporated to dryness to obtain a red oil (2.235 g) (the amounts measured by the assay procedure: I, 70 mg; II, 65 mg). The oil was treated with acetic anhydride (1 ml) and pyridine (5 ml). The mixture was worked up as usual to obtain II diacetate (43 mg).

CONJUGATE. Feces (251 g), collected during the month after the beginning of administration from male rats which received I orally in doses of 50 mg/kg per day, were homogenized in ether (300 ml) and stirred for 30 min. The extraction was repeated twice in the same manner. The residue (136 g) free from lipids was extracted with three 260-ml portions of methanol. The extracts were evaporated to dryness to give a red oil (65 g). To the residue was added 1.5 N HCl in 90% methanol (500 ml). The suspension was kept overnight at room temperature with stirring. The resulting mixture was extracted with three 300-ml portions of ethyl acetate after the addition of water (500 ml). The residue from the extracts was dissolved in chloroform (10 ml) and insoluble materials were filtered off. The filtrate was poured on a column packed with silica gel (70 g), and then chloroform (700 ml) was poured. The eluate was evaporated to dryness to obtain a yellow oil (301 mg). The oil was purified by thin-layer chromatography using benzene–ethyl acetate (3:1). The portions of silica gel corresponding to authentic VI and VIII were extracted with ether. The oil from the extracts was purified by thin-layer chromatography on a silica gel plate containing paraffin using acetone–water (2:3). The portions of silica gel corresponding to authentic VI and VIII were washed with hexane, and then extracted with ether to obtain VI and VIII as yellow oils. Amounts measured by gas–liquid partition chromatography: VI, 225 μg; VIII, 84 μg.

Metabolites in Liver. Liver (168 g) of male rats to which I was administered orally in doses of 150 mg/kg per day, was extracted with four 780-ml portions of acetone–ethanol (1:1). The combined extracts were evaporated to dryness to obtain a red oil (14.9 g) (amounts measured by the analysis procedure: I, 1381 mg; II, 1201 mg). The oil was worked up similarly to the case of the feces to give VI and VIII. Amounts measured by gas–liquid partition chromatography: VI, 42.6 μg; VIII, 16.8 μg.

Isolation of Cholesteryl Ester XV of XIV. Adrenals (354 mg) of male rats, to which I was administered orally in doses of 2000 mg/kg per day for 1 month, were extracted with four 10-ml portions of acetone–ethanol (1:1) by the Potter–Elvehjem apparatus. The oil (38 mg) from the extracts was purified by thin-layer chromatography with benzene. The portion of silica gel corresponding to I was extracted with ether. The residue from the extracts was divided by thin-layer chromatography on a silica gel plate containing paraffin using 90% acetone into I (R_F 0.5, 142 μg), Q-9 (R_F 0.2, 13.4 μg), and XV (R_F 0.0). XV was further purified by thin-layer chromatography on a similar plate using acetone to obtain a yellow oil (R_F 0.21, 500 μg): XV, λ_{max} 275 mμ; mass spectrum *m/e* 690 (M + 2 − R′ + 1), 688 (M − R′ + 1), 590, 522, 454, 386, 368 (M − XIV), 318, 235 (fragment A[2]), 197 (fragment B[2]).

Hydrolysis of XV. A solution of XV (500 μg), pyrogallol (200 mg), and 50% KOH (0.6 ml) in ethanol (3 ml) was refluxed for 1 hr. Water (10 ml) was added to the mixture, it was acidified with HCl, oxidized with 5% methanolic $FeCl_3$ (4 ml), and extracted with four 5-ml portions of chloroform. The residue from the extracts was purified by thin-layer chromatography using ether–hexane–acetic acid (60:40:1). A yellow band of R_F 0.42 gave a yellow oil, XIV (200 μg). Cholesterol (XVII) was obtained from the part of R_F 0.43–0.50: XIV, λ_{max} 274 mμ; infrared spectrum 2700–2500, 1695 (C=CCOOH), 1665, 1650, 1610 cm^{-1} (quinone).

Methylation of XIV. A yellow oil (200 μg) obtained by methylation of XIV with diazomethane was purified by thin-layer chromatography using hexane–ether (3:1). From a yellow band of R_F 0.44, XVI was obtained as a yellow oil (200 μg): XVI, λ_{max} 275 mμ; infrared spectrum 1710 (C=CCOOCH$_3$); mass spectrum *m/e* 704 (M + 2), 702 (M), 671 (M − OCH$_3$), 235 (fragment A), 197 (fragment B).

Isolation of Cholesteryl Ester XIX of XVIII. A yellow oil (26 mg) obtained from ovaries (250 mg) of rats to which I was administered orally in doses of 2000 mg/kg per day for 1 month, was treated in a similar manner to that of XV to give I (R_F 0.5, 182 μg), Q-9 (R_F 0.2, 14.2 μg), and a yellow oil XIX (100 μg).

XVIII was obtained from XIX by a similar alkaline hydrolysis to that of XV, and XX was obtained by the methylation of XVIII in a similar manner to that of XIV: XVIII, λ_{max} 275 mμ; infrared spectrum 2800–2500, 1710 (COOH), 1665, 1650, 1610 cm^{-1} (quinone); XIX, λ_{max} 275 mμ; XX, λ_{max} 275 mμ; infrared spectrum 1735 (COOCH$_3$), 1665, 1650, 1610 cm^{-1} (quinone); mass spectrum *m/e* 706 (M + 2), 704 (M), 673 (M − OCH$_3$), 235 (fragment A), 197 (fragment B).

Determination of Cholesterol. Cholesterol was determined by gas–liquid partition chromatography in the portion separated by thin-layer chromatography

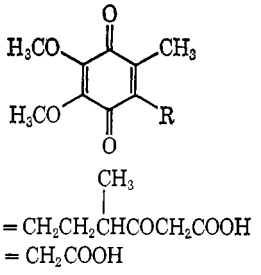
APPARATUS AND CONDITION. A nitrogen gas chromatograph apparatus equipped with a hydrogen ionization detector (Ohkura gas chromatograph, Model 2100) and a Pyrex glass column (0.35 × 122 cm) packed with 6% QF-1 on gas chrom

[2] These fragments are quoted in Morimoto *et al.* (1967).

Q (60–80 mesh) were used. The gas flow rates of nitrogen, hydrogen, and air were adjusted to 100, 40, and 400 ml per min, respectively. The temperatures of the evaporator and the column were kept at 200°. The t_R value was 14 min.

Discussion

Recently, the biosynthetic route of ubiquinone has been made almost clear (reviewed by Olson, 1966) but little has been known about its metabolism as well as those of other isoprenoid quinones. The authors have isolated several metabolites of I which retain the quinone ring and identified their structures. In comparison with the metabolism of steroids, fatty acids, and prostaglandins, the metabolic pathway of I can be said to be as follows: the degradation of I in mitochondria starts with ω-*cis*-methyl oxidation to form the carboxylic acids, XIV and XVIII and is followed by β oxidation to give VII. VII is then subjected to β oxidation and reduction giving a keto acid A. A is further oxidized to V. Whether IV is an artifact or not, is not clear at present because A would be artificially decarboxylated to IV by heating. However, the fact that IV and V were obtained supports the existence of A as a metabolic intermediate. The localization of the β oxidation enzymes in mitochondria supports the view described above. The β oxidation of V to B should be formally possible. Accordingly, the presence of B in the rat urine treated with hydrochloric acid was investigated by thin-layer chromatography and gas–liquid partition chromatography under the conditions which were established by using an authentic sample (Watanabe *et al.*, 1970), but it could not be detected.



A, R = $CH_2CH_2CHCOCH_2COOH$ (with CH_3 branch)
B, R = CH_2COOH

Examples of the enzymatic reduction of an ω double bond in an isoprenoid chain are seen in lanosterol and desmosterol which are converted into dihydrolanosterol and cholesterol, respectively (Kandutsch and Russel, 1960; Stokes and Fish, 1960; Steinberg and Avigan, 1960). It was also shown that the degradation of a cholesterol derivative starts with ω-methyl oxidation to a carboxylic acid *via* an alcohol, and is followed by β oxidation to give a cholic acid derivative (reviewed by Danielsson, 1968). The β oxidation of the cholic acid derivative was evidently prevented, thus demonstrating the hindering effect of the bulky steroid nucleus (Lindstedt and Tryding, 1957). Such hindrance to β oxidation was also found in a fatty acid (Bergström *et al.*, 1954) and prostaglandins (Hamberg, 1968). Similarly, the resistance of V to β oxidation seems to be attributable to its bulky quinone nucleus. NADH–ubiquinone reductase (Hatefi *et al.*, 1962) and succinate–ubiquinone reductase (Ziegler and Doeg, 1962) are known to be present in mitochondria. Accordingly, V and VII will be reduced to the corresponding hydroquinones by these enzymes and then conjugated with the microsomal enzymes (Bergström *et al.*, 1960) to give the disulfates. The presence of the cholesteryl esters XV and XIX can be understood from the observation that fatty acids are mostly present as cholesteryl esters in adrenals (Hanahan, 1960).

TABLE VI: Ubiquinone-7 (I) and Its Hydroquinone (II) in the Content of Gastrointestinal Tract.[a]

Content	Compound I (mg)	Compound II (mg)	II/I + II (%)
Stomach	2.3	1.0	30
Small intestine	1.2	0.2	15
Large intestine	1.9	7.9	81

[a] A rat to which the standard food powder (9 g) supplemented with I (18 mg) was administered.

It was reported that natural isoprenoid quinones, such as the vitamin K group and tocopherol group compounds were metabolized yielding γ-lactones (Wiss and Gloor, 1966). However, according to the present study, the lactones were assumed to be artifacts formed from the acids corresponding to VII during purification. Simon *et al.* (1956a) suggested a glucuronide conjugate in the metabolism of α-tocopherol, but the authors could not detect glucuronic acid by the color reaction (Hanson *et al.*, 1944) in the hydrolysate of the conjugate fraction of I metabolites.

In general, intaken foreign compounds enter the body mostly by absorption from the gastrointestinal tract and are taken to the liver where they are metabolized. Their metabolites are then excreted in the bile to be voided in the feces and in the urine (reviewed by Parke, 1968). Since I fed to rats was found mostly in the liver and similar ratios of V:VII were observed in hydrolysates of the liver, feces, and urine, I should be metabolized to V and VII mainly in the liver and then converted into their conjugates. The amount excreted in the feces was very small compared with that in the urine and the metabolites were found to be excreted mostly in the urine. In order to investigate sex and species differences of the metabolites, the amounts of the conjugates were measured as VI and VIII in the urine of male and female rats, buck rabbits, and an adult man. Sex difference was not recognized, but slight species difference was found though no definite conclusion was drawn from this small number of experiments.

The high ratio of II to I in the contents of the large intestine (Table VI), where they were found in large quantities, will suggest the reduction of I with sulfhydryl compounds in that portion. This is supported by the former observation (H. Morimoto, I. Imada, M. Sasaki, and H. Sugihara, 1966, unpublished data) that I is readily reduced with hydrogen sulfide or organic sulfhydryl compounds. But, the possibility of enterobacterial reduction cannot be excluded. It is necessary to further clarify the physiological significance of the ubiquinone metabolites, and to study the metabolites having neither a quinone nor hydroquinone structure.

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