

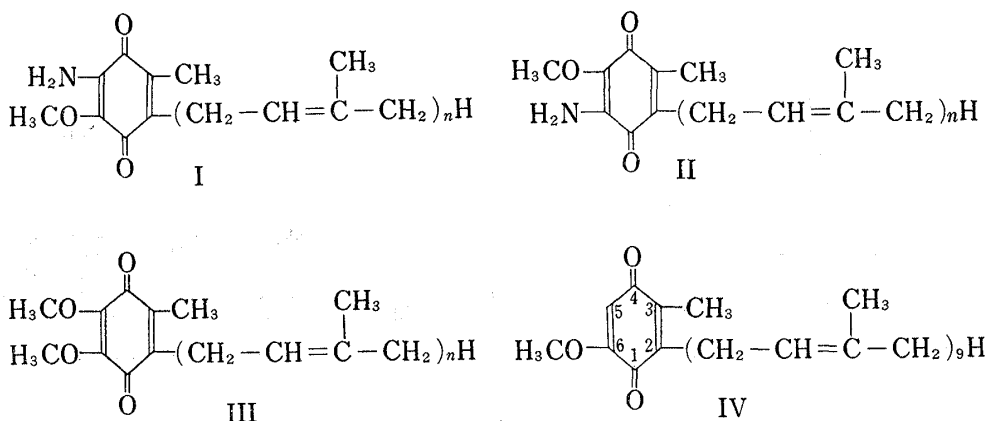
Rhodoquinone-9 from the Muscle of *Ascaris lumbricoides* var. *suis*¹⁾HIKARU OZAWA, MASASHI SATO,^{2a)} SHINSAKU NATORI,^{2b)}
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An aminoquinone was isolated from the muscle of *Ascaris lumbricoides* var. *suis* and characterized as rhodoquinone-9 (I, $n=9$) by the spectral data and by the identification with the synthetic specimens from ubiquinone-9 (III, $n=9$) and from 5-demethoxyubiquinone (IV). Biological significance of occurrence of the quinone in the worm is also discussed.

Rhodoquinone-10 was first isolated from *Rhodospirum rubrum*³⁾ and was proved to be the derivative (I, $n=10$) of ubiquinone-10 (III, $n=10$) in which one of the methoxyl groups is replaced by an amino group.⁴⁾ Either rhodoquinone-9 (I, $n=9$), the shorter isoprenologue of I ($n=10$), or its isomer, isorhodoquinone-9 (II, $n=9$), was isolated from *Euglena gracilis*, though the distinction between the two has not been carried out.⁵⁾ The occurrence of rhodoquinones in nature has been hitherto confined to only these few microorganisms in contrast to the ubiquitous existence of ubiquinones in nature.

During the survey on ubiquinones in parasitic nematodes, the authors obtained the evidence that the mitochondrial fraction of *Ascaris* muscle contained a rhodoquinone analogue in the place of ubiquinones.⁶⁾ The quinone was later isolated in a crystalline form^{1a)} and was characterized as rhodoquinone-9 (I, $n=9$) by unequivocal methods.^{1b)} This paper describes the details of the work.



- 1) The preliminary communications of this work have been published,^{1a,b)} a) H. Ozawa, M. Sato, S. Natori, and H. Ogawa, *Experientia*, **1969**, 484; b) H. Ogawa, S. Natori, M. Sato, and H. Ozawa, *Tetrahedron Letters*, **1969**, 1969.
- 2) Location: a) Aoba, Aramaki, Sendai; b) Kamiyoga-1-chome, Setagaya-ku, Tokyo; c) Present address: Department of Domestic Sciences, Sagami Women's University, Sagamihara, Kanagawa.
- 3) J. Glover and D.R. Threlfall, *Biochem. J.*, **85**, 14p (1952).
- 4) H.W. Moore and K. Folkers, *J. Am. Chem. Soc.*, **87**, 1409 (1965); **88**, 567 (1966).
- 5) R. Powls and F.W. Hemming, *Phytochem.*, **5**, 1235, 1249 (1966).
- 6) M. Sato and H. Ozawa, *J. Biochem.*, **65**, 867 (1969).

The muscle of adult round worms, *Ascaris lumbricoides* var. *suis*, was homogenized and extracted with ethanol-ether (3:1). The hexane-soluble portion of the extract was fractionated by column chromatography of silica gel and the violet band thus obtained was further purified by the repetition of preparative thin-layer chromatography (TLC). Finally 5 mg of deep violet crystals of mp 66.5—67° were obtained from 1600 g of the muscle.

Mass spectrum suggested the molecular formula, $C_{53}H_{81}O_3N$ (M^+ 779.618 m/e , calcd. 779.622). Infrared (IR) bands (3470, 3330, 1643, 1600 cm^{-1} , 3050—2850 cm^{-1}) suggested an aminoquinone with a long aliphatic chain for the compound and the ultraviolet (UV) absorptions (λ_{max}^{EtOH} $m\mu$ (log ϵ) 285 (4.03), 515 (3.08)) are superimposable with those of I and II.^{4,5)}

TABLE I. High-Resolution Mass Measurement for Rhodoquinone-9 (I, $n=9$, natural)

m/e	Observed	Calculated	Assignment
779	.618	.622	$C_{53}H_{81}O_3N$ M^+
764	.596	.598	$C_{52}H_{78}O_3N$ a
302	.178	.176	$C_{12}H_{24}O_3N$ b
220	.097	.097	$C_{21}H_{14}O_3N$ c
182	.080	.082	$C_9H_{12}O_3N$ d

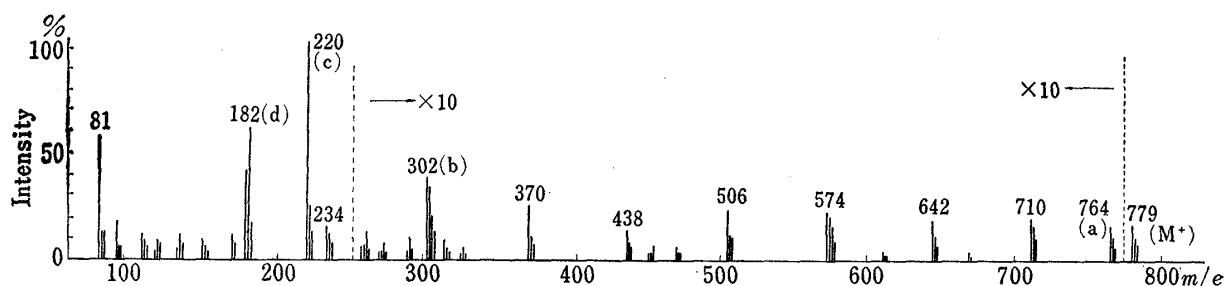
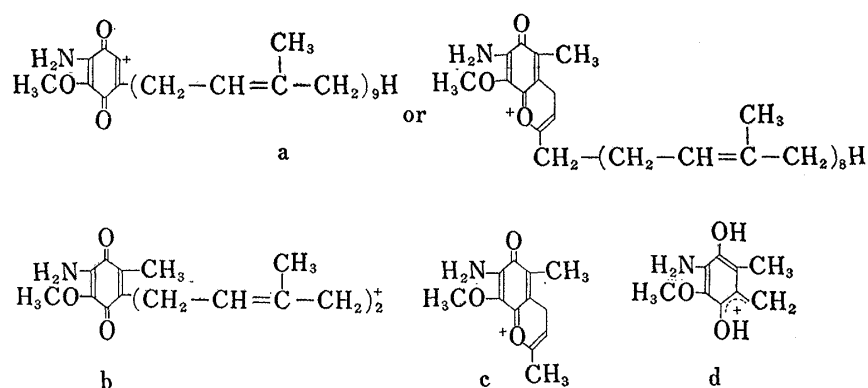


Fig. 1. Mass Spectrum of Rhodoquinone-9 from *Ascaris lumbricoides* var. *suis*

The most conclusive evidence for the structure was obtained by the mass spectrum (Table I and Fig. 1). As shown in the figure the quinone shows the typical cracking pattern of multiprenyl quinones, *i.e.* ubiquinones (III).^{7,8)} The ion formed by the loss of a methyl (a), fragments formed by the successive loss of eight prenyl groups ($M^+ - 69$, $M^+ - 69 - 68 \times n$ ($n=1-7$)), the pyrilium ion (c), and the benzylium ion of the quinol (d). The milli-mass numbers of the ions (Table I), along with other spectral data, strongly suggested that the

7) R.F. Muraca, J.S. Whittick, G.D. Daves, Jr., P. Friis, and K. Folkers, *J. Am. Chem. Soc.*, **89**, 1505 (1967).

8) B.C. Das, M. Lounasmaa, C. Tendill, and E. Lederer, *Biochem. Biophys. Res. Commun.*, **21**, 318 (1965).

quinone must have one naphrenyl chain, one methyl, and CH_3ON residues on the quinone ring, and hence might be either I ($n=9$) or II ($n=9$).

The relative positions of the methoxyl and the amino groups in I ($n=10$) was first determined by the derivation into the corresponding chromenol.⁴⁾ However the method is not applicable for a small amount of the sample like in this case. At this stage of our work the synthesis of I ($n=10$ and 3 (hexahydro)) and II ($n=10$ and 3 (hexahydro)) by the unequivocal method,⁹⁾ and the distinction between the two by the solvent shifts appeared in the separation of the ring methyl and the ring methylene¹⁰⁾ were reported. At the same time the separation of the mixture of I ($n=10$) and II ($n=10$) prepared by the ammonolysis of III ($n=10$) into the two components by TLC using the technique of multiple development was also reported.⁹⁾

Ammonolysis of III ($n=9$) in methanol-ether according to the procedure described for the higher homologue^{4,5,9)} afforded the mixture, mp $38-42^\circ$, of I ($n=9$) and II ($n=9$), which showed nearly the same spectral data and R_f value in TLC as the natural quinone. Further separation of the mixture was carried out by preparative TLC using the multiple developing technique. Finally two quinones, mp $50-52^\circ$ from the upper zone and mp $42-52^\circ$ from the lower,¹¹⁾ were obtained in the ratio of *ca.* 1:6. They showed very similar spectral properties except the difference in the finger print region in IR (Fig. 2) and some signals in nuclear

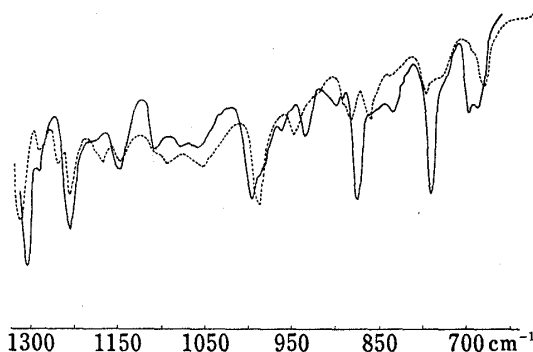


Fig. 2. Infrared Spectra of Rhoquinone-9 and Isorhoquinone-9 (in KBr)

————: rhoquinone-9
 - - - - -: isorhoquinone-9

TABLE II. Chemical Shift (τ) and Separation of Ring Methyl and Ring Methylene Resonance (ppm) of Synthetic Rhoquinone (I) and Isorhoquinone (II)

Comp.	Solvent	Type of proton (τ)			Ring methyl- ring methylene (ppm)
		Methoxyl	Ring methylene	Ring methyl	
I, $n=9$	CDCl_3	6.05	6.79	8.21	1.42
II, $n=9$	CDCl_3	6.07	6.78	—	—
I, $n=10^a$	CCl_4	6.18	6.92	—	— (1.17) ^b
II, $n=10^a$	CCl_4	6.18	6.95	—	— (1.10) ^b
I, $n=9$	benzene	6.23	6.77	8.18	1.41
II, $n=9$	benzene	6.28	6.93	8.10	1.17
I, $n=10^a$	benzene	6.35	6.89	8.24	1.35 (1.36) ^b
II, $n=10^a$	benzene	6.36	6.99	8.16	1.17 (1.17) ^b
I, $n=9$	pyridine	6.05	6.59	8.15	1.56
II, $n=9$	pyridine	6.10	6.75	8.20	1.45
I, $n=10^a$	pyridine	6.17	6.67	8.01	1.34 (1.33) ^b
II, $n=10^a$	pyridine	6.18	6.77	—	— (1.16) ^b

a) The value shown in ref. 10).

b) The value calculated from the observed value in I ($n=4$) or II ($n=4$) in ref. 10).

- 9) G.D. Daves, Jr., J.J. Wilczynski, P. Friis, and K. Folkers, *J. Am. Chem. Soc.*, **90**, 5587 (1968).
 10) J.J. Wilczynski, G.D. Daves, Jr., and K. Folkers, *J. Am. Chem. Soc.*, **90**, 5593 (1968).
 11) Due to the scarcity of the samples further purification to obtain the specimens showing higher mp was impossible. It was suggested that the ubiquinone analogues prepared by nuclear prenylation are generally contaminated with *cis*-isomers in the nearest double bond to the ring and show lower mp.⁹⁾ Our starting material might be the case.

magnetic resonance (NMR) in benzene and pyridine. The solvent shifts method¹⁰⁾ was applied for the assignment of the two. Since the ring methyl and the ring methylene resonances in the quinone showing higher *Rf* value are more separated than those of the lower *Rf* quinone in benzene and pyridine (Table II), they are respectively assigned as I and II. The natural quinone from the worm showed the identity with the former (I) by IR and TLC.

Imamoto and Senoh¹²⁾ isolated 5-demethoxyubiquinone-9¹³⁾ (IV) from *Pseudomonas ovalis* and differentiated from the 6-demethoxy isomer¹³⁾ by the comparison of UV and chromenol formation in the synthetic model compounds. 1,4-Addition of ammonia to IV in methanol-ether⁹⁾ gave the quinone (I, *n*=9), which was proved to be identical with the natural quinone. Thus the relative position of the methoxyl and the amino groups has been firmly established, and rhodoquinone-9 (I, *n*=9) from *Ascaris*, 5-demethoxyubiquinone (IV) from *Pseudomonas*, and ubiquinone-9 (III, *n*=9) were directly correlated.

By the courtesy of Dr. Hemming we have obtained the small sample of the quinone from *Euglena*.⁵⁾ Unfortunately the sample has been decomposed in the storage and the direct comparison becomes impossible. However the comparison of the IR chart with ours suggests that the quinone is also identical with I (*n*=9).

Ascaris lumbricoides is a rare animal in its strictly anaerobic existence and it is the only metazoa in which rhodoquinone has been detected. Comparative study of quinones between aerobic and anaerobic helminth, *i.e.* *Metastrongylus elongatus* and *Ascaris lumbricoides*, has shown that the former contains ubiquinone but the latter rhodoquinone exclusively.⁶⁾

Preliminary study on the distribution of rhodoquinone-9 in *Ascaris lumbricoides* provided the evidence that the level of rhodoquinone in the muscle and digestive tract is 15 γ /g of wet tissue, which is comparable to the level of ubiquinone in mammalian tissues,¹⁴⁾ and furthermore, in intracellular distribution, rhodoquinone in the mitochondrial fraction accounts for more than 50% of the total in the muscle (unpublished data). These facts strongly suggest that rhodoquinone may play functional role in the worm in the place of ubiquinone. The study on the functional role of rhodoquinone in the helminth is now in progress.

Experimental¹⁵⁾

The Isolation of Rhodoquinone-9 (I, *n*=9) from the Muscle of *Ascaris lumbricoides* var. *suis*—Adult round worms, *Ascaris lumbricoides* var. *suis*, were collected freshly from the slaughter house. Worms were cut open longitudinally and freed from intestine, eggs, *etc.* The muscular layer was then scraped from the cuticle and washed several times with 0.9% NaCl solution. Muscle strips thus obtained were stored at -15° or used for the extraction immediately. The muscle strips (1600 g) were homogenized in a Waring blender with a small amount of ethanol under ice-cooling and then extracted with ethanol-ether (3:1) for 15 hrs at room temperature. The combined extracts were evaporated under reduced pressure to 1/10 of the original volume and then extracted with equal volume of hexane. The hexane extracts were evaporated to dryness under nitrogen stream. The residue was applied on a column of silica gel (Mallinckrodt), eluted with hexane to remove the lipid fraction, and then with hexane-benzene (1:1) to obtain the violet quinone band. The quinone fraction was collected and purified by the repetition of preparative TLC on Silicagel G plates using chloroform or a mixture of hexane-chloroform as the solvent. Finally the violet band was extracted with ether and recrystallized from methanol in a cold room to deep violet crystals (5 mg) of mp $66.5-67^{\circ}$; M^+ 779.618 *m/e* (Calcd. for $C_{53}H_{81}O_9N$, 779.622). UV $\lambda_{\text{max}}^{\text{EtOH}}$ $m\mu$ (log ϵ); 285 (4.03), 515 (3.08). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} ; 3470, 3330, 3050—2850, 1643, 1600. Mass spectrum (*cf.* Table I and Fig. 1).

12) S. Imamoto and S. Senoh, *Tetrahedron Letters*, **1967**, 1237; *idem*, *Nippon Kagaku Zasshi*, **89**, 316 (1968).

13) The numbering after ref. 7—10, 12.

14) F.L. Crane, "Biochemistry of Quinones," ed. by R.A. Morton, Academic Press, New York and London, 1965, p. 183.

15) Melting points were determined in a Yanagimoto melting point apparatus and are not corrected; the UV spectra were taken on a Hitachi EPU-2A Spectrophotometer; the IR spectra were measured on a Nihon Bunko DS-301 Spectrophotometer in KBr discs; the NMR spectra were run on a JEOL JNM-6-60HL (60 Mc) using tetramethyl silane as the internal standard; the high resolution mass spectra were determined on a JEOL JMS-01SG mass spectrometer at the ionization potential (75 eV) and the sample temperature (*ca.* 190°).

Thin-Layer Chromatography of the Hexane Extract—The absence of ubiquinones in the extract was confirmed by TLC on Silicagel G and Wakogel B-5 plates using ubiquinone-9 and ubiquinone-5 as the standards. The hexane extract before the purification by column chromatography showed two purple spots and four colorless spots, showing fluorescence under ultraviolet light, on Silicagel G plate using chloroform-hexane (1:1) as the developer. The purple spot showing lower *R_f* value is that of rhodoquinone (I, *n*=9). The upper purple spot, though it was unsuccessful in isolating in a pure form due to its instability, showed nearly the same UV absorption as I (*n*=9) and changed into it in the course of further purification, *e.g.* repetition of TLC or column chromatography. The reversed-phase TLC¹⁶⁾ was also examined and applied for the identification.

Synthesis of Rhodoquinone-9 (I, *n*=9) and Isorhodoquinone-9 (II, *n*=9) from Ubiquinone-9 (III, *n*=9)—The synthesis and the separation were followed by the reported method.⁹⁾ Ubiquinone-9 (III, *n*=9) (mp 44°, 1.0 g) was dissolved in anhyd. methanol-anhyd. ether (1:1, 700 ml). Dry ammonia gas was passed through the solution for 3 hrs and the mixture was kept standing at room temperature for 2 days. After that when the color of the solution changed to violet-red, the solvent was evaporated under reduced pressure. The residue was dissolved in hexane and passed through a short column of silica gel (Mallinckrodt). The elution by mixtures of hexane-benzene with increasing amount of benzene afforded a yellow band, containing the starting material, and a violet band, containing rhodoquinone-9 (I, *n*=9) and isorhodoquinone-9 (II, *n*=9). The latter band was separated and applied on thin-layer plates of Silicagel G by the multiple development procedure⁹⁾ using chloroform as the developer. Repetition of the development gave two bands, the upper of which, after recrystallization from methanol, gave violet crystals (5 mg) of mp 50–52° and the lower, violet crystals (30 mg) of mp 42–52° from methanol. IR and UV spectra of the naturally occurring quinone were superimposable with those of the former, but were different from those of the latter in the finger print region in IR as shown in Fig. 2. NMR spectra shown in Table II indicated that the former is I (*n*=9) and the latter, II (*n*=9).

Synthesis of Rhodoquinone-9 (I, *n*=9) from 5-Demethoxyubiquinone-9¹³⁾ (IV)—5-Demethoxyubiquinone-9 (IV) (mp 45–50.5°, 19 mg), isolated from *Pseudomonas ovalis*,¹²⁾ in ether-methanol (1:2, 30 ml) was cooled to 0°, and ammonia was passed through the solution for 1.5 hrs.⁹⁾ The reaction mixture was allowed to stand for 4 hr at room temperature. The solvent was then removed and the residue was subjected to preparative TLC on Silicagel G plates developed in chloroform. An upper yellow band afforded the starting material and the lower violet band, violet crystals (2.4 mg) of mp 58–67° after recrystallization from methanol. The comparison by IR, UV, and TLC with the quinone from *Ascaris* showed the identity.

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The authors are glad to adopt a suggestion by a referee of this paper for the revision of some parts of this paper.

16) H. Wagner, L. Hörhammer, and D. Dengler, *J. Chromatography*, **7**, 211 (1962).