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68. **Hideko Ogawa and Shinsaku Natori**: Hydroxybenzoquinones from Myrsinaceae Plants. I. Reconfirmation of the Structure of Maesaquinone and Isolation of Acetylmaesaquinone from *Maesa japonica* MORITZI.*[1]

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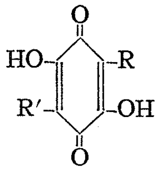
The occurence of hydroxybenzoquinones in higher plants, such as Myrsinaceae plants, and in fungi has long been reported.[1] Recently, wide distribution of ubiquiones and the related compounds and their role in electron transport system have been extensively studied.[2] Structural studies in our laboratory on helicobasidin (I),[3] a mold benzoquinone, and the examination of the ubiquinone-like activity of these hydroxybenzoquinone derivatives[4] urged us further studies on hydroxybenzoquinones in Myrsinaceae plants.

In 1939, Hiramoto[5,6] isolated maesaquinone from the fruits of *Maesa japonica* MORITZI (イズセンリョウ) (Myrsinaceae) and established the structure as IIa from the degradations and the synthesis of the dihydro derivative (IIb). Although Hiramoto's evidences for the structure are assumed to be quite ample, Thomson[7] pointed out that, in view of the uncertainties in the identification of these series of compounds, further confirmation by modern methods was desireble. Unexpectedly, maesaquinone (IIa) and the dihydro derivative (IIb) showed remarkable ubiquinone-like activity[4]; thus it becomes necessary to confirm the structure (IIa) for further studies on structure-activity relationship of these series of compounds.



I : R=1,2,2-trimethylcyclopentyl, $R'=CH_3$
IIa : $R=-(CH_2)_{13}CH=CH(CH_2)_3CH_3$, $R'=CH_3$
IIb : $R=-(CH_2)_{18}CH_3$, $R'=CH_3$

Maesaquinone (IIa), orange plates, m.p. 123°, $C_{26}H_{42}O_4$, was isolated from the fruits of *Maesa japonica* MORITZI according to Hiramoto's procedure[5] and the substitution pattern on benzoquinone nucleus was first examined by infrared, ultraviolet, and nuclear magnetic resonance spectra of IIa and the dihydro compound (IIb). Ultraviolet absorptions of IIa and IIb show the maxima both at 294 mμ and were superimposable with those of 2,5-dihydroxy- and 2,6-dihydroxy-alkylbenzoquinones (Table I).[3,8,9] Infrared spectra of IIa and IIb show the characteristic carbonyl absorptions at 1610 cm^{-1} and 1609 cm^{-1} in potassium bromide discs and 1635 cm^{-1} and 1633 cm^{-1} in chloroform solutions,

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1) R.H. Thomson : "Naturally Occuring Quinones," Butterworths, London (1957).

2) "A Chiba Foundation Symposium on Quinones in Electron Transport," J. & A. Churchill, London (1961).

3) S. Natori, H. Ogawa, H. Nishikawa : This Bulletin, **11**, 1343 (1963); *ibid.*, **12**, 236 (1964).

4) H. Ozawa, K. Momose, S. Natori, H. Ogawa, K. Yamaguchi : Biochem. Biophys. Acta, **86**, 397 (1964).

5) M. Hiramoto : Proc. Imp. Acad. Japan, **15**, 220 (1939); Yakugaku Zasshi, **59**, 665 (1939).

6) *Idem.* : *Ibid.*, **62**, 460, 464 (1942).

7) Same as reference 1), p. 26 (1957).

Table I. Ultraviolet and Infrared Spectra of Maesaquinone and Related Compounds

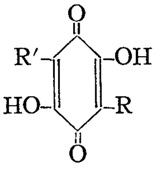
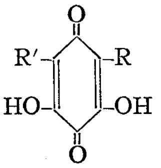
	R	R′	UV λ_{max}^{EtOH} mμ (log ε)			IR (cm^{-1}) ν_{O-H} solid (KBr)	IR (cm^{-1}) ν_{O-H} soln. ($CHCl_3$)	IR (cm^{-1}) ν_{C-O} solid (KBr)	IR (cm^{-1}) ν_{C-O} soln. ($CHCl_3$)
	CH_3	$-\underset{CH_3}{\underset{\vert}{C}}=\underset{CH_3}{\underset{\vert}{CH}}$	—	287 (4.24)	436 (2.40)	3310	3365	1617	1642
	〃	$-CH(CH_3)_2$	—	293 (4.31)	435 (2.36)	3319	3327	1616	1640
	〃	1,2,2-trimethylcyclopentyl	210 (4.13)	297 (4.15)	377, 430 (2.61, 2.47)	3300	3327	1609	1638
	$C_{10}H_{21}$	$C_{10}H_{21}$	209 (4.14)	295 (4.29)	429 (2.30)	3325	3374	1614	1630
	CH_3	CH_3	—	297 (4.26)	426 (2.26)	3417	3476	1660, 1641	1653, 1645
	maesaquinone (Ⅱa)		209 (4.24)	294 (4.36)	440 (2.58)	3310	3340	1610	1635
	dihydromaesaquinone (Ⅱb)		—	294 (4.37)	440 (2.58)	3315	3360	1609	1633

Table Ⅱ. Nuclear Magnetic Resonance Spectra of Maesaquinone and Related Compounds[a]

	Ⅱa	Ⅱb	Ⅶa	Ⅶb	
$-CH_2-\mathbf{CH_3}$	9.10	9.06	9.16	9.10	3H, t, J=6 c.p.s.
$-\mathbf{CH_2}-$	8.2~8.8 (centered at 8.71)	8.4~8.8 (8.74)	8.5~9.0 (8.74)	8.2~9.0 (8.72)	ca. 26~34H, m
$=\underset{\vert}{C}-\mathbf{CH_3}$	8.05	8.06	8.09	8.05	3H, s
$=\underset{\vert}{C}-\mathbf{CH_2}-CH_2-$	7.4~8.0	—	7.4~8.0	—	4~6H, m
$-CO-\mathbf{CH_3}$	—	—	7.69	7.67	3H, s
$-H_2C-\mathbf{CH}=\mathbf{CH}-CH_2-$	4.68	—	4.70	—	2H, t, J=5 c.p.s.
$-\mathbf{OH}$	2.37	2.40	3.01	3.02	2H for Ⅱa and Ⅱb, 1H for Ⅶa and Ⅶb, s

[a] NMR spectra were run on a Varian A-60 machine (60 Mc.) in $CDCl_3$ solution for Ⅱa and Ⅱb or CCl_4 solution for Ⅶa and Ⅶb using $(CH_3)_4Si$ as an internal standard and recorded in τ values.

respectively (Table I).[*3,3,8,9] These features, as was discussed in the previous paper,[3] indicated that Ⅱa is a 2,5-dihydroxybenzoquinone derivative.

Nuclear magnetic resonance spectra of Ⅱa and Ⅱb (Table Ⅱ) also gave a support for the structure. The absence of quinonoid ring proton and the presence of an olefinic

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[*3] Although Bycroft and Roberts[8] and Nakata, *et al.*[9] attributed the splitting of the carbonyl bands in 2,6-dihydroxybenzoquinones and the strong unit band in 2,5-dihydroxybenzoquinones to hydrogen bond formation of carbonyl groups with hydroxyl groups, mesomeric effects of the substituents at β position to the carbonyls are not negligible. For example 2-methoxy-6-alkylbenzoquinones show two bands at 1640~1680 cm^{-1} region.[3] In NMR spectra hydroxyl protons in I, Ⅱa, Ⅱb and their related compounds generally appear at 1.7~3.1 τ in $CDCl_3$ or CCl_4 solution, which suggests that the hydrogen bonds are far weaker than those of hydroxy-naphthoquinones and -anthraquinones having hydroxy groups at peri-positions, forming conjugated chelates.

8) B.W. Bycroft, J.C. Roberts: J. Org. Chem., **28**, 1429 (1963).

9) H. Nakata, K. Sakai, I. Morimoto, Y. Hirata: Tetrahedron, **20**, 2319 (1964).

methyl group ($8.05\,\tau$, 3H, s) clearly show the presence of a methyl substituent on the quinone nucleus, which was first overlooked by Hiramoto.[5,6] Another acyclic side chain is a normal chain ($9.10\,\tau$, 3H, t, $J=6$ c.p.s.) with an ethylenic double bond, in which the two protons are nearly identically situated and coupled with the adjacent methylene groups ($4.68\,\tau$, 2H, t, $J=5$ c.p.s.).

Next, the carbon numbers and the position of the bouble bond in the side chain were reconfirmed by alkaline hydrogen peroxide oxidation and the examination of the products by gas chromatography. An unsaturated acid of C_{20} (IIIa), m.p. 33~35.5°, formed by the oxidation of IIa,[5,6] the corresponding saturated acid (IIIb),[5,6] and volatile (IV) and nonvolatile (V) acids, formed by ozonisation of IIa and the following oxidation with silver oxide,[5,6] were derived to their methyl esters and compared their retention times with those of the authentic specimens. IIIb, IV, and V were proved to be identical with arachidic acid, valeric acid and tridecanedicarboxylic acid,[*4] respectively. These facts also coincided with the conclusion of Hiramoto.[6]

Dihydromaesaquinone (IIb) has already been synthesised by Hiramoto.[6] Since the synthetic procedure is steady but rather tedious, an improved synthesis of IIb by Fieser's method[10] was attempted. The peroxide, prepared from arachydoyl chloride, was coupled with 3,6-dihydroxytoluquinone[11] to give dihydroxymethylnonadecylbenzoquinone, m.p. 133~134°, which was proved to be identical with IIb.

All these facts clearly indicate that no ambiguity now exists on the structure (IIa) of maesaquinone, established by Hiramoto.[5,6]

Comparison of the infrared spectra of IIa and IIb, the leucoacetates (VIa and VIb) of IIa and IIb,[5] and eicosenoic acid (IIIa) and arachidic acid (IIIb), showed that they are distinguishable each other by the presence acid the absence ci the ν_{C-H} 2980~3000 cm^{-1} but they are superimposable at other region down to 680 cm^{-1}. The lack of the absorption, $\delta_{C=C-H}$ (*trans*) 960~980 cm^{-1}, indicated that the double bond might be *cis*.

In the course of the isolation of maesaquinone (IIa), we noticed that the purification procedure was simplified by the treatment of the crude extract with alkaline solution as suggested by Hiramoto.[5] Actually, thin-layer chromatography and ultraviolet spectra of the extract before further treatment, showed that the extract was chiefly composed of a proto-type compound, which easily changed to IIa by treatment with alkali. Extraction of the fruits by ethyl acetate, followed by repeated purification by thin-layer chromatography with silica-gel G treated with oxalic acid solution, afforded yellow waxy solid (VIIa), m.p. 28~40°, in a pure state. Catalytic hydrogenation of VIIa afforded the dihydro compound (VIIb), yellow needles of m.p. 74~75°. VIIa and VIIb changed into IIa and IIb, respectively, by the treatment with alkali or even by standing at several days in ethanolic solution. Acetylation of VIIa and VIIb afforded maesaquinone diacetate (VIIIa) and dihydromaesequinone diacetate (VIIIb) respectively.

Ultraviolet absorption maxima of VIIa and VIIb (λ_{max}^{EtOH} 275 mμ) appear just between those of IIa and IIb (λ_{max}^{EtOH} 294 mμ) and those of VIIIa and VIIIb (λ_{max}^{EtOH} 267 mμ) (Fig. 1). Infrared spectra of VIIa and VIIb show the presence of hydroxyl group (3350 cm^{-1}) and enolic ester group (1770, 1180 cm^{-1}); the latter is lacking in IIa and IIb and the former, in VIIIa and VIIIb. Furthermore, nuclear magnetic resonance spectra of VIIa and VIIb are superimposable with IIa and IIb, respectively, except the appearance of the signal for $COCH_3$

[*4] The identity of the dicarboxylic acid with the authentic specimen was not reported by Hiramoto.[5,6] The authors thank Dr. S.C. Bhattacharyya, National Chemical Laboratory, Poona, India, for his kind donation of the authentic specimen of tridecanedicarboxylic acid (B.B. Ghatgey, U.G. Najak. K.K. Chakravarti, S.C. Bhattacharyya : Chem. & Ind. (London), **1960**, 1334).

10) L.F. Fieser, E.M. Chamberlin : J. Am. Chem. Soc., **70**, 73 (1948).

11) T. Zincke : Ber., **16**, 1558 (1883); W.A. Anslow, J.N. Ashley, H. Raistrick : J. Chem. Soc., **1938**, 439.

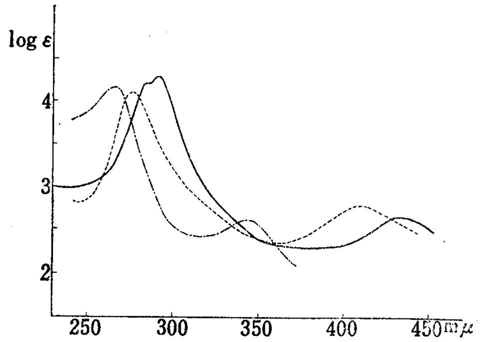
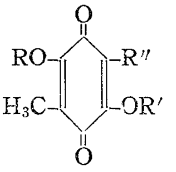


Fig. 1. Ultraviolet Spectra of IIa, IIb, VIIa, VIIb, VIIIa and VIIIb (EtOH soln.)

——— IIa, IIb
– – – – VIIa, VIIb
—·—·— VIIIa, VIIIb

(7.69 τ for VIIa and 7.67 τ for VIIb, 3H, s) and the decrease of the intensity of **OH** signal (3.01 τ for VIIIa and 3.02 τ for VIIIb, 1H, s) (Tablo II).

All these facts, along with analytical figures, clearly indicated that the proto-type compound is nothing other than monoacetate of maesaquinone. Quantitative treatment as shown in experimental part revealed that maesaquinone (IIa) is contained in the fresh fruits mainly as its acetate (VIIa). Since the content is nearly the same after the treatment of the fresh fruits with boiling water, existence of the quinone as its hydroquinone glucoside as in the case of juglone[12] and muscarfin[13] is inconceivable. Existence of hydroxyquinones as their alcoholic esters in the plants has been reported in the case of shikonin,[14] alkannin,[15] and royleanone.[16]



VIIa : R or R' = CH_3CO, R' or R = H, R'' = $C_{19}H_{37}$
VIIb : 〃 , 〃 , R'' = $C_{19}H_{39}$
VIIIa : R = R' = CH_3CO, R'' = $C_{19}H_{37}$
VIIIb : 〃 , R'' = $C_{19}H_{39}$

Experimental*5

Maesaquinone (IIa)[5]——The fruits of *Maesa japonica* were collected at Izusan, Atami-shi, Shizuoka Prefecture, in November 1962 and 1963. Ethereal extracts were treated with KOH soln. and, finally, crystallized from benzene-EtOH to afford IIa, orange plates of m.p. 123°, in 7% yield. *Anal.* Calcd. for $C_{26}H_{42}O_4$: C, 74.59; H, 10.12. Found : C, 74.82; H, 10.15.

Dihydromaesaquinone (IIb), Maesaquinone Diacetate (VIIIa), Dihydromaesaquinone Diacetate (VIIIb), Maesaquinone Leucotetraacetate (VIa), and Dihydromaesaquinone Leucotetraacetate (VIb)——They were prepared accoding to the direction of Hiramoto[5] ; IIb, m.p. 134°; VIIIa, m.p. 43°; VIIIb, m.p. 89°; VIa, m.p. 101.5°; and VIb, m.p. 121°.

Eicos-15-enoic Acid (IIIa)——Alkaline hydrogen peroxide oxidation product[5] (IIIa) of IIa was crystallized from acetone in a cold room to colorless powder of m.p. 33~35.5°. *Anal.* Calcd. for $C_{20}H_{38}O_2$: COOH, 14.52. Found : 14.75. IIIa was catalitically hydrogenated to afford arachidic acid (IIIb).[5,6] IIIa was ozonized and the products were treated with Ag_2O.[5] The acidic reaction mixture was steam-distilled to separate the volatile acid (IV) from the non-volatile acid (V). IIIb, IV and V were derived to their methyl esters and examined by gas chromatography. Results were shown in Table III.

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*5 Melting points were determined on a Yanagimonto micro-melting point apparatus. Infrared spectra were taken on a Koken Model 301 Infrared Spectrophotometer. UV spectra were measured in EtOH or hexane solution on a Cary Model 11 or a Hitachi EPS-2 Recording Spectrophotometer.

12) C. Daglish : Biochem. J., **47**, 452, 458, 462 (1950); H. W. Ruelius, A. Gauhe : Ann., **571**, 69 (1951); R.H. Thomson : J. Chem. Soc., **1950**, 1337.

13) F. Kögl, H. Erxleben : Ann., **479**, 11 (1930).

14) R. Majima, C. Kuroda : Nippon Kagaku Kaishi, **39**, 1051 (1918); Acta Phytochim.. **1**, 43 (1922); Y. Kimura, M. Takido, S. Kananishi : The paper presented at the Annual Meeting of Pharmacognostical Society of Japan, Kanazawa (September, 1964).

15) K. Brand, A. Lohmann : Ber., **68**, 1487 (1935).

16) O.E. Edwards, G. Feniak, M. Los : Canad. J. Chem., **40**, 1540 (1962).

Table III. Gas Chromatography of Fatty Acids

Column	Column temp. (C°)	Sample	Retention time (min.)
Polysuccinate (Shimadzu), 30～60 mesh	195	Methyl ester of IIIb and methyl arachidate	18.6
〃	68	Methyl ester of IV and methyl valerate	4.5
3.5% SE 30 on Chromosorb W, 60～80 mesh	183	Methyl ester of V and methyl tridecanedicarboxylate	9.85

Shimadzu G.C. IB type; carrier gas, N_2; flow rate, 76 ml./min.; column length, 1.5 m.

Synthesis of Dihydromaesaquinone (IIb)——A mixture of arachidic acid (1.4 g.) and thionyl chloride (2.0 g.) was heated on a water-bath for 1 hr. The excess reagent was removed by evaporation in vacuum with benzene and the acid chloride in petr. ether (20 ml.) was shaken vigolously with Na_2O_2 (8 g.) and cracked ice (24 g.). The mixture was extracted with (50 ml.) and the organic layer was separated, washed, and dried. The peroxide solution (2 mM by titration) was run into the solution of 3,6-dihydroxytoluquinone[11] (150 mg.) in AcOH (20 ml.), maintained at 70°, in such a rate as evaporation of the low-boiling solvents. The reaction mixture was then refluxed for 30 min. and allowed to stand overnight. The precipitate and the residue after evaporation were combined and run through a column of $CaHPO_4$ in benzene solution. Each fraction was examined by thin-layer chromatography and the fraction of the objective compounds was rechromatographed through a column of $CaHPO_4$ in hexane soln. Recrystallisation from EtOH gave orange-red leaflet of m.p. 133～134°. A mixed fusion, IR spectra, and thin-layer chromatography showed the identity with dihydromaesaquinone (IIb). *Anal.* Calcd. for $C_{26}H_{44}O_4$: C, 74.22; H, 10.55. Found : C, 74.14; H, 10.49.

Acetylmaesquinone (VIIa)——Dried fruits of *Maesa japonica* were extracted successively with hexane and AcOEt. Hexane extracts, after purification through a column of $CaHPO_4$, afforded measaquinone (IIa) in 1% yield. Thin-layer chromatography of AcOEt extract on silica-gel G treated with 3% oxalic acid solution, using benzene as the solvent, exhibited five spots, colorised with NH_3 : Rf 0.48 (maesaquinone (IIa)), 0.35 (acetylmaesaquinone (VIIa)), 0.15, 0.1, and 0.04. The second band, predominant in amounts, was collected by repetition of preparative thin-layer chromatography at the same condition as above. The band was extracted with ether, the solvent was evaporated, and the residue was extracted with hexane. The hexane extract, after concentration, was kept standing in a refrigirator (−15°) for several weeks. The yellow waxy deposit (VIIa) was collected; yield, 2.5% of the dried fruits. Since VIIa was easily soluble in almost all organic solvents and unstable to heating, attempts to purify by recrystallization or distillation were unsuccessful. Thus purification by thin-layer chromatography was repeated till it showed a completely single spot. VIIa liquifies at 28～32° and becomes a clear solution at 41°. *Anal.* Calcd. for $C_{28}H_{44}O_5$: C, 73.00; H, 9.63. Found : C, 73.41; H, 9.09.

Dihydroacetylmaesaquinone (VIIb)——VIIa was catallytically reduced in ether solution in the presence of Pt-catalyst. After the evaporation of ether, the residue was dissolved in MeOH, added with a drop of acidic $FeCl_3$ solution, and left standing at a room temperature for 5 hr. The reaction mixture was evaporated, dissolved in ether, washed with H_2O, and evaporated. Crystallization from 90% MeOH gave yellow needles of m.p. 74～75° in a theoretical yield. *Anal.* Calcd. for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found : C, 73.08; H, 10.18.

Formation of IIa and IIb from VIIa and VIIb——i) VIIa or VIIb was warmed in 5% NaOH soln. at 80° for 10 min. After acidification, the mixture was extracted with ether, evaporated, and the residue was crystallized from EtOH to give IIa, m.p. 122°, from VIIa and IIb, m.p. 134°, from VIIb. The identify was confirmed by IR spectra, thin-layer chromatography, and mixed fusions.

ii) 98% EtOH solution of VIIa or VIIb was kept standing at room temperature for 2 weeks. The deposit was collected and recrystallized from EtOH to afford IIa from VIIa and IIb from VIIb.

Acetylation of VIIa and VIIb——VIIa and VIIb were acetylated with Ac_2O and a drop of H_2SO_4 at a room temperature. Recrystallization from MeOH afforded the acetates, m.p. 43° and 89°, respectively, which were identified with diacetates (VIIIa and VIIIb) of maesaquinone (IIa) and dihydromaesaquinone (IIb) by IR spectra, thin-layer chromatography, and mixed fusions.

Determination of IIa and VIIa in Fresh Fruits——Fresh fruits of *Maesa japonica,* directly or after the treatment with boiling H_2O, were extracted with EtOH, AcOEt, benzene, or ether, the extracts were evaporated, and applied to thin-layer chromatography. The bands, corresponding to IIa and VIIa, were collected, eluted, and determined by absorbancies at 294 and 275 mμ, respectively. The contents were same with or without the treatment of boiling H_2O. The results were shown in Table IV.

Table IV. Contents of IIa and VIIa in the Fruits (calculated as % of the dried weight of fruits)

	VIIa	IIa
Ethanol extract	9.5	0.1
Ethyl acetate extract	5.1	0.4
Benzene extract	2.5	0.3
Ether extract	2.5	0.5

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Summary

The structure (IIa) of maesaquinone, a benzoquinone from *Maesa japonica* Moritzi, proposed by Hiramoto,[5,6] was further confirmed by spectral evidences, gas chromatography of the oxidation products, and an improved synthesis of the dihydro derivative (IIb). Isolation of monoacetate (VIIa) of IIa from the fruits suggested that VIIa is the chief component of the fruits.

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