Regular Article

Naphthoquinone Derivatives from *Diospyros maritima*

Matsutake Higa, Yuji Takashima, Hiroto Yokaryo, Yuta Harie, Toshimasa Suzuka, and Kazuhito Ogiwara*

Department of Chemistry, Biology, and Marine Science, Faculty of Science, University of the Ryukyus; 1 Senbaru, Nishihara-cho, Okinawa 903–0213, Japan.

Received February 28, 2017; accepted May 24, 2017

From the chloroform extract of the fresh fruits of *Diospyros maritima* BLUME (Ebenaceae), five new naphthoquinone derivatives, 2,7'-dimethyl-2',3-bijuglone (27), 2,7'-dimethyl-3,3'-bijuglone (28), 2,7'-dimethyl-6,8'-bijuglone (29), 7,7'-dimethyl-3,3'-ethylidenebijuglone (30), and 2',7-dimethyl-3,6'-ethylidenebijuglone (31), were isolated, in addition to twenty-one known naphthoquinone derivatives: plumbagin (4), droserone (5), 2,3-epoxyplumbagin (8), 3,3'-biplumbagin (9), chitranone (10), 3,8'-biplumbagin (11), elliptinone (12), martinone (13), isozeylanone (14), methylene-3,3'-biplumbagin (15), ethylidene-3,3'-biplumbagin (16), ethylidene-3,6'-biplumbagin (17), ethylidene-6,6'-biplumbagin (18), 7-methyl- β -dihydrojuglone (19), 7-methyljuglone (20), 2,3-epoxy-7-methyljuglone (21), neodiospyrin (22), mamegakinone (23), ehretione (24), isoxylopyrin (25) and β -dihydroplumbagin (26). The structures of the new compounds were established by spectral analysis. The quinones obtained from the chloroform extract of the fruits were compared with previously reported quinones obtained from ethanol extracts. The quinones in the fruits were categorized in three groups: quinones from ethanol extract only, quinones from chloroform extract only, and quinones from both extracts. The six naphthoquinones, 19–21, 25, 26, and 29, were examined for their ichthyotoxic activity and germination inhibitory activity. Quinones 19–21, 26, and 29 showed ichthyotoxic activity against Japanese killifish (*Oryzias latipes* var.) at 10 ppm; quinones 19 to 21 and 26 showed germination inhibitory activity toward lettuce seeds (*Lactuca sativa* L. var. Great Lakes) at 100 ppm.

Key words *Diospyros maritima*; Ebenaceae; naphthoquinone; bijuglone; ethylidenebijuglone

Diospyros maritima BLUME (Ebenaceae) is a shrub that grows in Southeast Asia. We previously reported the isolation of eighteen naphthoquinone derivatives, 1–18, from the ethanol extract of the fruits of this plant^{1,2)} (Chart 1). The naphthoquinone derivatives isolated to date from the genus *Diospyros* are commonly assumed to be derived from a precursor equivalent to 4,5-dihydroxy-2-methylnaphthalene or a biogenetic analog.³⁾ 3-Methylplumbagin (3), 3-(2-hydroxyethyl)plumbagin (6), 6-(1-ethoxyethyl)plumbagin (7), methylene-3,3'-biplumbagin (15), ethylidene-3,3'-biplumbagin (16), ethylidene-3,6'-biplumbagin (17), and ethylidene-6,6'-biplumbagin (18) isolated from this plant contain an alkyl group that is not found in the naphthoquinone derivatives isolated from other *Diospyros* species. Because it is possible to isolate rare and novel quinones using different extraction solvents, we employed chloroform as the extraction solvent instead of ethanol to examine the naphthoquinone derivatives in the chloroform extract of the fresh fruits. Here, we describe the isolation and structure elucidation of five new naphthoquinone derivatives, 27–31, from the chloroform extract; we also discuss the classification of naphthoquinone derivatives obtained only from the ethanol extract of the fresh fruits of this plant, only from the chloroform extract, and from both extracts.

Results and Discussion

Structure Elucidation A chloroform extract of the fresh fruits of *D. maritima* afforded, after chromatographic separation, eight known naphthoquinone derivatives: 7-methyl- β -dihydrojuglone (19), 7-methyljuglone (20), 2,3-epoxy-7-methyljuglone (21), neodiospyrin (22), mamegakinone (23), ehretione (24), isoxylopyrin (25), β -dihydroplumbagin (26),

and five new quinones (27 to 31) (Chart 2), in addition to quinones 4, 5, and 8–18, which were previously isolated from the ethanol extract.

The new quinone 27 was obtained as red prisms with a melting point (mp) of 184–185°C. Its molecular formula was determined to be C₂₂H₁₄O₆ by high-resolution electrospray ionization (HR-ESI)-MS, showing the pseudomolecular ion [M–H][–] at *m/z* 373.0711 (calcd for C₂₂H₁₃O₆: 373.0712). The IR ($\nu_{\max}$ (KBr) cm^{–1}: 1667, 1643, 1610 (sh) and UV ($\lambda_{\max}$ (CHCl₃) nm (log ϵ): 255 (4.29), 434 (3.90)) spectra showed the characteristic bands of juglone (5-hydroxy-1,4-naphthoquinone) derivatives. The MS spectrum showed fragment ion peaks at *m/z* 134 and 106, suggesting the presence of the 7-methyljuglone (20) moiety; the fragment ion peaks at *m/z* 120 and 92 exhibited the presence of the plumbagin (4) moiety.^{4,5)} The ¹H-NMR spectrum showed the presence of one aromatic methyl [δ 2.47 (3H, s)], one quinonoid proton [δ 6.86 (1H, s)], two aromatic protons [δ 7.14 (1H, m), 7.51 (1H, d, *J*=1.5 Hz)], and one hydrogen-bonded hydroxyl [δ 11.84 (1H, s)] attributed to the 7-methyljuglone moiety, one quinonoid methyl [δ 2.13 (3H, s)], three aromatic protons [δ 7.28 (1H, dd, *J*=1.0, 8.0 Hz), 7.65 (1H, t, *J*=8.0 Hz), 7.71 (1H, dd, *J*=1.0, 7.5 Hz)], and another hydrogen-bonded hydroxyl [δ 11.75, (1H, s)] were attributed to the plumbagin moiety. From these results, the quinone 27 is a dimer that consists of plumbagin (4) and 7-methyljuglone (20). The position of the dimeric linkage was established by ¹H-NMR spectrometry as follows. The absence of allylic coupling of the quinonoid methyl with the quinonoid proton in 27 indicates that the 3-position of the plumbagin moiety is occupied by a substituent. Because only one quinonoid proton is observed, the 2- or 3-position of the

* To whom correspondence should be addressed. e-mail: kogihara@sci.u-ryukyu.ac.jp

Chart 1. Structures of Naphthoquinones Isolated from the EtOH Extracts of *Diospyros maritima* Chart 2. Structures and Some Derivatives of Naphthoquinones Isolated from the CHCl₃ Extracts of *Diospyros maritima*

7-methyljuglone moiety is occupied by a substituent. From the above results, the dimeric linkages in **27** must be located at the 3-position of the plumbagin moiety and the 2- or 3-position of the 7-methyljuglone moiety. Complete assignments of the signals in the ¹H- and ¹³C-NMR spectra were established by one-dimensional (1D) and two-dimensional (2D)-NMR

spectral analyses. The heteronuclear multiple bond correlation heteronuclear multiple bond connectivity (HMBC) spectrum of **27** (Fig. 1) showed long-range correlations between the quinonoid proton signal due to the 7-methyljuglone moiety and the carbon signals at C-3 and C-10', which reveals that the 2-position of the 7-methyljuglone moiety is occupied by

Fig. 1. HMBC Correlations for Compounds 27–31

a substituent. These HMBC data indicate that the location of the dimeric linkage of **27** is C-3 and C-2'. Based on the above evidence, quinone **27** was characterized as 2,7'-dimethyl-2',3-bijuglone.

The new quinone **28** was obtained as red-brown prisms with mp 170°C (dec.). Its molecular formula was determined to be $C_{22}H_{14}O_6$ (m/z 373.0695 $[M-H]^-$, calcd for $C_{22}H_{13}O_6$: 373.0712), which is the same as quinone **27**, by HR-ESI-MS. The IR and UV spectra of **28** were almost superimposable with those of **27**. The MS spectrum was similar to that of **27**. The 1H - and ^{13}C -NMR spectra closely coincided with those of **27**, except for signals due to a quinonoid proton and a carbon atom. These results suggested that **28** is a dimer consisting of compounds **4** and **20**, similar to **27**, and that the dimeric linkages in **28** must be located at the 3-position of the plumbagin moiety and the 2- or 3-position of the 7-methyljuglone moiety. The HMBC spectrum of **28** (Fig. 1) showed long-range correlations between the quinonoid proton signal due to the 7-methyljuglone moiety and carbon signals at C-3 and 9', which reveals that the 3-position of the 7-methyljuglone moiety is occupied by a substituent. These HMBC data indicate that the location of the dimeric linkage of **28** is C-3 and C-3'. Thus, quinone **28** was characterized as 2,7'-dimethyl-3,3'-bijuglone.

Quinones **27** and **28** are mixed dimers of **4** and **20**. Although many symmetric dimers of quinones **4** and **20** have been reported from *Diospyros* species to date, ehretione (**24**) isolated from *Diospyros ehretioides* WALL.⁶⁾ is only one example of a mixed dimer of quinones **4** and **20**.

Compound **29** was obtained as orange leaflets with mp 176–178°C. Its molecular formula was determined to be $C_{22}H_{14}O_6$ (m/z 373.0705 $[M-H]^-$, calcd for $C_{22}H_{13}O_6$: 373.0712) by HR-ESI-MS. The IR ($\nu_{\max}$ (KBr) cm^{-1} : 1665, 1636, 1614) and UV ($\lambda_{\max}$ (CHCl₃) nm (log ϵ): 256 (4.31), 435 (3.83)) spectra showed the characteristics of juglone derivatives. The 1H -NMR spectrum showed the presence of one quinonoid methyl (δ 2.23 (3H, d, $J=1.5$ Hz)), one aromatic methyl (δ 2.10 (3H, s)), one quinonoid proton (δ 6.84 (1H, q, $J=1.5$ Hz)), two coupled quinonoid protons (δ 6.72 (1H, d, $J=10.0$ Hz), 6.91 (1H, d, $J=10.0$ Hz)), one pair of *ortho*-coupled aromatic protons (δ 7.29 (1H, d, $J=8.0$ Hz), 7.74 (1H,

d, $J=8.0$ Hz)), one aromatic proton (δ 7.26, (1H, s)), and two hydrogen-bonded hydroxyls (δ 12.21, 12.43 (each 1H, s)). The presence of the three quinonoid protons and two aromatic methyl groups indicates that compound **29** is a dimer composed of **4** and **20**; it also suggests that linkages exist between the aromatic rings, in contrast to **27** and **28**. The position of the dimeric linkage was established by 1H -NMR spectrometry as follows. The presence of allylic coupling of the quinonoid methyl group with the quinonoid proton and the presence of *ortho*-coupled aromatic protons show that the position of the dimeric linkage in the plumbagin moiety must be C-6 or C-8. The presence of two coupled quinonoid protons and the presence of only one aromatic proton reveal that the position of the dimeric linkage in the 7-methyljuglone moiety must be C-6' or C-8'. The HMBC spectrum of **29** (Fig. 1) showed long-range correlations between the aromatic proton H-8 and C-1 and between H-7 and C-8', which indicates that the location of the dimeric linkage of the plumbagin moiety is C-6. The HMBC spectrum of **29** also showed long-range correlations between the aromatic proton H-6' and C-8' and C-10'. No correlation between the aromatic proton H-6' and C-1' was observed. These HMBC data demonstrate that the location of the dimeric linkage of the 7-methyljuglone moiety is C-8'. Thus, the location of the dimeric linkage of **29** was determined to be C-6 and C-8'; therefore, compound **29** was characterized as 2,7'-dimethyl-6,8'-bijuglone.

Compound **30** was obtained as an orange powder with mp 223–225°C. Its molecular formula was determined to be $C_{24}H_{18}O_6$ (m/z 401.1043 $[M-H]^-$, calcd. for $C_{24}H_{17}O_6$: 401.1025) by HR-ESI-MS. The IR ($\nu_{\max}$ (KBr) cm^{-1} : 1668, 1636, 1614) and UV ($\lambda_{\max}$ (CHCl₃) nm (log ϵ): 256 (4.21), 432 (3.75)) spectra showed the characteristic peaks of juglone derivatives. The MS spectrum showed the molecular ion at m/z 402; fragment ions at m/z 134 and 106 demonstrated the presence of the 7-methyljuglone moiety.^{4,5)} The 1H -NMR spectrum showed the presence of two aromatic methyls (δ 2.43 (6H, s)), two quinonoid protons (δ 6.72 (2H, d, $J=1.5$ Hz)), four aromatic protons (δ 7.07, 7.43 (2H, d, $J=1.5$ Hz)), two hydrogen-bonded hydroxyls (δ 11.89 (2H, s)), and one ethylidene group (δ 4.68 (1H, brq, $J=7.0$ Hz, $>CHCH_3$), 1.45 (3H, d, $J=7.0$ Hz,

$>\text{CHCH}_3$) in **30**. From the above results, compound **30** is a symmetric dimer of **20** linked by an ethylidene bridge. Because only two quinonoid protons were observed in addition to all four aromatic protons, the position of the linkage must be C-2,2' or C-3,3'. Thus, compound **30** is ethylidene-2,2'-di-7-methyljuglone or ethylidene-3,3'-di-7-methyljuglone. The HMBC spectrum (Fig. 1) showed long-range correlations between the methyl protons of the ethylidene group and C-3,3'. These HMBC correlations indicate that the ethylidene group is located at C-3 and C-3'. Based on the above evidence, the location of the dimeric linkage of **30** was determined to be C-3 and C-3'; thus, **30** was characterized as 7,7'-dimethyl-3,3'-ethylidenebijiuglone.

Compound **31** was obtained as orange needles with mp 215–216°C (dec.) and $[\alpha]_D^{26} +0.42^\circ$ ($c=4.0$, CHCl_3). Its molecular formula was determined to be $\text{C}_{24}\text{H}_{18}\text{O}_6$ (m/z 401.1030 $[\text{M}-\text{H}]^-$, calcd for $\text{C}_{24}\text{H}_{17}\text{O}_6$: 401.1025) by HR-ESI-MS. The IR (ν_{max} (KBr) cm^{-1} : 1667, 1639, 1614) and UV (λ_{max} (CHCl_3) nm (log ϵ): 260 (4.33), 430 (3.88)) spectra showed the characteristic peaks of juglone derivatives. The ^1H -NMR spectrum showed the presence of the plumbagin moiety (one quinonoid methyl [δ 2.19 (3H, d, $J=1.5\text{ Hz}$)], a quinonoid proton [δ 6.80 (1H, q, $J=1.5\text{ Hz}$)], two *ortho*-coupled aromatic protons [δ 7.54, δ 7.62 (each 1H, d, $J=7.5\text{ Hz}$)], one hydrogen-bonded hydroxyl [δ 12.49 (1H, s)], one 7-methyljuglone moiety (one aromatic methyl [δ 2.42 (3H, s)], another quinonoid protons [δ 6.66 (1H, d, $J=1.5\text{ Hz}$)], two *meta*-coupled aromatic protons [δ 7.04 (1H, m), 7.41 (1H, d, $J=1.5\text{ Hz}$)], another hydrogen-bonded hydroxyl [δ 11.93 (1H, s)], and one ethylidene group (δ 4.83 (1H, br q, $J=7.0\text{ Hz}$, $>\text{CHCH}_3$), 1.55 (3H, d, $J=7.0\text{ Hz}$, $>\text{CHCH}_3$)) in **31**. These results indicate that **31** is a mixed dimer of **4** and **20** linked by an ethylidene bridge. Because one quinonoid proton coupled with a quinonoid methyl and two *ortho*-coupled aromatic protons were observed, the position of the dimeric linkage in the plumbagin moiety must be C-6' or C-8'. Because only one quinonoid proton and two *meta*-coupled aromatic protons were observed, the position of the dimeric linkage in the 7-methyljuglone moiety must be C-2 or C-3. The HMBC spectrum (Fig. 1) showed long-range correlations between the methyl protons of the ethylidene group and C-3 and C-6'. These HMBC correlations indicate that the ethylidene group is located at C-3 and C-6'. Based on the above evidence, the location of the dimeric linkage of **31** was determined to be C-3 and C-6'; thus, **31** was characterized as 2,7'-dimethyl-3,6'-ethylidenebijiuglone.

β -Dihydroplumbagin (**26**) was obtained as pale yellow leaflets (hexane), mp 80–82°C, $[\alpha]_D^{23} -88.1^\circ$ ($c=0.78$, CHCl_3). Compound **26** was previously isolated from *Juglans nigra* and *J. regia* by Binder *et al.*⁷; however, they did not describe its optical properties. Therefore, the absolute configuration at C-2 of **26** was determined as follows. Quinone **26** was reduced with zinc in 80% acetic acid to afford the *cis*-tetralone (**32**) as a pale yellow oil, $[\alpha]_D^{25} +14.3^\circ$ (CHCl_3), and the *trans*-tetralone (**33**) as colorless needles with mp 102°C, $[\alpha]_D^{25} 0^\circ$ (CHCl_3). The ^1H -NMR spectrum of the *cis*-tetralone (**32**) was almost superimposable on that of isoshinanolone (4*R*,8-dihydroxy-3*R*-methyl-3,4-dihydro-1(2*H*)-naphthalenone).^{3,8} The *cis*- and *trans*-isomers can be distinguished by comparing the coupling constants of the carbonyl protons of the two isomers (*cis*: $J=2.5\text{ Hz}$, *trans*: $J=7.5\text{ Hz}$).³ The optical rotations for *cis*-isomer (**32**) were reported by two research groups, but

the rotation values were significantly different between both reports (-7° and $+24^\circ$).^{3,8} Therefore, the *cis*-isomer (**32**) was treated with benzoyl chloride in pyridine to afford its dibenzoate (**34**) as a pale yellow semi-solid, $[\alpha]_D^{25} +71.5^\circ$ (CHCl_3). The signals in the ^1H -NMR spectrum and the circular dichroism (CD) curve of the dibenzoate (**34**) were almost superimposable with those in previous reports.^{3,8} Thus, *cis*-tetralone (**32**) must be isoshinanolone, and the absolute configuration at C-2 of **26** was established to be *R*.

Categorization of Isolates by Extraction Solvent By changing the extraction solvent, the quinones in the fruit of *D. maritima* were categorized in three groups: quinones isolated only from the ethanol extract, quinones isolated only from the chloroform extract, and quinones isolated from both extracts.

The naphthoquinone derivatives isolated only from the chloroform extract of the fresh fruits are listed as **19–26**, in addition to five new quinones, **27–31**. The chloroform extract newly afforded seven naphthoquinone derivatives, 7-methyl- β -dihydrojuglone (**19**), 7-methyljuglone (**20**), 7-methyl-2,3-epoxyjuglone (**21**), neodiospyrin (**22**), mamegakinone (**23**), ehretione (**24**), isoxylopyrin (**25**), and β -dihydroplumbagin (**26**), in comparison with naphthoquinones isolated from the ethanol extract. 7-Methyljuglone (**20**) has been shown to be readily converted into 2- and 3-methoxy-7-methyljuglone at room temperature in methanol.⁹ Therefore, quinones **20–25**, which contain 7-methyljuglone moieties, are expected to be unstable or labile in ethanol. 7-methyl- β -dihydrojuglone (**19**) is quite rare; its isolation has been reported only from *Diospyros hebecarpa* by Cooke and Dowd in 1952.¹⁰ When quinones **19** and **26** were refluxed in chloroform, no new products were obtained. However, these quinones were converted completely into the corresponding naphthoquinones, 7-methyljuglone (**20**) and plumbagin (**4**), respectively, by refluxing in 50% aqueous ethanol for 12 h. Quinones **19** and **26** may be isolated from other *Diospyros* species by extraction of the fresh plant with chloroform. Quinone **26** is believed to be the biogenetic precursor of zeylanone and isozeylanone (**14**), which have been isolated from *Plumbago zeylanica*.¹¹ However, quinone **26** has not been isolated from any species belonging to the genus *Plumbago*. β -Dihydrojuglone (**35**) has been shown to be involved in the biosynthesis of juglone (**36**) in the genus *Juglans* (Juglandaceae).¹² Therefore, quinones **19** and **26** may be intermediates in the biosynthesis of 7-methyljuglone (**20**) and plumbagin (**4**), respectively, in the genus *Diospyros*.

The naphthoquinone derivatives isolated only from the ethanol extract of the fresh fruits were listed as **1, 2, 3, 6**, and **7**, and those from both extracts were as **15–18**, in addition to quinones **4, 5**, and **8–14**. Meanwhile, quinones **1, 2, 3, 6**, and **7** were isolated only from the ethanol extract. 3-Chloroplumbagin (**2**) has been also isolated from *Plumbago zeylanica*¹³ and *Drosera intermedia*.¹⁴ Lillie *et al.* suggested that **2** is an artifact formed by the reaction of plumbagin-2,3-epoxide (**8**) with chloroform.⁴ Quinone **2** was obtained as a byproduct of the conversion of **4** with NaBO_3 and HCl into **8**.¹⁵ The reaction mechanism can be described as follows: compound **8** underwent $\text{S}_{\text{N}}2$ substitution with HCl followed by dehydration to afford **2**. It is known that chloroform is decomposed into COCl_2 and HCl by daylight. Therefore, **8** was refluxed in chloroform containing a small amount of ethanol¹⁶ under daylight for 48 h; however, no quinone **2** was obtained initially. If this treatment is continued for a long time (more

than 48 h), **2** may be detected. However, this is longer than the time required for chromatographic separation. Compound **2** would first be isolated from the chloroform extract if it was formed by this reaction. Thus, it is shown that the chlorine atom of **2** did not originate from the chloroform used for partition between solvents during the isolation procedure. 3-Bromoplumbagin (**1**) is easily obtained by treatment of **4** with bromine.^{1,15} Therefore, it is possible that **1** is an artifact formed during ethanolic extraction, although the origins of the bromine are not clear. If quinones **1** and **2** are artifacts, it is suggested that the quinonoid moiety of **4** can be readily substituted with chlorine and bromine and converted into quinones **1** and **2** by reaction in ethanol. However, quinones **1** and **2** may not be extracted from the fruits using chloroform, as the solubilities of the precursors, which are expected to be equivalent to 4,5-dihydroxy-2-methylnaphthalene or the biogenetic analogs of **1** and **2** in chloroform, are lower than their solubilities in ethanol. 3-Methylplumbagin (**3**) has also been isolated from the acetone extracts of *Juglans nigra* and *J. regia* (*Juglandaceae*)⁷; however, the possibility that **3** is an artifact has not been suggested. 3-(2-Hydroxyethyl)plumbagin (**6**) and 6-(1-ethoxyethyl)plumbagin (**7**) contain ethanolic moieties, as does the extraction solvent. However, it is believed to be unlikely that **6** and **7** are artifacts formed during ethanolic extraction, as experimental evidence for this has not been demonstrated.

The naphthoquinone derivatives isolated from both the chloroform and ethanol extracts of the fresh fruits were listed as **15–18**, in addition to **4**, **5**, and **8–14**. Methylene-3,3'-biplumbagin (**15**), ethylidene-3,3'-biplumbagin (**16**), ethylidene-3,6'-biplumbagin (**17**), and ethylidene-6,6'-biplumbagin (**18**) were isolated from both extracts. This fact indicates that quinones **15–18** are not artifacts formed during ethanol extraction. In other words, the results clearly excluded the possibility that quinones **15–18** were formed during the isolation procedure. Gunaherath *et al.* suggested that the biosynthesis of quinone **15** in the plant may involve the participation of formaldehyde.¹⁷ The origin of the ethylidene group of quinones **16–18** is not yet clear.

Bioassay of Quinones Two bioassays, ichthyotoxicity and seed germination tests, were performed with six compounds (**19–21**, **25**, **26**, and **29**). Compounds **19–21**, **26**, and **29** displayed ichthyotoxic activity against Japanese killifish (*Oryzias latipes* var.) when tested at 10 ppm. Compound **25** did not display any ichthyotoxic activity. Compounds **19–21** and **26** showed germination inhibitory activity against lettuce seeds (*Lactuca sativa* L. var. Great Lakes) when tested at 100 ppm. Compounds **25** and **29** did not show any germination inhibitory activity. The ichthyotoxic and germination inhibitory activities of **22–24**, **27**, **28**, **30**, and **31** could not be determined due to the low quantities of the samples or low solubility in the solvent.

Experimental

General Procedures Melting points were measured on a Yanagimoto MP-S3 micro melting point apparatus and are uncorrected. Specific rotations were recorded using a JASCO DIP-1000 digital polarimeter. Spectral data were obtained using the following instruments: CD on a JASCO MOE-1 spectropolarimeter; IR on JASCO A-302, Shimadzu FTIR-8200A, and JASCO FT/IR-4100 spectrophotometers;

UV on a Hitachi 100–50 spectrophotometer and a JASCO V-630 spectrophotometer; EI-MS on a Hitachi RMU-6L mass spectrometer and a Hitachi M-2500 apparatus (70 eV, direct inlet system); HR-ESI-MS on a JEOL JMS-T100LP mass spectrometer; ¹H, ¹³C, and 2D-NMR on a Hitachi R-24 NMR spectrometer (¹H: 60 MHz), a JEOL α500 NMR spectrometer (¹H: 500 MHz, ¹³C: 125 MHz), and a Bruker Avance III 500 NMR spectrometer (¹H: 500 MHz, ¹³C: 125 MHz). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. The symbols s, d, t, q, dd, dq, m, and br denote singlet, doublet, triplet, quartet, doublet of doublets, double quartet, multiplet, and broad, respectively. Column chromatography (CC) and flash-column chromatography (FC) were carried out with Wakogel C-300 (Wako Pure Chemical Industries, Ltd.) and Kieselgel 60N 40–50 μm (Merck), respectively. Preparative TLC (PTLC) was performed on a pre-coated Kieselgel 60F₂₅₄ plate (Merck). HPLC was performed with a Shimadzu LC-8A.

Extraction and Isolation The fresh fruits (82.0 kg) of *D. maritima*, collected at Chinen, Okinawa in April 1988, were extracted with CHCl₃ (25 L), after crushed by using a Waring blender in the same solvent, in the day. The extract was evaporated to dryness, and the residue was partitioned between CHCl₃ and H₂O. The CHCl₃ layer was evaporated, and the residue (963.0 g) was divided into C₆H₆-soluble and C₆H₆-insoluble portions (247 g). The C₆H₆-soluble portion was subjected to CC on silica gel with a gradient of C₆H₆–EtOAc to afford nine fractions (I to IX). The procedure was finished in a day and the fractions I to IX were stored at –5 or –20 °C until chromatographic separations and purifications.

Fraction I (30.11 g) contained only non-quinonoid material. Fraction II (408.26 g) was purified by FC on silica gel (hexane–C₆H₆ gradient) to yield **4** (321.1 g) and **8** (726 mg). Fraction III (76.35 g) was purified by FC on silica gel (hexane–C₆H₆ gradient) and PTLC on silica gel (5:5 hexane–C₆H₆) to yield **26** (9.78 g), **19** (26.50 g), **15** (45 mg), **20** (5.27 g), **21** (87 mg), **22** (143 mg), **23** (780 mg), **9** (105 mg), and **24** (378 mg). Fraction IV (22.08 g) was purified by FC on silica gel (hexane–C₆H₆ gradient) to yield **17** (150 mg). Fraction V (24.43 g) was purified by FC on silica gel (hexane–C₆H₆ gradient) to yield **27** (322 mg), **16** (99 mg), **10** (399 mg), **14** (210 mg), **28** (482 mg), **11** (99 mg), and **18** (161 mg). Fraction VI (38.65 g) was purified by FC on silica gel (hexane–C₆H₆ gradient) to yield **30** (58 mg), **31** (294 mg), and **12** (1.43 g). Fraction VII (10.20 g) was purified by FC on silica gel (hexane–C₆H₆ gradient) and PTLC (C₆H₆) to yield **13** (268 mg), **29** (420 mg), and **25** (484 mg). Fraction VIII (1.59 g) was purified by FC on silica gel (hexane–C₆H₆ gradient) to yield **5** (531 mg). Fraction IX (18.33 g) contained only non-quinonoid material.

Compounds **4**, **5**, and **8–18** were identified by direct comparison with authentic samples.

7-Methyl-β-dihydrojuglone (**19**)

Yellow-green leaflets (C₆H₆), mp 113–114 °C (lit.⁹) 112–113 °C). IR ν_{max} (KBr) cm^{–1}: 1680, 1635, 1605. UV λ_{max} (CHCl₃) nm (log ε): 241 (4.19), 277 (3.84), 350 (3.77). ¹H-NMR (CDCl₃) δ: 2.41 (3H, s, CH₃-7), 3.01 (4H, s, –CH₂CH₂–), 7.02 (1H, m, H-6), 7.31 (1H, m, H-8), 12.09 (1H, s, OH-5). MS *m/z* (rel. int. %): 190 [M]⁺ (100), 175 (9), 162 (13), 147 (7), 134 (54), 106 (13). Compound **19** was identified by direct comparison with synthetic sample.¹¹

Table 1. ^1H -NMR Spectral Data for Compounds **27–31** (500 MHz, CDCl_3 , δ)

	27	28	29	30	31
H-2				6.72 (d, 1.5)	6.66 (d, 1.5)
H-3			6.84 (q, 1.5)		
H-6	7.28 (dd, 1.0, 8.0)	7.29 (dd, 1.0, 8.5)		7.07 (m)	7.04 (m)
H-7	7.65 (t, 8.0)	7.66 (t, 8.0)	7.29 (d, 8.0)		
H-8	7.71 (dd, 1.0, 7.5)	7.71 (dd, 1.0, 7.5)	7.74 (d, 8.0)	7.43 (d, 1.5)	7.41 (d, 1.5)
H-2'		6.84 (s)	6.72 (d, 10.0)	6.72 (d, 1.5)	
H-3'	6.86 (s)		6.91 (d, 10.0)		6.80 (q, 1.5)
H-6'	7.14 (m)	7.12 (m)	7.26 (s)	7.07 (m)	
H-7'					7.54 (d, 7.5)
H-8'	7.51 (d, 1.5)	7.51 (d, 1.5)		7.43 (d, 1.5)	7.62 (d, 7.5)
CH_3 -2	2.13 (s)	2.15 (s)	2.23 (d, 1.5)		
CH_3 -2'					2.19 (d, 1.5)
CH_3 -7				2.43 (s)	2.42 (s)
CH_3 -7'	2.47 (s)	2.47 (s)	2.10 (s)	2.43 (s)	
OH-5	11.75 (s)	11.75 (s)	12.21 (s)	11.89 (s)	11.93 (s)
OH-5'	11.84 (s)	11.72 (s)	12.43 (s)	11.89 (s)	12.49 (s)
$>\text{CHCH}_3$				4.68 (brq, 7.0)	4.83 (brq, 7.0)
$>\text{CHCH}_3$				1.45 (d, 7.0)	1.55 (d, 7.0)

7-Methyljuglone (20)

Orange needles (C_6H_6), mp 108°C (lit.³) 120–121°C). The ^1H -NMR spectral data were identical to those in ref. 3.

2,3-Epoxy-7-methyljuglone (21)

Orange-red plates (C_6H_6), mp 95–96°C (lit.¹⁶) 94–95°C). The ^1H -NMR spectral data were identical to those in ref. 16.

Neodiospyrin (22)

Orange-red needles (C_6H_6), mp 221–223°C (dec.) (lit.¹⁸) 205–210°C (dec.)). The ^1H -NMR spectral data were identical to those in ref. 18.

Mamegakinone (23)

Orange-red needles (C_6H_6), mp 250–255°C (dec.) (lit.¹⁹) 256°C (dec.)). The ^1H -NMR spectral data were identical to those in ref. 19.

Ehretione (24)

Orange needles (CHCl_3), mp 242–243°C (lit.⁶) 232°C (dec.)). The ^1H -NMR spectral data were identical to those in ref. 6.

Isoxylopyrin (25)

A yellow powder (C_6H_6), mp >300°C (lit.²⁰) >350°C). The ^1H -NMR spectral data were identical to those in ref. 21.

(–)-2*R*- β -Dihydroplumbagin (26)

Pale yellow leaflets (hexane), mp 80–82°C. $[\alpha]_{\text{D}}^{23}$ –88.1° ($c=0.78$, CHCl_3). IR ν_{max} (KBr) cm^{-1} : 1685, 1640, 1605. UV λ_{max} (CHCl_3) nm (log ϵ): 242 (4.08), 266 (3.74), 349 (3.74). MS m/z (rel. int. %): 190 $[\text{M}]^+$ (76), 175 (100), 162 (17), 147 (11), 120 (37), 92 (22). ^1H -NMR (CDCl_3) δ : 1.31 (3H, d, $J=6.0$ Hz, Me-2), 2.1–3.5 (3H, m, H-2,3,3), 7.0–7.8 (3H, m, H-6,7,8), 12.01 (1H, s, OH-5). Compound **26** was identified by direct comparison with synthetic sample.¹¹⁾

2,7'-Dimethyl-2',3-bijuglone (27)

Red plates (C_6H_6), mp 184–185°C. HR-ESI-MS m/z : 373.0711 ($[\text{M}-\text{H}]^-$, calcd for $\text{C}_{22}\text{H}_{13}\text{O}_6$: 373.0712). IR ν_{max} (KBr) cm^{-1} : 1667, 1643, 1610 sh. UV λ_{max} (CHCl_3) nm (log ϵ): 255 (4.29), 434 (3.90). MS m/z (rel. int. %): 374 $[\text{M}]^+$ (100), 359 (27), 357 (24), 346 (7), 187 (6), 134 (3), 120 (8), 106 (5), 92 (14). ^1H -NMR: Table 1. ^{13}C -NMR: Table 2.

2,7'-Dimethyl-3,3'-bijuglone (28)

Orange-red plates (C_6H_6), mp 170°C (dec.). HR-ESI-MS m/z : 373.0695 ($[\text{M}-\text{H}]^-$, calcd for $\text{C}_{22}\text{H}_{13}\text{O}_6$: 373.0712). IR ν_{max} (KBr) cm^{-1} : 1667, 1633, 1610 sh. UV λ_{max} (CHCl_3) nm (log ϵ):

Table 2. ^{13}C -NMR Spectral Data for Compounds **27–31** (125 MHz, CDCl_3 , δ)

	27	28	29	30	31
C-1	183.51	183.45	184.65	184.31	184.76
C-2	147.36	147.55	149.92	134.96	135.06
C-3	139.92	139.55	135.35 ^{a)}	152.96	153.82
C-4	187.73 ^{a)}	187.72	190.60	188.16	188.79
C-5	161.51	161.53	158.61	162.09	161.98
C-6	124.51	124.52 ^{a)}	136.00	124.23	124.10
C-7	136.58	136.60	135.42 ^{a)}	148.78	148.43
C-8	119.59	119.62	119.67	120.42	120.14
C-9	131.84	131.60	131.22	131.63	131.72
C-10	114.66	114.61	115.03	113.05	113.14
C-1'	182.30	183.51	184.86	184.31	184.53
C-2'	144.47	138.80	140.23	134.96	149.81
C-3'	138.34	143.77	137.98	152.96	135.40
C-4'	188.38 ^{a)}	187.16	190.37	188.16	190.50
C-5'	161.83	162.17	161.89	162.09	158.67
C-6'	124.51	124.54 ^{a)}	125.32	124.23	138.70
C-7'	148.89	149.11	148.38	148.78	133.62
C-8'	121.40	120.77	129.48	120.42	119.12
C-9'	131.30	131.81	129.07	131.63	130.72
C-10'	113.07	112.75	113.93	113.05	114.92
CH_3 -2	14.78	14.80	16.57		
CH_3 -2'			21.26		16.48
CH_3 -7				22.20	22.16
CH_3 -7'	22.28	22.28		22.20	
$>\text{CHCH}_3$				30.53	31.65
$>\text{CHCH}_3$				17.86	18.21

a) Signals with the same superscript may be interchanged.

254 (4.27), 434 (3.87). MS m/z (rel. int. %): 374 $[\text{M}]^+$ (100), 359 (10), 357 (17), 346 (5), 187 (4), 134 (8), 120 (7), 106 (13), 92 (14). ^1H -NMR: Table 1. ^{13}C -NMR: Table 2.

2,7'-Dimethyl-6,8'-bijuglone (29)

Orange leaflets (hexane), mp 176–178°C. HR-ESI-MS m/z : 373.0705 ($[\text{M}-\text{H}]^-$, calcd for $\text{C}_{22}\text{H}_{13}\text{O}_6$: 373.0712). IR ν_{max} (KBr) cm^{-1} : 1665, 1636, 1614. UV λ_{max} (CHCl_3) nm (log ϵ): 256 (4.31), 435 (3.83). MS m/z (rel. int. %): 374 $[\text{M}]^+$ (100), 357 (19), 346 (9), 329 (21), 319 (3), 303 (6), 278 (5), 250 (6), 166

(4), 140 (4). $^1\text{H-NMR}$: Table 1. $^{13}\text{C-NMR}$: Table 2.

7,7'-Dimethyl-3,3'-ethylidenebijuglone (30)

Orange powder (C_6H_6), mp 223–225°C. HR-ESI-MS m/z : m/z 401.1043 ($[\text{M}-\text{H}]^-$, calcd for $\text{C}_{24}\text{H}_{17}\text{O}_6$: 401.1025). IR ν_{max} (KBr) cm^{-1} : 1668, 1636, 1614. UV λ_{max} (CHCl_3) nm (log ϵ): 256 (4.21), 432 (3.75). MS m/z (rel. int. %): 402 $[\text{M}]^+$ (100), 387 (47), 359 (5), 216 (5), 201 (3), 188 (5), 135 (6), 134 (4), 106 (7), 77 (7), 57 (3), 43 (4). $^1\text{H-NMR}$: Table 1. $^{13}\text{C-NMR}$: Table 2.

2',7-Dimethyl-3,6'-ethylidenebijuglone (31)

Orange needles (C_6H_6), mp 215–216°C, $[\alpha]_{\text{D}}^{25}$ (26) +0.42° ($c=4.0$, CHCl_3). HR-ESI-MS m/z : 401.1030 ($[\text{M}-\text{H}]^-$, calcd for $\text{C}_{24}\text{H}_{17}\text{O}_6$: 401.1025). IR ν_{max} (KBr) cm^{-1} : 1667, 1639, 1614. UV λ_{max} (CHCl_3) nm (log ϵ): 260 (4.33), 430 (3.88). MS m/z (rel. int. %): 402 $[\text{M}]^+$ (100), 384 (27), 374 (4), 356 (6), 227 (8), 201 (5), 135 (3), 106 (2), 40 (14). $^1\text{H-NMR}$: Table 1. $^{13}\text{C-NMR}$: Table 2.

Reflux of 2,3-Epoxyplumbagin (8) in CHCl_3 A solution of **8** (50 mg) in CHCl_3 containing 7% EtOH (50 mL) was refluxed for 48 h in daylight. The reaction mixture was examined by HPLC (Shim-pack SIL (H), n -hexane– C_6H_6 (7:3) containing 0.2% AcOH, UV 430 nm). Compound **2** was not detected in the reaction mixture.

Reflux of 7-Methyl- β -dihydrojuglone (19) and β -Dihydroplumbagin (26) in CHCl_3 and in 50% EtOH Compounds **19** (20 mg) and **26** (20 mg) were refluxed in chloroform (10 mL) and in 50% aqueous EtOH (10 mL) for 12 h. The resulting mixtures were examined by HPLC (Shim-pack SIL (H), C_6H_6 containing 0.2% AcOH, UV 340, 430 nm). No change was observed in the reaction mixtures of **19** and **26** in CHCl_3 . In the reaction mixtures of **19** and **26** in 50% aqueous EtOH, both **19** and **26** disappeared and **20** and **4** were detected, respectively.

Reduction of (–)- β -Dihydroplumbagin (26) To 26 (100 mg) in 80% acetic acid (20 mL) was added zinc (5 g); the mixture was refluxed for 2 h. The zinc was removed by filtration. After the addition of water (100 mL), the mixture was extracted with EtOAc. The extract was washed with 1% NaHCO_3 and water and dried over Na_2SO_4 . After evaporation of the solvent, the residue was subjected to preparative TLC (CHCl_3 – C_6H_6 (2:1)). The yellow fluorescent band afforded a mixture of *cis*- and *trans*-tetralones. The mixture was separated into *cis*-tetralone (**32**) (18 mg, 18%) and *trans*-tetralone (**33**) (35 mg, 34%) by HPLC (Shim-pack PREP SIL (H), CHCl_3 containing 0.2% AcOH, UV 340 nm).

***cis*-Tetralone (32)**

Pale yellow oil, $[\alpha]_{\text{D}}^{25}$ (23) +14.3° ($c=0.105$, CHCl_3). IR ν_{max} (neat) cm^{-1} : 3450, 1635. UV λ_{max} (CHCl_3) nm (log ϵ): 259 (3.93), 332 (3.59). MS m/z (%): 192 (M^+ , 100), 177 (13), 174 (11), 163 (9), 159 (5), 150 (51), 122 (40), 121 (83). $^1\text{H-NMR}$ (CDCl_3) δ : 1.13 (3H, d, $J=6.5$ Hz, $-\text{CHCH}_3-$), 2.2–3.2 (3H, m, $-\text{CH}_2\text{CH}(\text{CH}_3)-$), 4.69 (1H, d, $J=2.5$ Hz, $-\text{CHOH}-$), 6.8–7.6 (3H, m, Ar H), 12.36 (1H, s, bonded OH).

***trans*-Tetralone (33)**

Colorless needles (C_6H_6), mp 102°C, $[\alpha]_{\text{D}}^{25}$ (30) 0° ($c=0.075$, CHCl_3). IR ν_{max} (KBr) cm^{-1} : 3200, 1630. UV λ_{max} (CHCl_3) nm (log ϵ): 260 (3.97), 334 (3.60). MS m/z (rel. int. %): 192 $[\text{M}]^+$ (100), 177 (15), 174 (10), 163 (9), 159 (7), 150 (40), 122 (29), 121 (57). $^1\text{H-NMR}$ (CDCl_3) δ : 1.18 (3H, d, $J=6.0$ Hz, $-\text{CH}(\text{CH}_3)-$), 2.1–3.2 (3H, m, $-\text{CH}_2\text{CH}(\text{CH}_3)-$), 4.47 (1H, brd, $J=7.5$ Hz, $-\text{CHOH}-$), 6.7–7.7 (3H, m, Ar H), 12.32 (1H, s,

bonded OH).

***cis*-Tetralone Dibenzoate (34)**

cis-Tetralone (**32**, 18 mg) in pyridine (1 mL) was treated with benzoyl chloride (0.5 mL). The reaction product was separated by PTLC (C_6H_6) as a pale yellow semi-solid (21 mg), $[\alpha]_{\text{D}}^{25}$ (22) +71.5° ($c=1.01$, CHCl_3). IR ν_{max} (neat) cm^{-1} : 1740, 1720, 1690. UV λ_{max} (CHCl_3) nm (log ϵ): 243 (4.03), 282 (3.21), 292 sh (3.12). MS m/z (%): 400 $[\text{M}]^+$ (5), 295 (1), 278 (3), 174 (3), 105 (100), 77 (18). $^1\text{H-NMR}$ (CDCl_3) δ : 1.17 (3H, brd, $J=6.5$ Hz, $-\text{CH}(\text{CH}_3)-$), 2.75 (3H, m, $-\text{CH}_2\text{CH}(\text{CH}_3)-$), 6.40 (1H, brs, $-\text{CH}(\text{OBz})-$), 7.1–8.4 (13H, m, Ar H). CD λ_{ext} (MeOH) nm ($\Delta\epsilon$): 290 (+1.3), 239 (+10.4), 230 (0.0), 222 (–6.2).

Ichthyotoxicity Tests According to a previous paper,¹⁾ ichthyotoxicity tests were performed on 150 mL of aqueous solution containing the test sample using male Japanese killifish (*Oryzias latipes* var.).

Seed Germination Tests According to the previous paper,¹⁾ seed germination tests were conducted in 10 mL of aqueous solution containing the test sample against Lettuce seeds (*Lactuca sativa* L. var. Great Lakes).

Conflict of Interest The authors declare no conflict of interest.

References

- Higa M., Ogihara K., Yogi S., *Chem. Pharm. Bull.*, **46**, 1189–1193 (1998).
- Higa M., Noha N., Yokaryo H., Ogihara K., Yogi S., *Chem. Pharm. Bull.*, **50**, 590–593 (2002).
- Tezuka M., Takahashi C., Kuroyanagi M., Satake M., Yoshihira K., Natori S., *Phytochemistry*, **12**, 175–183 (1973).
- Lillie T. J., Musgrave O. C., Skoyles D., *J. Chem. Soc., Perkin Trans. 1*, 2155–2161 (1976).
- Bowie J. H., Cameron D. W., Williams D. H., *J. Am. Chem. Soc.*, **87**, 5094–5099 (1965).
- Lillie T. J., Musgrave O. C., Skoyles D., *J. Chem. Soc., Perkin Trans. 1*, **1976**, 2546 (1976).
- Binder R. G., Benson M. E., Flath R. A., *Phytochemistry*, **28**, 2799–2801 (1989).
- Gunaherath G. M. K. B., Gunatilaka A. A. L., Sultanbawa M. U. S., Balasubramaniam S., *Phytochemistry*, **22**, 1245–1247 (1983).
- Tezuka M., Kuroyanagi M., Yoshihira K., Natori S., *Chem. Pharm. Bull.*, **20**, 2029–2035 (1972).
- Cooke R. G., Dowd H., *Aust. J. Sci. Res.*, **5A**, 760–767 (1952).
- Sankaram A. V. B., Rao A. S., Shoolery J. N., *Tetrahedron*, **35**, 1777–1782 (1979).
- Müller W. U., Leistner E., *Phytochemistry*, **17**, 1735–1738 (1978).
- Sidhu G. S., Sankaram A. V. B., *Tetrahedron Lett.*, **1971**, 2385–2388 (1968).
- Bendz G., Lindberg G., *Acta Chem. Scand.*, **22**, 2722–2723 (1968).
- Ogihara K., Yamashiro R., Higa M., Yogi S., *Chem. Pharm. Bull.*, **45**, 437–445 (1997).
- Lillie T. J., Musgrave O. C., *J. Chem. Soc., Perkin Trans. 1*, **1980**, 1161–1164 (1980).
- Gunaherath G. M. K. B., Gunatilaka A. A. L., Thomson R. H., *J. Chem. Soc., Perkin Trans. 1*, **1988**, 407–410 (1988).
- Zakaria M. B., Jeffreys J. A. D., Waterman P. G., Zhong S. M., *Phytochemistry*, **23**, 1481–1484 (1984).
- Yoshihira K., Tezuka M., Natori S., *Chem. Pharm. Bull.*, **19**, 2308–2313 (1971).
- Thomson R. H., “Naturally Occurring Quinones III Recent Advances,” 3rd ed., Chapman & Hall, London–New York, 1984, p. 182.
- Khan M. R., Timi D., *Fitoterapia*, **70**, 320–321 (1999).