[Chem. Pharm. Bull. 19(11)2308—2313(1971)]

UDC 547.655.02.05 : 581.192 : 582.925

Naphthoquinone Derivatives from the Ebenaceae. II.[1] Isodiospyrin, Bisisodiospyrin, and Mamegakinone from *Diospyros lotus* L. and *D. morrisiana* Hance[2]

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(Received April 23, 1971)

7-Methyljuglone (II), mamegakinone (III), isodiospyrin (IV), and bisisodiospyrin (V) were isolated from the roots of *Diospyros lotus*. (IV) and (V) were also isolated from the roots of *D. morrisiana*. The structure of (IV) was confirmed by the nuclear Overhauser effects observed in the methyl ether (IV'). Bisisodiospyrin (V) is a naphthoquinone tetramer corresponding to the symmetrical dimer of isodiospyrin (IV) linking at 2'- or 3'-position. The plants contain betulin, betulinic acid, oxyallobetulin, taraxerol, lupeol, and ursolic acid.

In the previous paper,[1] the revised structure of diospyrol (I) from *Diospyros mollis* Griff., 7,7'-dimer of 1,8-dihydroxy-3-methylnaphthalene, and the chemistry of the related compounds were reported. In this paper the structures of two dimers and one tetramer of 7-methyljuglone are reported.

Diospyros lotus L. (Ebenaceae) (Japanese name: mamegaki)[4] is a shrub growing in southern part of Japan. Chloroform extracts of the dried roots of the plant yielded, after column and preparative layer chromatographic separations, four naphthoquinones, 7-methyljuglone (II), a new quinone of mp 256° (decomp.) (III), isodiospyrin (IV), and a new quinone of mp >320° (V), besides three triterpenoids, betulinic acid (VI), oxyallobetulin (VII), and taraxerol (VIII). 7-Methyljuglone (II) has already been isolated from several species of the genus *Diospyros*[5,6] and is assumed to be the precursor of other congeners. The quinone (III), named mamegakinone after the Japanese name of the plant, showed the ultraviolet (UV) and infrared (IR) spectra characteristic of juglone derivatives. The nuclear magnetic resonance (NMR) spectrum (Table 1) suggested the structure of 3—3' or 2—2' dimer of 7-methyljuglone (III or IX). The former was first obtained by us by the oxidation of diospyrol[1,7] and then isolated from the dried bark and fruits of *D. mollis* Griff.[1] The direct comparison of the quinone (III) with a sample of the 3—3' dimer showed the identity. The 2—2' dimer (IX) has recently been isolated as a constituent of *Drosera ramentacea* Burch (Droseraceae).[8]

Isodiospyrin (IV) was first isolated from *Diospyros chloroxylon*[6,9] and then from *D. mespiliformis* and *D. virginiana*[10] and was proposed to be 8—6' dimer of 7-methyljuglone from the spectral data.[6,10] Although our specimen showed the higher $[\alpha]_D$ value than the reported

1) Part 1: K. Yoshihira, M. Tezuka, P. Kanchanapee, and S. Natori, *Chem. Pharm. Bull.* (Tokyo), **19**, 2271 (1971).
2) Preliminary communication: K. Yoshihira, M. Tezuka, and S. Natori, *Tetrahedron Letters*, **1970**, 7.
3) Location: *Kamiyoga-1-chome, Setagaya-ku, Tokyo.*
4) In this series of works the classification according to J. Ohwi ("*Flora of Japan*," Shibundo, Tokyo (1965)) is adopted.
5) R.G. Cooke and H. Dowd, *Nature*, **169**, 974 (1952).
6) G.S. Sidhu and K.K. Prasad, *Tetrahedron Letters*, **1967**, 2905.
7) K. Yoshihira, S. Natori, and P. Kanchanapee, *Tetrahedron Letters*, **1967**, 4857.
8) V. Krishnamoorthy, and R.H. Thomson, *Phytochemistry*, **8**, 1591 (1969).
9) G.S. Sidhu, and K.K. Prasad, *Tetrahedron Letters*, **1970**, 1739.
10) A.L. Fallas, and R.H. Thomson, *J. Chem. Soc.* (*C*), **1968**, 2279.

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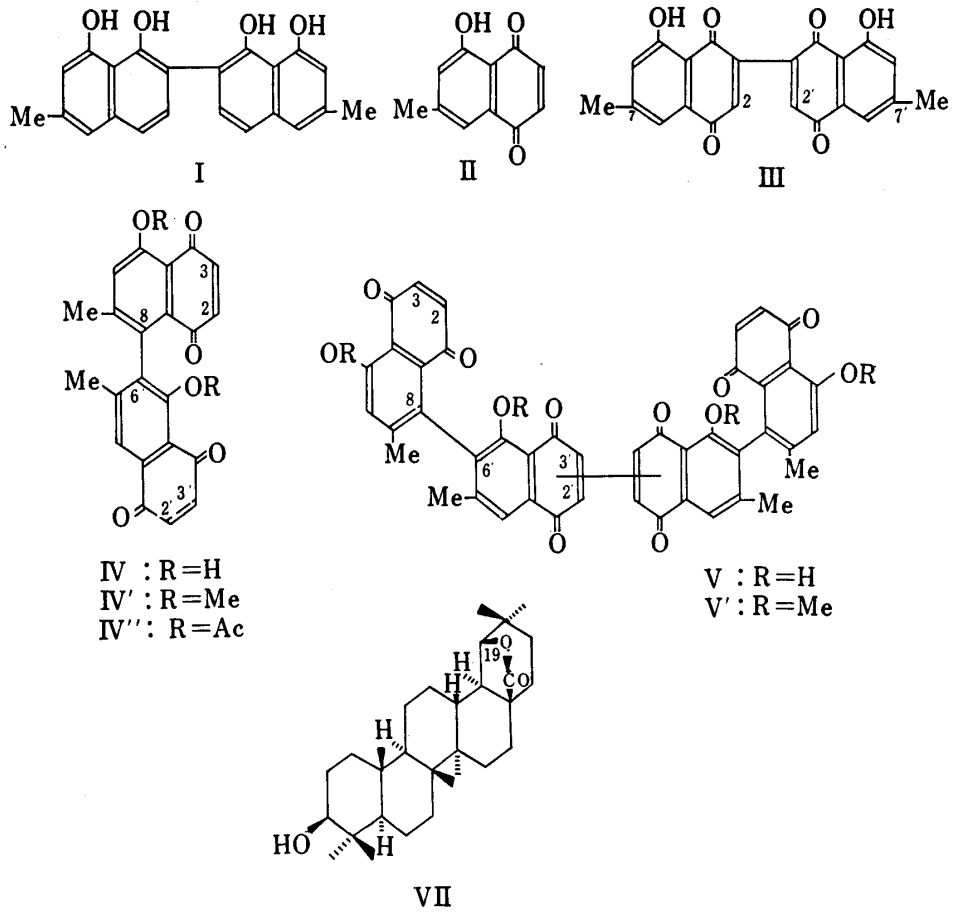

Chart 1

one[10] (see Experimental), the identity was firmly established by direct comparisons with an authentic sample by a mixed fusion, IR, and thin-layer chromatography (TLC). In order to remove the ambiguity existed in the assignment of the structure (IV) detailed examination of the NMR spectra has been carried out. Despite the lack of symmetry, the two quinonoid protons in simple 1,4-naphthoquinones substituted at 5-, 6-, 7-, and/or 8-positions usually have the same chemical shift and appear as an unresolved singlet.[11] This was the case in one of the two pairs of quinonoid protons of IV[10] and its dimethyl ether (IV′)[6,10] in the previous works. In the case of IV we also obtained the same result but the spectrum of IV′ measured in deuteriochloroform at 100 Mc clearly showed two pairs of doublets of both J=10 Hz (see Table 1), though the chemical shifts of two protons of the one pair were quite close. The fact clearly disclosed the absence of the linkage at the quinonoid positions in the both units. As was pointed out by the previous work[6] alternative structures involving 6-methyljuglone and/or 8-methyljuglone moiety could also be written for the compound to satisfy the NMR observations. The possibility was excluded by the nuclear Oberhauser effects (NOE) observed in IV′. As shown in Fig. 1 (see also Table 1) irradiation of the higher methyl signal (δ 1.99) increased the area and hight of the signal of the lower benzenoid ring proton at *peri*-position (δ 7.87), while that of the lower methyl signal (δ 2.04) influenced the higher benzenoid ring proton not in *peri*-position (δ 7.33). The latter ring proton also showed the effect by the irradiation of the lower methoxyl signal (δ 4.06), while no effect was shown by the irradiation of the higher methoxyl signal (δ 3.47). These observations made clear the relative positions of the substituents and the linkage and the structure (IV) was

11) R.E. Moore and P.S. Scheuer, *J. Org. Chem.*, **31**, 3272 (1966).

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proved to be only one structure to suffice these results. The rather large difference of chemical shifts between C-2 and C-3 protons was assumed to be due to the bulky substituent at C-8 and the respective assignment as shown in Table 1 was made taking account of the ring current of another quinone moiety. Thus the assignment of each signals in IV and IV′ was established. Isodiospyrin (IV) is optically active and the optical rotatory disperison (ORD) curve is shown in Fig. 2. The optical activity has been retained after boiling in xylene for 15 hr.

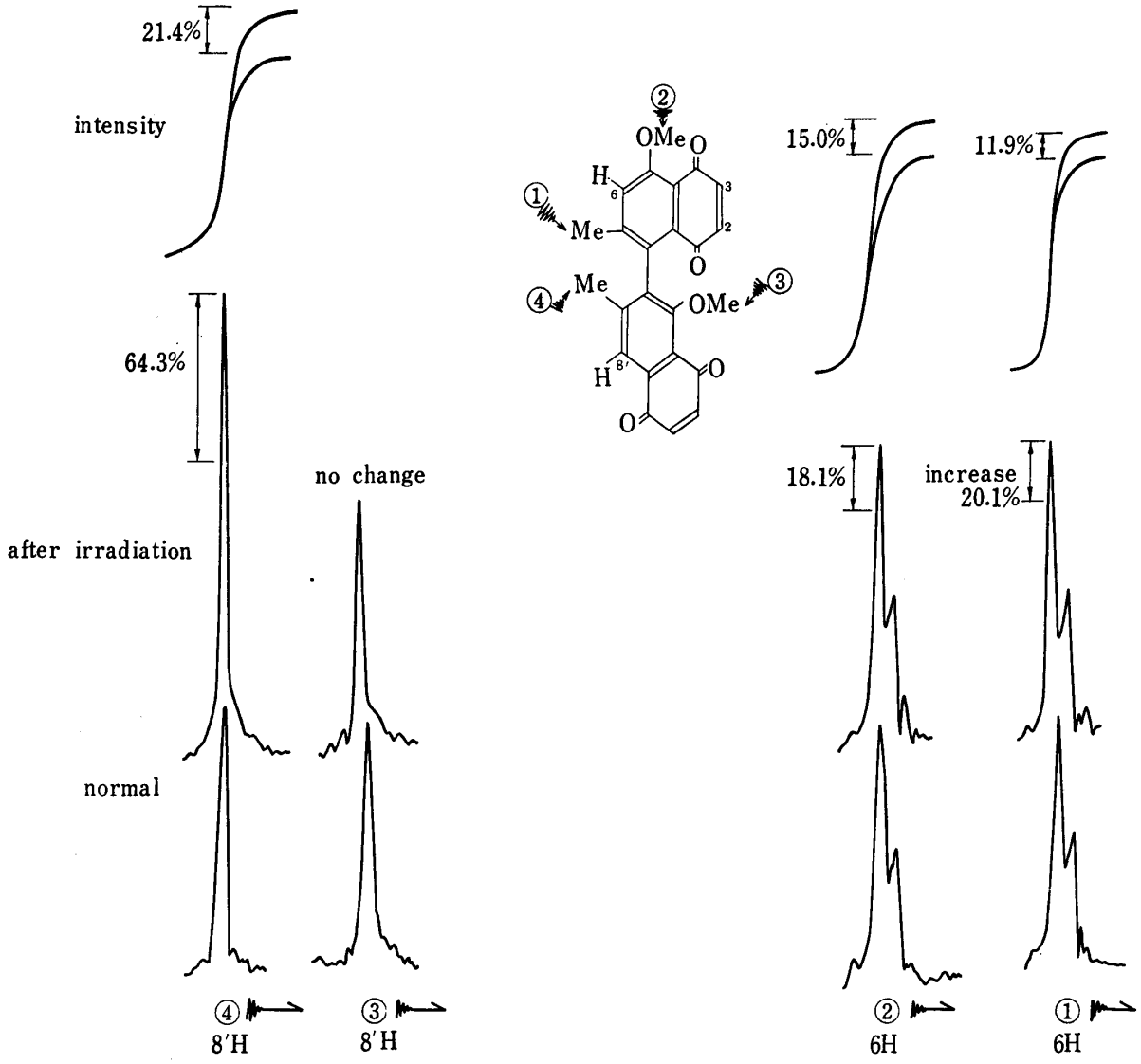

Fig. 1. Nuclear Overhauser Effects Observed in Isodiosyprin Dimethyl Ether (IV′)

The new quinone (V), mp >320°, $[\alpha]_D^{21}$ −678° ($CHCl_3$), shows the molecular ion at 746.142 *m/e*, suggesting the formula, $C_{44}H_{26}O_{12}$ (Calcd. 746.143). The molecular weight was also supported by the osmometric determination. Since V and its tetramethyl ether (V′) show UV (V, $\lambda_{max}^{CHCl_3}$ mμ (log ε) 257, 444 (4.69, 4.25); V′, $\lambda_{max}^{CHCl_3}$ mμ (log ε) 260, 405 (4.87, 4.20)) (Fig. 3) and IR (V, ν_{max}^{KBr} cm^{-1} 1658, 1639, 1601; V′, ν_{max}^{KBr} cm^{-1} 1655, 1583) characteristic of 7-methyljuglone (II) and its methyl ether respectively, V is assumed to be a 7-methyljuglone tetramer from the molecular formula. The NMR spectra of V and V′ (Table 1) are quite informative, showing striking similarity with those of IV and IV′ of the established assignment and indicating the symmetrical feature of the molecule of V. In the spectra of V and V′

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one pair of quinonoid protons showing AB quartet appears as in the case of IV and IV' while one of the two nearly identical quinonoid proton signals at 2'- and 3'-positions in IV and IV' is lacking and the other shifts to lower field (δ 6.98→7.09), the shift being analogous to that of II to III (6.98→7.18). All these findings disclosed the structure of the quinone as a symmetrical dimer of isodiospyrin (IV) linking at 2'- or 3'-position, in which the latter is more likely from the biogenetical grounds. The quinone (V) was designated as bisisodio-

Table I, NMR Spectra of 7-Methyljuglone and its Derivtives

compound	2- and 2'-H	3- and 3'-H	5- and 5'-OH OCH_3 or $OCOCH_3$	6- and 6'-H	7- and 7'-CH_3	8- and 8'-H
II	6.98 s	6.98 s	11.91 s	7.14 d (J=1.5)	2.36 s	7.50 d (J=1.5)
III[a]	7.18 s	—	—[b]	7.22 d (J=0.8)	2.51 s	7.63 d (J=0.8)
IV	6.77 d (J=10)	6.94 d (J=10)	12.12 s[c]	7.35 s	2.05 s	—
	6.98 s	6.98 s	12.50 s[c]	—	2.02 s	7.67 s
IV'[b]	6.66 d (J=10)	6.83 d (J=10)	4.06 s	7.33	2.04 s	—
	6.92 d[c] (J=10)	6.87 d[c] (J=10)	3.47 s	—	1.99 s	7.87 s
IV''	6.88 d (J=10)	6.97 d (J=10)	2.49 s	7.44 s	2.09 s[c]	—
	6.80 s	6.80 s	2.17 s[c]	—	2.03 s[c]	8.10 s
V	6.78 d (J=10)	6.96 d (J=10)	12.00 s[c]	7.33 s	2.06 s	—
	7.09 s[c]	—	12.51 s[c]	—	2.06 s	7.73 s
V'	6.70 d (J=10)	6.88 d (J=10)	4.07 s	7.34 s	2.07 s	—
	7.05 s[c]	—	3.48 s	—	2.01 s	7.95 s

(δ value in ppm from the internal standard TMS in $CDCl_3$ at 60 MHz unless otherwise specified; coupling constant in Hz)

a) determined in $CDCl_3$+CF_3COOH *b*) not observed by the addition of CF_3COOH

c) Assignment is tentative. *d*) determined at 100 MHz

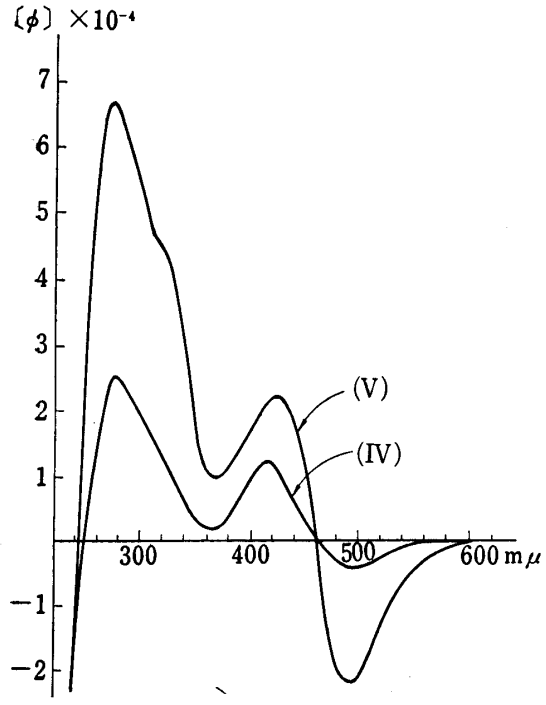

Fig. 2. ORD Curves of Isodiospyrin (IV) and Bisisodiospyrin (V) (in Dioxane)

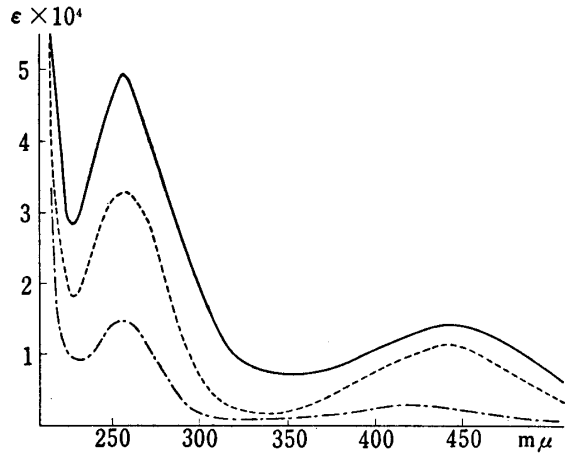

Fig. 3. UV Spectra of 7-Methyljuglone (II), Isodiospyrin (IV), and Bisisodiospyrin (V) (in $CHCl_3$)

———— : bisisodiospyrin (V)
---------- : isodiospyrin (IV)
—·—·— : 7-methyljuglone (II)

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spyrin after the established structure. The quinone (V) is also optically active and shows the same sign of ORD curve with nearly the twice of amplitude of V as shown in Fig. 2. Although the present knowledge of the correlation of the ORD curve with the absolute configurations is insufficient,[12] the curves demonstrate that the two halves of the molecule of V retain the same absolute configuration as that of IV. Recently naphthoquinone trimers such as xylospyrin from *Diospyros chloroxylon*[9] and trianellinone from *Dianella revoluta* (Liliaceae)[13] were reported but, as far as the authors are aware, this is the first example of a naphthoquinone tetramer.

The three triterpenoids, betulinic acid (VI), oxyallobetulin (VII) and taraxerol (VIII), were isolated and identified with authentic samples. Isolation of VII as the natural product is quite rare[14] and, as far as the authors are aware, this is the first example of the isolation from plants.[15] In the NMR spectrum of VII the proton at C-19 bearing the lactone group appears in a singlet at δ 3.96 (in VII-acetate, δ 3.97), supporting the stereochemistry[14] of the lactone ring.

From chloroform extract of the dried wood of the same plant, taraxerol (VIII), lupeol (X), and ursolic acid (XI) were isolated but no sign of the presence of naphthoquinone derivatives was witnessed.

Diospyros morrisiana Hance (Ebenaceae) (Japanese name: tokiwagaki) is also a subtropical shrub growing in southern part of this country. Extraction of the dried roots with chloroform, followed by the same procedure as in the case of *D. lotus*, afforded isodiospyrin (IV) and bisisodiospyrin (V), along with lupeol (X) and betulinic acid (VI). Fom this plant 7-methyljuglone (II) was not identified.

Experimental[16]

Extraction of the Roots of *Diospyros lotus* ——The dried roots (2.5 kg), collected at Tsumura Botanic Garden, Tokyo, in November, 1968, were extracted with boiling $CHCl_3$ for 25 hr and the solvent was evaporated to leave the extract (51 g). The extract (40 g) was dissolved in benzene and applied on a column of silica gel (2.3 kg). The elution with benzene afforded four fractions. The first, after further purification by preparative layer chromatography and recrystallization from hexane, afforded orange needles of mp 124° (II) (34 mg). UV λ_{max}^{EtOH} mμ (log ε): 253 (4.18), 428 (3.60). IR ν_{max}^{KBr} cm^{-1}: 1663, 1630 (s), 1591, 1384, 1368, 1343, 1305 (s), 1280 (s), 1230 (s), 1109, 1053, 1022, 988, 884, 850 (s), 777, 758. NMR (Table 1). The comparison with an authentic sample of 7-methyljuglone by IR, TLC and a mixed fusion showed the identity.

The second fraction was purified by the repetition of preparative layer chromatography and recrystallization from chloroform gave orange-red crystalline powder of mp 256° (decomp.) (III) (8 mg). UV $\lambda_{max}^{CHCl_3}$ mμ (log ε): 255 (4.30), 445 (3.87). IR ν_{max}^{KBr} cm^{-1}: 1655, 1621 (s), 1578, 1380, 1304 (s), 1254 (s), 1217 (s), 1178, 1137, 863, 740. NMR (Table 1). The compound was designated as *mamegakinone* and the identity with the sample derived from diospyrol[1,7] and that isolated from *D. mollis*[1] was confirmed.

The third fraction was treated with active carbon and recrystallized from ethanol to give colorless leafless of mp 285° (20 mg). IR ν_{max}^{KBr} cm^{-1}: 3446, 2910, 1440, 1378, 1035, 997, 812. NMR (in $CDCl_3$) δ: 0.82 (3H, s), 0.93 (6H, s), 0.94 (6H, s), 0.96 (3H, s), 0.99 (3H, s), 1.10 (3H, s), 3.20 (1H, m), 5.45 (1H, q, J=3.7, 7.5 Hz). Comparison with the authentic sample of taraxerol (VIII) showed the identity.

12) K. Mislow, M.A.W. Glass, R.E. O'Brien, P. Rutkin, D. Steinberg, and C. Djerassi, *J. Am. Chem. Soc.*, **82**, 4740 (1960); S. Yamada, and H. Arimoto, *Tetrahedron Letters*, **1968**, 3967.

13) R.G. Cooke and J.G. Down, *Tetrahedron Letters*, **1970**, 583.

14) G.S. Davy, T.G. Halsall, and E.R.H. Jones, *J. Chem. Soc.*, **1951**, 2696; G.S. Davy, T.G. Halsall, E.R.H. Jones, and G.D. Meakins, *J. Chem. Soc.*, **1951**, 2702; D.H.R. Barton, W. Carruthers, and K.H. Overton, *J. Chem. Soc.*, **1956**, 788; R. Ikan, and J. McLean, *J. Chem. Soc.*, **1960**, 893; S.C. Pakrashi, and T.B. Samanta, *Indian J. Chem.*, **7**, 522 (1969).

15) Since there exists the possibility of the formation of VII from VI in the course of chromatographic separation, the presence of VII in the extract before the chromatography was confirmed by thin-layer chromatography.

16) Melting points were determined in a Yanagimoto melting point apparatus and are not corrected. Unless otherwise specified silica gel (Mallinckrodt) was used for column chromatography. For acid-treatment the silica gel was washed with 3% CH_3COOH and dried. For thin-layer chromatography silica gel G with or without the treatment with 3% oxalic acid solution was employed.

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The fourth fraction was further purified by preparative layer chromatography and recrystallized from benzene to yield dark red needles of mp 228° (lit.[10] mp 226—228°) (IV) (8.0 g). $[\alpha]_D^{20}$ −150° (c=0.27, $CHCl_3$) (lit.[10] $[\alpha]_D^{23}$-16.6±1° (c=0.27, $CHCl_3$)). UV $\lambda_{max}^{CHCl_3}$ mμ (log ε): 257 (4.53), 440 (3.99). IR ν_{max}^{KBr} cm^{-1}: 1660, 1638 (s), 1363, 1338 (s), 1279 (s), 1237, 1202, 1155, 1110, 1090, 1040, 840. NMR (Table 1). The compound was identified with *isodiospyrin*[10] by direct comparison.

Further elution of the column with benzene–EtOAc (95:5) afforded two fractions. The first fraction was applied on preparative layer and/or column chromatography to give orange powder (V) of mp>320° (130 mg) (from benzene). $[\alpha]_D^{21}$ −678° (c=0.14, $CHCl_3$). M^+ 746.142 *m/e*, mol, wt. (by osmometer) 655 (in pyridine, 65°), mol. wt. Calcd. for $C_{44}H_{26}O_{12}$, 746.143. Mass Spectrum *m/e*: 746 (M^+), 729, 470, 373, 280, 234, 226, 206, 196, 149, 111, 109, 105, 97, 95, 91. UV $\lambda_{max}^{CHCl_3}$ mμ (log ε): 257 (4.69), 444 (4.25), IR ν_{max}^{KBr} cm^{-1}: 1658, 1639 (s) 1601 (s), 1361 (s), 1340, 1268 (s), 1233, 1213, 1126, 1107, 852. NMR (Table 1). The quinone was designated as *bisisodiopyrin* (V).

The second fraction was again passed through a column of alumina and then purified by preparative layer chromatography. It gave two bands and the one gave colorless needles of mp 296° from EtOH (48 mg), ν_{max}^{KBr} cm^{-1}: 3460, 2940, 1680, 1449, 1373, 1234, 1188, 1043, 880. The comparison with the sample of *betulinic acid* (VI) showed the identity. Methyl ester, mp 225° (from hexane); acetate methyl ester, mp 203—205° (from MeOH). The second band was recrystallized from EtOH to give oxyallobetulin (VII) as colorless needles of mp>310° (25 mg). IR ν_{max}^{KBr} cm^{-1}: 2930, 2863, 1755, 1442, 1388, 1378, 1155, 1116, 1067, 1043, 1025, 964, 920. NMR (in $CDCl_3$) δ: 0.76 (s, 3H), 0.85 (s, 3H), 0.87 (s, 3H), 0.92 (s, 3H), 0.98 (s, 6H), 1.03 (s, 3H), 3.18 (m, 1H), 3.96 (s, 1H). Acetate, mp>310° (from acetone). IR ν_{max}^{KBr} cm^{-1}: 2930, 2860, 1751, 1720, 1437, 1369, 1245, 1150, 1115, 1066, 1023, 1017, 980, 963, 942, 917. NMR (in $CDCl_3$) δ: 0.85 (3H, s), 0.87 (9H, s), 0.92 (3H, s), 0.95 (3H, s), 1.03 (3H, s), 2.06 (3H, s), 3.97 (1H, s), 4.50 (1H, m). The acetate showed the identity with sample prepared from betulinic acid acetate.[14]

Isodiospyrin Dimethyl Ether (IV′)——mp 239—240° (from MeOH) (lit.[9] mp 235°, lit.[10] mp 233—234°). UV λ_{max}^{EtOH} mμ (log ε): 218 (4.60), 255 (4.58), 392 (3.86). IR ν_{max}^{KBr} cm^{-1}: 2940, 1655 (s), 1610, 1582 (s), 1455, 1332 (s), 1302, 1287, 1248, 1228, 1108, 1098, 1066, 1054 (s), 1043, 845. NMR (Table 1).

Isodiospyrin Diacetate (IV″)——Prepared by Ac_2O–H_2SO_4. Yellow needles of mp 245° from MeOH. UV $\nu_{max}^{CHCl_3}$ mμ (log ε): 255 (4.67), 348 (3.95). IR ν_{max}^{KBr} cm^{-1}: 1770 (s), 1664 (s), 1610, 1590 (s), 1365, 1331 (s), 1261, 1193 (s), 1102, 1097, 1040, 1026, 913, 848. NMR (Table 1).

Bisisodiospyrin Tetramethyl Ether (V′)——The mixture of V (60 mg), Ag_2O (100 mg), and CH_3I (1 ml) in $CHCl_3$ (10 ml) was refluxed for 4 hr. After cooling the reaction mixture was filtered and evaporated. The residue was applied on thin-layer plates and the main product was collected and recrystallized from CCl_4 to give orange crystals of mp 220—223° (decomp.) (8 mg). UV $\lambda_{max}^{CHCl_3}$ mμ (log ε): 260 (4.87), 405 (4.20). IR ν_{max}^{KBr} cm^{-1}: 2930, 1655 (s), 1583 (s), 1328 (s), 1278, 1248 (s), 1228 (s), 1112, 1050, 850. NMR (Table 1).

Extraction of the Woods of *Diospyros lotus*——The dried woods (2.5 kg) were extracted with hot $CHCl_3$ and the extract was treated with hexane (fraction A) and then with EtOH (fraction B). From fractino A, taraxerol (VIII), mp 285° (75 mg), was obtained and identified. Fraction B afforded a mixture of fatty acids (210 mg). Ethanol-insoluble part was dissolved in benzene and passed through a column of silica gel. The benzene eluate yielded taraxerol (VIII, 80 mg) and lupeol (X, 600 mg), mp 186° (from hexane). IR ν_{max}^{KBr} cm^{-1}: 3420, 2950, 2870, 1445, 1378, 1040, 828. NMR (in $CDCl_3$) δ: 0.78 (3H, s), 0.81 (3H, s), 0.86 (3H, s), 0.99 (6H, s), 1.07 (3H, s), 1.73 (3H, br. s) 3.25 (1H, m), 3.55 (1H, s), 4.64 (1H, d), 4.75 (1H, d). The identity with the authentic sample was confirmed by TLC, IR, and NMR. The acetate, mp 201° (from MeOH or $CHCl_3$), was prepared and compared with an authentic sample.

Further elution of the column with benzene–EtOAc (1:1) afforded a mixture of triterpene-carboxylic acids (460 mg), mainly composed of ursolic acid (XI). The acetate, mp 275° (from MeOH), ν_{max}^{KBr} cm^{-1}: 1710, 1242, the methyl ester, mp 115° (from EtOH), and the methyl ester acetate, mp 244—247° (from EtOH), were prepared and identified with the authentic samples.

Extraction of the Roots of *Disopyros morrisiana*——The dried roots (2.0 kg) colected at Tanegashima, Kagoshima Prefecture, in May, 1969, were extracted with boling $CHCl_3$ and the extract (30 g) was chromatographed on a column of silica gel (2.0 kg). The column was successively eluted with benzene and benzene–EtOAc (9:1) and each fraction was further purified by column chromatography and/or preparative layer chromatography. The first fraction after treatment with active carbon afforded lupeol (X), colorless needles (260 mg) of mp 186° (from hexane).

The second fraction afforded isodiospyrin (IV), mp 230° (48 mg).

The third fraction afforded bisisodiospyrin (V), mp>320° (470 mg).

The fourth fraction was treated with active carbon, recrystallized from MeOH to give colorless needles of mp 296° (400 mg) and identified with betulinic acid (VI).

Acknowledgement The authors thank Mr. I. Sasaki, Tsumura Laboratory, and Mr. M. Taki, Tanegashima Station for Medicinal Plants of this Institute, for the collection of plant materials. They are grateful to Professor R.H. Thomson, University of Aberdeen, for the authentic sample of isodiospyrin. They are indebted to Central Research Laboratory, Sankyo Co., Ltd. for the determination of the NOE.

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