Novel Naphthoquinones from *Heterophragma adenophyllum*

by **Amir Reza Jassbi***[a])[b]), **Pahup Singh**[c]), **Sonakshi Jain**[c]), and **Satoshi Tahara**[b])

[a]) Department of Phytochemistry, Medicinal Plants Research Institute, Shahid Beheshti University, Evin, Tehran, Iran (fax: +98-21-2418679; e-mail: a-jassbi@cc.sbu.ac.ir)

[b]) Laboratory of Ecological Chemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan

[c]) Department of Chemistry, University of Rajasthan, Jaipur-302004, India

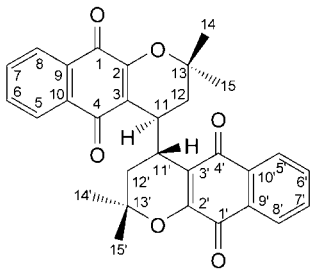
A new symmetric naphthoquinone dimer, dilapachone (**1**), and a novel asymmetric naphthoquinone dimer, adenophyllone (**2**), were isolated from the heartwood of *Heterophragma adenophyllum*. Their structures were elucidated by spectroscopic means including high-resolution mass, UV, IR, 1D- and 2D-NMR spectroscopy.

Introduction. – In pursuing our interest in the quinone constituents [1], we examined *Heterophragma adenophyllum* SEEM (Syn. *Haplophragma adenophyllum* (WALL.) P.DOP.) (Bignoniaceae), an ornamental tree with a white fissured bark grown in tropical and subtropical climates. *Heterophragma* is a small genus of trees distributed in Southeast Asia and Africa. Its root is prescribed as drink in viper bite, and its wood tar is used in various skin diseases [2]. Previous work on this plant led to the isolation of lapachol, dehydro-α-lapachone, tecomaquinone-I, dehydro-iso-α-lapachone, β-sitosterol, tectol, α-lapachone, β-lapachone, and β-amyrin [3–6]. β-Lapachone could be shown to be a potent inhibitor of transcriptase activity from myeloblastosis virus and *Rauscher* murine leukaemia virus. In addition, it affects eukaryotic DNA-dependent DNA-polymerase activity [7]. Several naphthoquinones including diosquinone and the trimeric naphthoquinone derivative conocurvone have shown cytotoxic and anti-HIV activities [8–10]. An antimicrobial naphthoquinone pigment, cribrarione, was isolated from *Cribraria purpurea* [11].

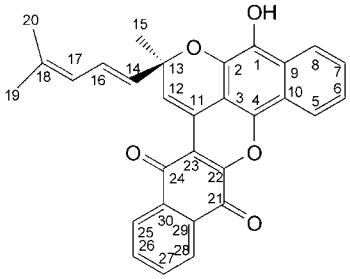
In this paper, we report the isolation and structure elucidation of two new naphthoquinone pigments, dilapachone (**1**)[1]) and adenophyllone (**2**), from the heartwood of *Heterophragma adenophyllum* SEEM. (Bignoniaceae).

2. Results and Discussion. – The heartwood shavings of *H. adenophyllum* (5 kg) were extracted with acetone. The acetone extract, after different liquid extractions and chromatography over neutral alumina, yielded compounds **1** and **2**.

Dilapachone (**1**) was obtained as a yellow powder. It displayed a molecular-ion peak at *m/z* 482.1721 in the HR-EI-MS, which suggests the molecular formula $C_{30}H_{26}O_6$ (M_r 482.1729). The IR absorptions at 1665 and 1641 cm^{-1} indicated the presence of a 1,4-quinonoid moiety, and the UV spectrum showed absorptions at 330, 280, 253 and 217 nm corresponding to those reported for the monomer compound, α-lapachone [12]. Detailed analysis of the spectral data established the structure of dilapachone



1 Dilapachone[1])



2 Adenophyllone[1])

(**1**) to be 3,3′,4,4′-tetrahydro-2,2,2′,2′-tetramethyl-4,4′-bi[2*H*-naphtho[2,3-*b*]pyran]-5,5′,10,10′-tetrone.

Fragment ions at *m/z* 242 and 241 in the MS of **1** may arise due to a *McLafferty* and allylic cleavage of the dimer to its monomer. The ^{1}H-NMR spectrum (*Table 1*) showed signals at δ 8.10 (*d, J* = 7.9 Hz), 7.68 (*dd, J* = 7.4, 7.4 Hz), 7.72 (*dd, J* = 7.4, 7.4 Hz), and 8.09 (*d, J* = 7.9 Hz) representing a disubstituted benzene ring. The second spin system comprised three signals at δ 4.35 (*ddd, J* = 6.6, 6.6, 13.8 Hz), 1.48 (*dd, J* = 13.8, 13.8 Hz), and 1.34 (*dd, J* = 6.6, 13.8 Hz), which, together with two geminal Me groups at δ 1.44 and 1.20, indicated a 4-substituted 3,4-dihydro-2,2-dimethyl-2*H*-pyran moiety in the molecule [12]. In the ^{13}C-NMR spectrum (*Table 1*), the presence of 15 signals suggested a highly symmetric dimer structure for the molecule. These included seven quaternary C-atoms, four aromatic CH groups, one CH for the aliphatic part, and one CH_2 and two Me groups. The signals at δ 29.6, 34.2, 78.4, 29.3, and 23.3 indicated a 3,4-dihydro-2*H*-pyran moiety originating from an *ortho*-prenylphenol in the molecule [13]. Considering the molecular formula, **1** should consist of two similar parts linked through C(11)[1]). The connectivity of the protons to the C-atoms was established by a HMQC experiment, and long-range couplings were detected by a HMBC experiment. The deshielding effect on H–C(11)[1]) may arise from the anisotropic effect of the quinone ring. The values *J* = 6.6, 6.6 and 13.8 Hz for H–C(11) and H–C(11′) are consistent with the proposed structure. In the NOESY plot, cross-peaks for H–C(11,11′)/Me(15,15′) and H–C(12,12′) were observed (δ 1.34). Me(14,14′) showed correlation with

Table 1. *^{1}H- and ^{13}C-NMR and HMBC Data for Compound* **1** *in $CDCl_3$.* Arbitrary numbering[1]).

	δ(H)	δ(C)	HMBC
C(1,1′)	–	179.7	
C(2,2′)	–	156.1	
C(3,3′)	–	122.7	
C(4,4′)	–	184.2	
H–C(5,5′)	8.10 (*d, J* = 7.9)	126.2	C(4), C(6), C(10)
H–C(6,6′)	7.68 (*dd, J* = 7.4, 7.4)	133.1	C(5), C(10)
H–C(7,7′)	7.72 (*dd, J* = 7.4, 7.4)	134.1	C(6), C(8)
H–C(8,8′)	8.09 (*d, J* = 7.9)	126.3	C(1), C(7), C(9)
C(9,9′)	–	132.3	
C(10,10′)	–	131.1	
H–C(11,11′)	4.35 (*ddd, J* = 6.6, 6.6, 13.8)	29.6	C(2), C(3), C(12)
H–C(12,12′)	1.48 (*dd, J* = 13.8, 13.8),	34.2	C(11), C(14)
	1.34 (*dd, J* = 6.6, 13.8)		C(3), C(11), C(14)
C(13,13′)	–	78.4	
Me(14,14′)	1.44 (*s*)	29.3	C(2), C(12), C(15)
Me(15,15′)	1.20 (*s*)	23.3	C(12), C(14)

[1]) Arbitrary numbering; for systematic names, see *Exper. Part.*

H–C(12,12′) (δ 1.48). Finally, Me(15,15′) correlated with H–C(12,12′) (δ 1.34) and Me(14,14′). All of these correlations can justify the presence of both rotamers of **1**. However, the minimized steric energy calculated by the MM2 program for the '*trans*' conformer (33.150 kcal/mol) was less than that calculated for the '*cis*' form (35.919 kcal/ mol).

Adenophyllone (**2**) was obtained as a gray purple powder. The molecular formula $C_{30}H_{22}O_5$ (M_r 462.1467) was determined in the HR-EI-MS by detecting a M^+ peak at *m/z* 462.1468. The UV spectrum exhibited an absorption at 239 nm, and the IR spectrum showed absorptions at 3450 and 1675 cm^{-1} establishing the presence of free OH and quinone C=O functionalities, respectively. Further spectral data established the structure of adenophyllone (**2**) to be 4-hydroxy-2-methyl-2-[(1*E*)-4-methylpenta-1,3-dienyl]-2*H*-3,9-dioxadibenzo[*a*,*de*]naphthacene-10,15-dione.

In the EI-MS of **2**, besides M^+ at *m/z* 462 (69%), the fragment ions at *m/z* 447 (100%), 419 (42%), and 381 (56%) suggested the loss of a Me group, an ^{i}Pr group, and a prenyl side chain (81 amu). In the ^{1}H-NMR spectrum (*Table 2*), two series of signals at δ 8.06 (*d*, J = 8.1 Hz), 7.47 (br. *t*, J = 8.1 Hz), 7.43 (br. *t*, J = 8.1 Hz), and 8.28 (*d*, J = 7.8 Hz) and at δ 8.13 (*d*, J = 7.3 Hz), 7.77 (br. *t*, J = 7.3 Hz), 7.73 (br. *t*, J = 7.3 Hz), and 8.16 (*d*, J =

Table 2. *^{1}H- and ^{13}C-NMR and HMBC Data for Compound* **2** *in $CDCl_3$.* Arbitrary numbering[1]).

	δ(H)	δ(C)	HMBC
HO–C(1)	5.74 (*s*)	131.7	C(1), C(2), C(9)
C(2)	–	135.3[a])	
C(3)	–	107.5	
C(4)	–	135.8[a])	
H–C(5)	8.06 (*d*, J = 8.1)	121.1	C(7), C(9), C(10)
H–C(6)	7.47 (br. *t*, J = 8.1)	126.8	C(5), C(7), C(9)
H–C(7)	7.43 (br. *t*, J = 8.1)	125.1	C(8), C(10)
H–C(8)	8.28 (*d*, J = 7.8)	121.3	C(6), C(7), C(9)
C(9)	–	125.0	
C(10)	–	119.3	
C(11)	–	115.8	
H–C(12)	7.08 (*s*)	122.5	C(3), C(11), C(13), C(15)
C(13)	–	79.8	
H–C(14)	5.67 (*d*, J = 15.0)	131.7	C(13), C(15), C(17)
H–C(15)	1.71 (*s*)	27.4	C(12), C(13), C(14)
H–C(16)	6.45 (*dd*, J = 11.0, 15.0)	126.0	C(13), C(17), C(18)
H–C(17)	5.75 (*d*, J = 11.0)	124.0	C(14), C(19), C(20)
C(18)	–	137.6	
H–C(19)	1.66 (*s*)	18.5	C(17), C(18), C(20)
H–C(20)	1.71 (*s*)	26.0	C(17), C(18), C(19)
C(21)	–	177.9	
C(22)	–	151.1	
C(23)	–	117.3	
C(24)	–	183.6	
H–C(25)	8.13 (*d*, J = 7.3)	126.5	C(24),C(27), C(30)
H–C(26)	7.77 (br. *t*, J = 7.3)	134.6	C(25), C(29)
H–C(27)	7.73 (br. *t*, J = 7.3)	133.5	C(28), C(30)
H–C(28)	8.16 (*d*, J = 7.3)	126.5	C(21), C(26), C(29)
C(29)	–	132.5	
C(30)	–	130.6	

[a]) Assignment may be interchanged.

7.3 Hz) indicated two different aromatic ring systems. The downfield-shifted signals at δ 5.67 (*d, J* = 15.0 Hz), 6.45 (*dd, J* = 11.0, 15.0 Hz), and 5.75 (*d, J* = 11.0 Hz) suggested the presence of a conjugated π system. In the ^{13}C-NMR spectra (*Table 2*, BB and DEPT) 30 C-atoms were detected, which accounted for 12 CH and 3 Me groups and 15 quaternary C-atoms. From the molecular formula, 20 degrees of unsaturation were deduced for **2**, which accounted for 2 C=O, 12 C=C bonds, and 6 rings. With the help of the HMQC experiment, the assignment of the ^{13}C-NMR data was established for all proton-bearing C-atoms. The above spin systems in the ^{1}H-NMR spectrum were connected to each other by using HMBC data. The cross-peaks between H–C(25) and H–C(28) with the two C=O groups at δ 183.6 and 177.9, respectively, indicated the presence of a naphthoquinone moiety in **2**. The connectivity of an aromatic proton at δ 7.08 (H–C(12)) with C(3), C(11), C(13), and C(15) on one hand and of H–C(14) with C(13) and C(15) on the other hand suggested that these protons are part of a prenyl branch in the molecule. The cross-peaks between HO–C(1) and C(1), C(2), and C(9), between H–C(5) and C(7), C(9) and C(10), and between H–C(8) and C(6), C(7), and C(9) suggested the presence of a naphthoquinol part in **2**. In the NOESY spectrum, the cross-peaks between H–C(12) and H–C(14), H–C(15), and H–C(16) confirmed the vicinity of H–C(12) to these protons. H–C(16) showed a cross peak with Me(19) and H–C(17) with Me(20), which established the assignment of the Me signals in the prenyl moiety of **2**.

Two of us (*S. J.* and *A. R. J.*) are thankful to the *U. G. C.*, New Delhi, and the *Japan Society for the Promotion of Science* for granting a junior and a post-doctoral fellowship, respectively. Our thanks are expressed to Dr. *Eri Fukushi* and Mr. *Kenji Watanabe* for their skillful measuring of the NMR and mass spectra.

Experimental Part

General. Column chromatography (CC): neutral alumina, deactivated with 10% aq. AcOH. Anal. TLC: *Merck* silica gel *60F$_{254}$* precoated glass plates. Optical rotation: *Jasco-DIP-370* digital polarimeter. UV spectra: *Hitachi-U-3210* spectrophotometer; λ_{max} (ε) in nm. IR Spectra: *FT-IR-Nicolet-Magna-550* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, NOESY, HMQC, and HMBC: 500/125-MHz *Bruker-AMX-500-FT* spectrometer; δ in ppm, *J* in Hz. MS: *JEOL JMS-SX102A* spectrometer; in *m/z* (rel. %).

Plant Material. The plant material was collected from the campus of University of Rajasthan, Jaipur, India, and duly identified by Prof. *P. S. Jain*, Department of Botany, University of Rajasthan, where a voucher specimen of *Heterophragma adenophyllum* is deposited with herbarium number RUBL 19887.

Extraction and Isolation. The heartwood shavings (5.0 kg) of the plant were extracted with acetone (3 ×) over a steam bath for 12 h. The resulting extract was evaporated (98 g) and the residue dissolved in Et_2O. The Et_2O soln. was treated with 2N Na_2CO_3 soln. The Na_2CO_3-insoluble portion (neutral fraction) was washed with H_2O, dried (Na_2SO_4), and evaporated. The dark brown extract (10 g) was subjected to CC (neutral alumina; petroleum ether, petroleum ether/$CHCl_3$): 1:1, $CHCl_3$): **1** (150 mg; with petroleum ether) and **2** (100 mg; with petroleum ether/$CHCl_3$ 1:1).

Dilapachone (*= 3,3′,4,4′-Tetrahydro-2,2,2′,2′-tetramethyl-4,4′-bi[2*H*-naphtho[2,3-*b*]-pyran]-5,5′,10,10′-tetrone;* **1**). Bright yellow powder. M.p. 222–223°. $[\alpha]_D^{26} = +12°$ ($CHCl_3$, *c* = 0.75). UV (MeOH): 218 (4223), 253 (7223), 280 (4128), 330 (1223). IR (KBr): 1665, 1641, 1660, 1592, 1572, 1389, 1201, 790, 680. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 482 (40), 480 (15), 467 (12), 242 (100), 241 (74), 439 (30), 227 (60), 223 (32), 199 (11), 195 (12), 165 (12), 152 (10), 105 (17), 77 (15), 76 (11).

Adenophyllone (*= 4-Hydroxy-2-methyl-2-[(1*E*)-4-methylpenta-1,3-dienyl]-2*H*-3,9-dioxadibenzo[*a,de*]-naphthacene-10,15-dione;* **2**). Gray purple powder. M.p. 226–227°. IR (KBr): 3450, 1675, 1600, 1550. UV: (MeOH): 239 (28300). ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 462 (69), 447 (100), 445 (29), 419 (42), 381 (56), 342 (17), 239 (13), 213 (11), 105 (18), 91 (18), 77 (20).

REFERENCES

[1] P. K. Gupta, P. Singh, *J. Asian Nat. Prod. Res.*, in press.
[2] R. N. Chopra, S. L. Nayar, I. C. Chopra, 'Glossary of Indian Medicinal Plants', C. S. I. R., New Delhi, 1956, p. 132.
[3] P. Singh, L. Prakash, K. C. Joshi, *Phytochemistry* **1972**, *11*, 1498.
[4] K. C. Joshi, L. Prakash, P. Singh, *J. Indian Chem. Soc.* **1973**, *50*, 561.

[5] K. C. Joshi, L. Prakash, P. Singh, R. T. Pardasani, G. Singh, *Planta Med.* **1979**, *37*, 60.
[6] S. Jain, P. Chauhan, P. Singh, *J. Indian Chem. Soc*. **2002**, *79*, 946.
[7] A. R. Schuerich, W. Wehrli, *Eur. J. Biochem*. **1978**, *84*, 197.
[8] B. A. Adeniyi, M. F. Robert, H. Chai, H. H. S. Fong, *Phytother. Res.* **2003**, *17*, 282.
[9] L. A. Decosterd, I. C. Parsons, K. R. Gustafson, J. H. Cardellina II, J. B. McMahon, G. M. Cragg, Y. Murata, L. K. Pannell, J. R. Steiner, J. Clardy, M. R. Boyd, *J. Am. Chem. Soc*. **1993**, *115*, 6673.
[10] J.-R. Dai, L. A. Decosterd, K. R. Gustafson, J. H. Cardellina II, G. N. Gray, M. R. Boyd, *J. Nat. Prod*. **1994**, *57*, 1511.
[11] A. Naoe, M. Ishibashi, Y. Yamamoto, *Tetrahedron* **2003**, *59*, 3433.
[12] H. Inouye, T. Okuda, T. Hayashi, *Chem. Pharm. Bull*. **1975**, *23*, 384.
[13] B. A. Dawson, M. Girard, D. Kindack, J. Fillion, D. V. C. Awang, *Magn. Reson. Chem*. **1989**, *27*, 1176.

Received October 2, 2003