Transformation of Lapachol to a Novel Naphthoquinone and Related Compounds

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Z. Naturforsch. **61b,** 73 – 77 (2006); received August 23, 2005

In boiling pyridine, lapachol (**1**) is transformed into novel asymmetric naphthoquinone derivative, quadrilone (**2**), and into dehydro-α-lapachone (**3**), and adenophyllone (**4**). Lapachol used in the present study was isolated from the heartwood of *Heterophragma quadriloculare* and found to be the most abundant naphthoquinone.

Key words: Heterophragma quadriloculare, Bignoniaceae, Naphthoquinones

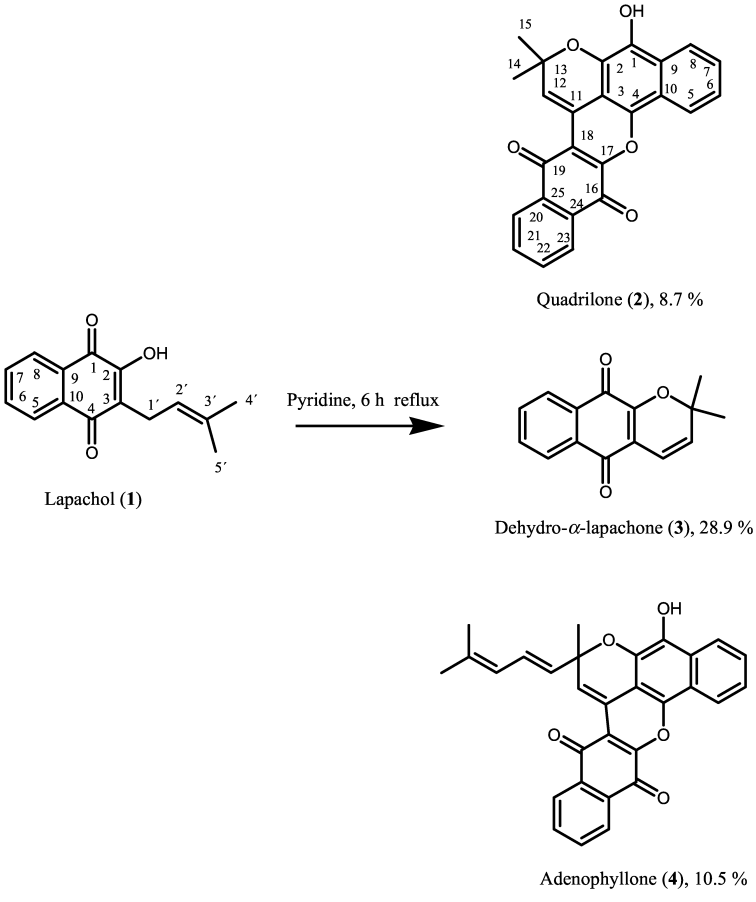
Introduction

Pursuing our interest in quinone constituents, we examined the chemical constituents of *Heterophragma quadriloculare*. *Heterophragma quadriloculare* (Roxb.) K. Schum. (syn. *H. roxburghii* D. C.) (Bignoniaceae) is a medicinal tree. A thick fluid-like tar extracted from the plant is reportedly used to treat skin disease [1]. The plant's medicinal value may result from the high content of allantoin present in its flower; this compound is said to be a dermatological agent [1]. The tree is grown in Deccan and southern region of India; its root is reportedly to be used against snake bite [1, 2]. *Heterophragma* is a small genus of trees distributed in Southeast Asia and Africa. Its root is prescribed against viper bite and its wood-tar is used to treat various skin diseases [3]. Previous work on this plant led to the isolation of allantoin, sitosterol, ursolic acid [1], and 24-methylchlost-5-en-3-*O*-α-D-glucoside $(1 \rightarrow 4)$-O-β-L-rhamnoside [2]. Investigation into the chemical constituents of the seeds of the plant led to identification of its sugars and amino acids [4], together with subulin, lupeol, β-sitosterol and stigmasterol [5]. Palmitic, stearic, linoleic and oleic acids were detected in the seed lipids of the plant [6].

To prepare dehydro-α-lapachone (**3**), lapachol (**1**) was refluxed in pyridine for seven hours [7]. Chromatography on silica gel showed three bands. One of them was identified as xiliodone (dehydro-α-lapachone, **3**); another compound was reported to be an antibiotic effective against gram negative bacteria of the genus *Brucella* [7]. Another report shows that dehydro-α-lapachone (**3**) forms from lapachol by $FeCl_3$ oxidation in the presence of pyridine and acetic anhydride [8]. In this paper we report the isolation of naphthoquinone pigments and identify lapachol as the major compound from the heartwood of *Heterophragma quadriloculare*. To investigate the pyridine catalyzed products of lapachol, the main pigment in the *Heterophragma* plants, we refluxed lapachol (**1**) in pyridine to give quadrilone (**2**), dehydro-α-lapachone (**3**), and adenophyllone (**4**). Adenophyllone (**4**) is a novel naphthoquinone previously isolated by our group from *H. adenophyllum* [9].

Results and Discussion

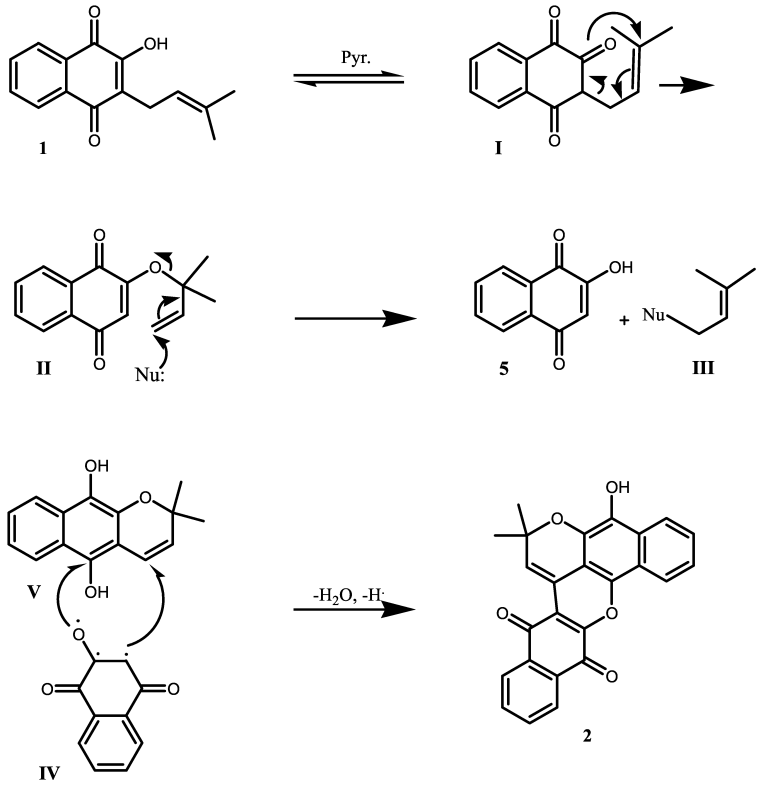
The heartwood shavings of *H. quadriloculare* (5 kg) were extracted with acetone. Lapachol (**1**) was purified from the extract. When **1** was refluxed in pyridine for six hours in a sand bath, a red mass resulted which was purified by different chromatography procedures to yield **2**, **3** and **4** (Scheme 1).



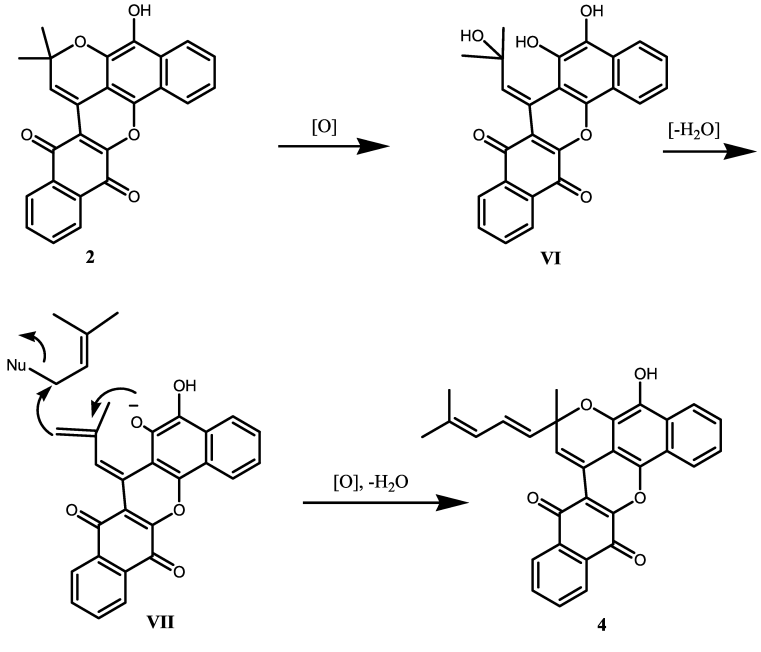
Scheme 1. Transformation of lapachol (**1**) to compounds **2**–**4**.

Quadrilone (**2**) was obtained from the silica column by eluting with benzene as a gray-purple powder, m. p. 232 °C. Its molecular formula, $C_{25}H_{16}O_5$ (exact mass: 396.0998), was determined using HREI mass spectrometry to detect a molecular ion peak at *m/z* 396.1085. The UV spectrum gave absorption at 385, 265, 238 and 203 nm. The IR spectrum showed peaks at 3555 and 1675 cm^{-1}, confirming the presence of hydroxyl and carbonyl functionalities in the molecule. In addition to the molecular ion at *m/z* 396 (69%) in the EI mass spectrum, the base peak at *m/z* 381 $[M\text{-}CH_3]^+$ suggested that a methyl was lost to gain the most stable fragment. The other fragments in the EIMS were 364 (5%), and 239 (5%).

The ^{1}H NMR spectrum of **2** was similar to that recorded for adeophyllone [9] (Table 1). It showed two series of signals at $\delta = 8.07$ (*d*, $J = 8.0$ Hz), 7.49 (dt, $J = 1.0$, 8.0 Hz), 7.47 (dt, $J = 1.0$, 8.0 Hz), 8.30 (*d*, $J = 8.0$ Hz) and 8.17 (dd, $J = 1.0$, 7.5 Hz), 7.79 (dt, $J = 1.0$, 7.5 Hz), 7.75 (d*t*, $J = 1.0$, 7.5 Hz), and 8.15 (dd, $J = 1.0$, 7.5 Hz), indicating the presence of two 1,2-disubstituted aromatic rings. Unlike adenophyllone, the spectrum contains only one up-field shifted signal at $\delta = 1.28$, which accounted for six protons. In the ^{13}C NMR spectrum (Table 1, BB and APT) 24 signals representing 25 carbons were detected, which accounted for nine methines, two methyls, and fourteen quaternary carbons. From the molecular formula, eighteen unsaturations were deduced for the molecule, and were accounted for two carbonyls, ten double bonds, and six rings. With the help of HMQC spectrum the assignment of the ^{13}C NMR spectral data were established for all proton-bearing carbons. The above spin systems in ^{1}H NMR spectrum are connected to each other using HMBC spectral data similar to that observed for adenophyllone [9]. Different pathways for the formation of dehydro-α-lapachone (**3**) from lapachol (**1**) were suggested by several authors, involving oxidative cyclyzation between C-2 and C-3' [10–12]. In addition to the pyridine-



Scheme 2. Suggested mechanism for the formation of quadrilone (**2**). The nucleophile may be pyridine or a conjugated base of a hydroxynaphthoquinone.



Scheme 3. Suggested mechanism for the formation of adenophyllone (**4**).

Table 1. ^{1}H NMR, ^{13}C NMR, and HMBC data for compound **2** in CDCl3.

Position	δ_H (Hz)	δ_C	HMBC
1, OH	5.71 s	135.7	
2	–	131.8	
3	–	107.5	
4	–	135.5	
5	8.07 d 8.0	121.0	C-4, C-6, C-10
6	7.47 dt 1.0, 8.0	125.1	C-5, C-10
7	7.49 dt 1.0, 8.0	126.8	C-8, C-9
8	8.30 d 8.0	121.3	C-1, C-7, C-9
9	–	124.8	
10	–	119.3	
11	–	115.9	
12	7.1 s	124.7	C-3, C-11, C-13, C-14 (C-15)
13	–	78.3	
14	1.28 s	28.4	C-12, C13, C-15
15	1.28 s	28.4	C-12, C13, C-14
16	–	178.1	
17	–	151.2	
18	–	116.9	
19	–	183.8	
20	8.15 dd 1.0, 7.5	126.3	C-19,C-21, C-25
21	7.75 dt 1.0, 7.5	133.5	C-20, C-25
22	7.79 dt 1.0, 7.5	134.6	C-23, C-24
23	8.17 dd 1.0, 7.5	126.6	C-16, C-22, C-24
24	–	132.5	
25	–	130.6	

catalyzed formation of **3** [7, 10], a modified Hooker oxidation in the presence of $KMnO_4$ resulted in the formation of 2-hydroxy-1,4-naphthoquinone (**5**) from lapachol [13]. Although we could not detect the presence of **5** in the reaction mixture, but the structure of quadrilone is composed of substructures of those similar to **3** and **5** (Scheme 2). An oxidative coupling between a quinone radical of **5** (**IV**) with hydroquinone form of **3** (**V**) may produce **2**, after dehydration and dehydrogenation of the resulting adduct (Scheme 2) [14]. To suggest the mechanism of the formation of **4** from **2**, a prenyl group in **II** or **III**, may be subjected to a nucleophilic attack by **VII** (Scheme 3). The resulting alkyl side chain may be oxidized at newly formed allylic position, followed by dehydration to form **4** (Scheme 3). The mechanism of the formation of **VI** in an autooxidative ring opening was suggested for **3** previously (Scheme 3) [11]. The above mechanisms of the formation of **2** and **4** were suggested according to the mechanistic investigation on related compounds [10 – 12, 14 – 17]. However to confirm the mechanisms of these transformations more detailed investigations seem to be essential. Compounds **1**, **3**, and **4** were identified by comparing the physical and spectral data with those published previously [9, 10].

Experimental Section

General procedure: ^{1}H and ^{13}C NMR spectral data, ^{1}H-^{1}H COSY, NOESY, HMQC, and HMBC experiments were measured with a Bruker Avance DRX 500 NMR. MS spectra were recorded on a Micromass MasSpec spectrometer. UV/Vis spectra were obtained on a JASCO model V-550 spectrophotometer. IR spectra were measured on an FTIR Bruker EQUINOX-55 spectrometer. Column chromatography was performed on silica gel (60 – 120 mesh, Merck). Analytical TLC was performed on Merck silica gel $60F_{254}$ pre-coated glass plates.

Plant material: The plant material was collected from the forest of Sagar, M. P., India, and identified by taxonomists at the Department of Botany, University of Rajasthan, Jaipur, India. A voucher specimen of *Heterophragma quadriloculare* has been deposited in the Department of Botany at the same university.

Extraction and isolation: The heartwood shavings of *H. quadriloculare* (5 kg) were extracted with acetone for 15 h. The extract was concentrated to dryness (90 g), taken up in Et_2O, and then extracted with 2N Na_2CO_3.The alkali-soluble fraction on acidification with 2N HCl yielded lapachol (41 g). When refluxed with pyridine for 6 h in a sand bath at 210 °C, lapachol (3 g) yielded a red mass (1.90 g), which was poured into ice-cold water and extracted with ether (2×50 ml) and chloroform (2×50 ml). The resulting orange needles (from chloroform) were dried over anhydrous sodium sulfate and subjected to column chromatographic separation over silica gel (60 – 120 mesh, Merck). On elution with petroleum-ether: benzene (3:1) gave dehydro-α-lapachone (860 mg, yield, 28.9%) as orange needles, m. p. 142 – 143 °C; petroleum ether: benzene (1:1) yielded adenophyllone (300 mg, yield 10.5%) as gray purple needles, m. p. 226 – 227 °C; pure benzene produced a novel asymmetric naphthoquinone dimmer, quadrilone (190 mg, yield 7.7%) m. p. 232 °C (**2**). Individual fractions were examined by TLC and further purified by preparative TLC over silica gel PF254, $60F_{254}$ E. Merck plates.

Quadrilone (=4-hydroxy-2,2-dimethyl-2H-3,9-dioxadibenzo[a,de]-naphthacene-10,15-dione; **2**): gray-purple powder (0.19 g, 7.7%, yield). – M. p. 232 °C. – UV/vis (MeOH): λ_{max} (lg ε) = 385 (5.73), 265 (6.73), 238 (6.72), 203 (6.76) nm. -IR (KBr): ν_{max} = 3555 (OH), 3070, 2968, 2926, 1675 (C=O), 1662 (C=O), 1594, 1560, 1454, 1414, 1361, 1328, 1306, 1269, 1210, 1065, 1027, 987 cm^{-1}. – MS (EI, 70 eV): *m/z* (%) = 396 (69) [M$^+$], 381(100) [M$^+$ – CH_3], 364 (5), 239 (5). – MS (HREI): m/z = 396.1085 [$C_{25}H_{16}O_5$, calcd. 396.0998]. For NMR data see Table 1.

Acknowledgements

ARJ and SJ are thankful to the Alexander von Humboldt foundation for a postdoctoral fellowship in Germany and to U. G. C., New Delhi, for financial support.

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