Pergamon

S0031-9422(96)00135-5

*Phytochemistry*, Vol. 42, No. 5, pp. 1315–1320, 1996

0031-9422/96 $15.00 + 0.00

# ANTIFUNGAL AND ANTIBACTERIAL NAPHTHOQUINONES FROM *NEWBOULDIA LAEVIS* ROOTS

STEFAN GAFNER, JEAN-LUC WOLFENDER, MALO NIANGA,* HELEN STOECKLI-EVANS† and KURT HOSTETTMANN‡

Institut de Pharmacognosie et Phytochimie, Université de Lausanne; B. E. P., CH-1015 Lausanne, Switzerland; *Laboratoire des Composés Naturels (LACONA), Conakry, République de Guinée; †Institut de Chimie, Université de Neuchâtel, 51 Av. de Bellevaux, CH-2000 Neuchâtel, Switzerland

(*Received in revised form* 23 *January* 1996)

**Key Word Index**—*Newbouldia laevis*; Bignoniaceae; roots; naphthoquinones; antifungal activity; antibacterial activity.

**Abstract**—From a dichloromethane extract of *Newbouldia laevis* roots, four new (6-hydroxydehydroiso-α-lapachone, 7-hydroxydehydroiso-α-lapachone, 5,7-dihydroxydehydroiso-α-lapachone and 3-hydroxy-5-methoxydehydroiso-α-lapachone) and six known naphthoquinones have been isolated. Their structures were established by spectroscopic methods (UV, EI mass spectrometry, $^1$H and $^{13}$C NMR) and that of 7-hydroxydehydroiso-α-lapachone was confirmed by X-ray crystallography. All naphthoquinones showed antifungal activity against *Cladosporium cucumerinum* and *Candida albicans*, and activity against the bacteria *Bacillus subtilis* and *Escherichia coli*.

## INTRODUCTION

*Newbouldia laevis* Seem. (Bignoniaceae) is a shrub or small tree growing in regions of wooden savanna and deciduous forest of west Africa. The plant is widely used in African traditional medicine [1]. Recently, anti-inflammatory activity of the chloroform-soluble portion of the methanolic extract of the stem bark was evidenced in pharmacological studies [2]. Previous phytochemical work led to the isolation of a tetrasaccharide [3], four indolic bases [4, 5] and three naphthoquinones [5].

As the dichloromethane extract of the root of *N. laevis* showed interesting activity in our antifungal and antibacterial tests, this extract was investigated in order to determine the nature of the active compounds.

## RESULTS AND DISCUSSION

The roots of *N. laevis* were extracted successively with dichloromethane and methanol. HPLC-UV and HPLC–mass spectrometric analysis of the dichloromethane extract allowed the detection of the alkaloids described by Adesanya *et al.* [4] and re-isolation of these compounds was thus avoided.

The dichloromethane extract was first fractionated by flash chromatography on silica gel. Further separations were performed by MPLC on RP-18 and gel filtration on Sephadex LH-20 (see Experimental) to afford the naphthoquinones **1–9** and **11**. Compound **10** was an artefact obtained by an attempt to methylate compound **7**.

The structures of the naphthoquinones were determined by UV, $^1$H and $^{13}$C NMR spectroscopy and EI–mass spectrometry. The UV spectra of compounds **1–11** exhibited bands characteristic for naphthoquinones [6]; the position of the absorption maxima depended on the type of substitution. The naphthoquinone structure was confirmed by signals for two carbonyl groups and eight aromatic carbons in the $^{13}$C NMR spectra.

In $^1$H NMR, the absence of signals in the quinoid proton region (δ 5.8–6.8) indicated **1–11** to be naphthoquinones substituted at the 3a- and 9a-positions. Furthermore, $^1$H NMR of **1–9** showed signals for an isopropenyl group (δ 1.8, *s*, 3H; δ 5.0, *s*, 1H and δ 5.1–5.2, *s* 1H), which were confirmed in $^{13}$C NMR by signals at δ 16.9–17.4 ($CH_3$), 113.4–114.2 ($CH_2$) and δ 139.1–141.9 (quaternary carbon). As only a small quantity of **6** was isolated, the $^{13}$C NMR spectrum could not be obtained. Proton spectra of **1–6** gave one proton between δ 5.3 and 5.5 (*dd*, 1H) and the protons of a $CH_2$ group were found between δ 2.9–3.1 (*dd*, 1H) and 3.2–3.4 (*dd*, 1H). Two protons with a low field shift (δ 5.3–5.4, *d*, 1H and δ 5.1–5.2, *s*, 1H) and the signal of a hydroxyl proton at δ 3.3–3.6 were observed in the $^1$H NMR spectra of **7–9**, thus showing that one proton of the $CH_2$ group in **1–6** was replaced

‡Author to whom correspondence should be addressed.

by a hydroxyl group. In $^{13}C$ NMR, naphthoquinones **1–5** showed a $CH_2$-carbon signal at $\delta$ 31.9–32.4 and a CH group at $\delta$ 88.2–89.0, while compounds with an aliphatic hydroxyl group (**7–9**) showed a signal between $\delta$ 74.7 and 75.0 for a CH group bearing an oxygen substituent and another low field CH group at $\delta$ 95.3–95.7. In $^1H$ NMR, signals of aromatic protons were found between $\delta$ 7.0 and 8.0. A signal at $\delta$ 4.0 (*s*, 3H) indicated an aromatic methoxyl group and a low field singlet ($\delta$ 11.6–12.4, 1H) was found for compounds with a chelated hydroxyl group (**1** and **7**). Aromatic methoxyl groups for **3** and **9** were confirmed in $^{13}C$ NMR by a signal at $\delta$ 56.5.

From the NMR information described above and by comparison with literature data [5, 7–12], **1–9** were found to be derivatives of dehydroiso-$\alpha$-lapachone. Compounds **1–6** had no substitution in position C-3, while **7–9** possessed a hydroxyl group in this position.

For **10**, signals of an isopropenyl group were also found, but shifted more towards low field ($\delta$ 2.14, *s*, 3H; $\delta$ 5.37, *s*, 1H and $\delta$ 5.96, *s*, 1H). Furthermore, three aromatic protons ($\delta$ 7.2–7.8) and a low field singlet ($\delta$ 6.84, 1H) were found.

For **11**, finally, two signals of four protons were found in the aromatic region between $\delta$ 7.62 and 8.14. Therefore, the aromatic moiety of **11** had to be unsubstituted. Furthermore, it showed signals for two methyl groups ($\delta$ 1.69 and 1.80, both *s*, 3H) a $CH_2$ group ($\delta$ 3.31, *d*, 2H) and one low field proton at $\delta$ 5.21 (*m*, 1H). In $^{13}C$ NMR, the methyl groups resonated at $\delta$ 17.9 and 22.6, the $CH_2$ group at $\delta$ 25.7 and the CH group at $\delta$ 119.6.

Fragmentation patterns in EI–mass spectrometry for **1–9** were very similar, but differences were found between compounds with position C-3 oxygenated and those without oxygenation at C-3. According to ref. [12], compounds without a hydroxyl group at C-3 showed a typical fragmentation pattern with peaks $[M]^+$, $[M-15]^+$, $[M-28]^+$, $[M-43]^+$ and $[M-71]^+$. Compounds with a hydroxyl group at C-3 showed fragments $[M]^+$, $[M-17]^+$, $[M-29]^+$ and $[M-57]^+$. Assignment of further fragments was based on cleavage of the dehydroiso-$\alpha$-lapachone nucleus ($m/z = 240$). Fragments at $m/z = 104$ $[C_7H_4O]^+$, $m/z = 105$ $[C_5H_5O]^+$ and $m/z = 133$ $[C_8H_5O_2]^+$ indicated an unsubstituted aromatic moiety [13]. One hydroxy group attached to the aromatic ring caused a shift of these fragments by 16 amu ($m/z = 120$, 121 and 149). Compounds with one methoxy group showed a shift of 30 amu ($m/z = 134$, 135 and 163), and a shift of 32 amu ($m/z$ 136, 137 and 165) indicated two hydroxyl groups attached to the aromatic ring.

By comparison of spectral data with literature [5, 7–12], **1–3** and **7** and **8** were identified as 5-hydroxydehydroiso-$\alpha$-lapachone (**1**), dehydroiso-$\alpha$-lapachone (**2**), 5-methoxydehydroiso-$\alpha$-lapachone (**3**), 3,8-dihydroxydehydroiso-$\alpha$-lapachone (**7**) and 3-hydroxydehydroiso-$\alpha$-lapachone (**8**). They have been isolated from other species of the Bignoniaceae earlier. The $^1H$ and $^{13}C$ NMR data showed **10** to be the dehydrated form of **7**. Its structure was identified as 2-isopropenyl-8-hydroxynaphtho[2,3-*b*]furan-4,9-quinone by comparison of spectral data with those of literature [12]. Compound **11** was determined by its UV, EI mass, $^1H$ and $^{13}C$ NMR data to be lapachol, which has been isolated from *N. laevis* earlier [5]. Compounds **4–6** and **9** are new natural products. Their structural elucidation is given below.

The EI mass spectral fragmentation pattern of **4** ($m/z = 256$) suggested it to be a derivative of **2** with one hydroxyl group located on the aromatic moiety. The $^1H$ NMR spectrum ($CD_3OD$) showed three aromatic protons forming an ABX system ($\delta$ 7.87, *d*, $J = 8.6$ Hz, 1H; $\delta$ 7.35, *d*, $J = 2.4$ Hz, 1H and $\delta$ 7.07, *dd*, $J = 8.5$ and 2.4 Hz, 1H). Furthermore, there was no signal of a chelated hydroxyl group in this spectrum. Therefore, the hydroxyl group was either in position C-6 or C-7. However, the position could not be determined by the data obtained by UV, EI-mass spectrometry, $^1H$ or $^{13}C$ NMR. The small amount of **4** prevented the use of the selective INEPT NMR technique to locate the hydroxyl group. Finally, crystals obtained from methanol–water were subjected to X-ray analysis (Fig. 1) and the position of the hydroxyl group was found to be at C-7. Thus, **4** is 7-hydroxydehydroiso-$\alpha$-lapachone.

Compound **5** showed the same mass ($m/z = 256$) and fragmentation pattern in the EI-mass spectrum as **4**,

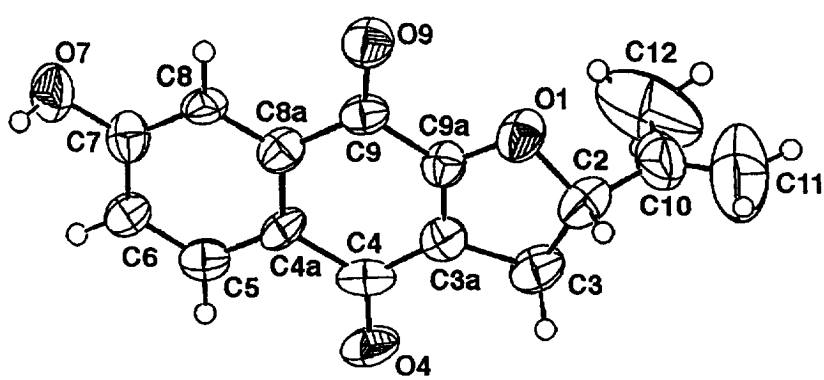



Fig. 1. PLUTON view of 7-hydroxydehydroiso-$\alpha$-lapachone (**4**).

Table 1. $^{13}C$ NMR spectral data for compounds **1–5** and **7–9** (50.30 MHz, $CDCl_3$)

| C | 1 | 2 | 3 | 4* | 5* | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| 2 | 89.0 | 88.5 | 88.2 | 89.6 | 89.2 | 95.7 | 95.4 | 95.3 |
| 3 | 31.4 | 31.9 | 32.4 | 32.9 | 33.1 | 74.7 | 74.9 | 75.0 |
| 3a | 123.5 | 124.0 | 125.9 | 126.2† | 126.2† | 124.5 | 123.8 | 121.5 |
| 4 | 188.1 | 182.2 | 182.5 | 183.4 | 183.9‡ | 182.7† | 182.6 | 182.1 |
| 4a | 114.8 | 133.0 | 120.0 | 125.2† | 134.4 | 133.1 | 132.9 | 119.3 |
| 5 | 161.1 | 126.0† | 159.5 | 129.4 | 117.5 | 119.3 | 126.2 | 161.6† |
| 6 | 125.7 | 134.2 | 119.0 | 121.3 | 156.0§ | 137.3 | 134.6 | 117.7‡ |
| 7 | 135.1 | 133.0 | 134.1 | 164.0‡ | 123.9 | 124.5 | 133.4 | 135.8 |
| 8 | 119.5 | 126.3† | 119.6 | 113.6 | 129.9 | 162.3‡ | 126.7 | 119.0‡ |
| 8a | 131.8 | 131.5 | 134.1 | 135.1 | 125.3† | 114.5 | 131.7 | 135.4 |
| 9 | 177.0 | 177.7 | 177.6 | 179.0 | 181.1‡ | 181.6† | 178.2 | 176.9 |
| 9a | 160.8 | 160.0 | 157.4 | 161.1‡ | 157.5§ | 160.7‡ | 160.9 | 160.5† |
| 10 | 141.4 | 141.7 | 141.9 | 144.1 | 144.4 | 139.1 | 139.2 | 139.3 |
| 11 | 114.2 | 114.0 | 113.7 | 113.7 | 113.4 | 114.2 | 114.1 | 113.9 |
| 12 | 16.9 | 16.9 | 16.9 | 17.0 | 17.0 | 17.3 | 17.3 | 17.2 |
| $OCH_3$ | | | 56.5 | | | | | 56.5 |

*Spectra in $CD_3OD$.

†,‡,§Assignments within the same column may be interchangeable.

thus indicating it to be a derivative of dehydroiso-α-lapachone with one aromatic hydroxyl group, either at C-6 or C-7. The $^1H$ NMR ($CD_3OD$) spectrum showed three aromatic protons with an ABX coupling pattern as in **4**, but all signals were shifted towards high field (δ 7.71, *d*, $J = 8.6$ Hz, 1H; δ 7.13, *d*, $J = 2.7$ Hz, 1H and δ 6.75, *dd*, $J = 8.6$ and 2.6 Hz, 1H). The $^{13}C$ NMR spectrum (Table 1) gave further evidence of a difference in structure between **5** and **4**. As **4** was determined by X-ray analysis to be 7-hydroxydehydroiso-α-lapachone, **5** is 6-hydroxydehydroiso-α-lapachone.

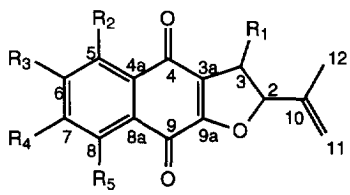
The EI mass spectrum of **6** ($m/z = 272$) showed the fragmentation pattern of a dehydroiso-α-lapachone derivative with two hydroxyl groups located on the aromatic moiety. In the $^1H$ NMR spectrum ($CD_3OD$), a pair of *meta*-coupled protons was found in the aromatic region (δ 7.10, *d*, $J = 2.5$ Hz, 1H and δ 6.56, *d*, $J = 2.2$ Hz, 1H), indicating **6** to be either 5,7-dihydroxy- or 6,8-dihydroxydehydroiso-α-lapachone. The structure was determined by the shift value of the chelated proton (δ 12.32, *s*, $CDCl_3$). Lillie and Musgrave [14] reported that the shift of the hydroxyl proton of 5-hydroxy-1,4-naphthoquinones (juglone type) in $CDCl_3$ is influenced by the substituents in position C-2 and C-3, but independent of the sample concentration. The shift of the proton of the chelated hydroxyl group in the model compounds is 11.64 ppm (8-hydroxydehydroiso-α-lapachone [12] or 12.23 ppm (5-hydroxydehydroiso-α-lapachone). According to Pretsch *et al.* [15], another hydroxyl group in a *meta* position has little influence on the shift of the chelated hydroxyl proton (−0.12 ppm). Consequently, a shift of 11.52 ppm for 6,8-dihydroxydehydroiso-α-lapachone and of 12.11 ppm in the case of 5,7-dihydroxydehydroiso-α-lapachone is calculated. Therefore, since the hydroxyl proton in **6** is at 12.32 ppm, this compound is 5,7-dihydroxydehydroiso-α-lapachone.

The structure of **9** was deduced from its EI mass spectrum and $^{13}C$ NMR spectrum to be a derivative of dehydroiso-α-lapachone with a hydroxyl group in position C-3 and one methoxyl substituent on the aromatic moiety ($m/z = 286$). The coupling of the aromatic protons in the $^1H$ NMR spectrum ($CDCl_3$, δ 7.75, *dd*, $J = 7.7$ and 1.6 Hz, 1H; δ 7.68, *dd*, $J = 7.8$ and 7.5 Hz, 1H and δ 7.28, *dd*, $J = 7.8$ and 1.7 Hz, 1H) indicated three adjacent protons forming an ABC system; thus, **9** was either 3-hydroxy-8-methoxy- or 3-hydroxy-5-methoxydehydroiso-α-lapachone. In the $^{13}C$ NMR spectra, no major difference was observed between compounds with an oxygen substituent in positions C-5 and C-8 (Table 1). The structure of **9** was determined by comparing the shifts and couplings of the 5- and 8-substituted dehydroiso-α-lapachones in the $^1H$ NMR spectrum ($CDCl_3$, see Table 2), which all showed an ABC coupling pattern on the aromatic moiety. While the coupling pattern of **7** was very different from that of **9**, compounds **1** and **3** showed a pattern very similar to that of **9**; therefore, this compound is 3-hydroxy-5-methoxydehydroiso-α-lapachone.

Naphthoquinones in general are well known for antibacterial, antifungal and antitumoural activities, and

Table 2. $^1H$ NMR data for the aromatic protons of compounds **1**, **3**, **7** and **9** ($CDCl_3$, 200 MHz, δ from TMS)

| | 1 | 3 | 7 | 9 |
|---|---|---|---|---|
| $H_5$ | | | 7.62, *m* | |
| $H_6$ | 7.24, *dd* ($J = 8.3, 1.5$) | 7.32, *dd* ($J = 8.4, 1.2$) | 7.24, *dd* ($J = 5.7, 5.7$) | 7.28 *dd* ($J = 7.8, 1.7$) |
| $H_7$ | 7.53, *dd* ($J = 8.3, 7.3$) | 7.58, *dd* ($J = 8.4, 7.6$) | 7.62, *m* | 7.68, *dd* ($J = 7.8, 7.5$) |
| $H_8$ | 7.63, *dd* ($J = 7.6, 1.5$) | 7.78, *dd* ($J = 7.6, 1.2$) | | 7.75, *dd* ($J = 7.7, 1.6$) |



**1** $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = OH$

**2** $R_1 = R_2 = R_3 = R_4 = R_5 = H$

**3** $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = OCH_3$

**4** $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = OH$

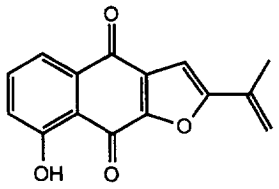
**5** $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = OH$

**6** $R_1 = R_3 = R_5 = H$, $R_2 = R_4 = OH$

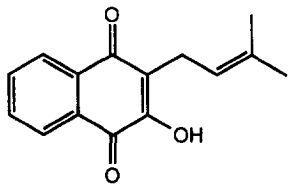
**7** $R_2 = R_3 = R_4 = H$, $R_1 = R_5 = OH$

**8** $R_2 = R_3 = R_4 = R_5 = H$, $R_1 = OH$

**9** $R_3 = R_4 = R_5 = H$, $R_1 = OH$, $R_2 = OCH_3$



**10**



**11**

**11** has been especially widely tested in various pharmacological studies [16–18]. Therefore, the activities of the isolated compounds (except **5** and **6**, due to lack of material) against *Candida albicans*, *Cladosporium cucumerinum*, *Bacillus subtilis* and *Escherichia coli* were determined by bioautographic TLC assays [19–21]. Activities against *C. albicans* and *B. subtilis* were further determined in an agar-dilution assay [22]. The results of the TLC tests and the agar-dilution assays are listed in Table 3. In spite of strong bioactivities, the medical use of naphthoquinones is limited. Owing to their supposed mechanisms of action, the compounds are likely to be toxic for all living organisms [17].

Previous phytochemical investigation of *N. laevis* reported the isolation of three naphthoquinones: lapachol (**11**), dehydro-$\alpha$-lapachone and 3-hydroxy-dehydroiso-$\alpha$-lapachone (**2**). Naphthoquinones of the lapachol-type were found throughout the Bignoniaceae. Dehydroiso-$\alpha$-lapachone and its derivatives have been isolated from the following genera: *Catalpa*, *Crescentia*, *Haplophragma*, *Markhamia*, *Newbouldia*, *Paratecoma*, *Radermachia* and *Tabebuia* [5, 7–12], which gives further proof of the chemotaxonomic unity of the family.

## EXPERIMENTAL

*General.* Mps: uncorr. $^1H$ and $^{13}C$ NMR spectra were measured in $CDCl_3$ or $CD_3OD$ at 200.06 and 50.30 MHz, respectively. TMS: int. standard. UV spectra were recorded in MeOH. TLC: silica gel 60 $F_{254}$ Al sheets (Merck). CC: silica gel (63–200 $\mu$m, Merck; 600 × 50 mm i.d.). MPLC: home-packed LiChroprep RP-18 columns (15–25 $\mu$m; 460 × 16 mm i.d. and 460 × 26 mm i.d.). Mps: Mettler-FP-80/82 hot stage apparatus. UV: Varian DMS 100S UV–Vis spectrophotometer. $^1H$ and $^{13}C$ NMR: Varian VXR 200. El-MS and D/CI-MS: Finnigan MAT TSQ-700 triple-

Table 3. Antifungal and antibacterial activities of naphthoquinones

| Compound | *Cladosporium cucumerinum* | *Candida albicans* | | *Bacillus subtilis* | | *Escherichia coli* |
|---|---|---|---|---|---|---|
| **1** | 0.02* | 0.1* | 10† | 0.02* | 1.25† | 0.06* |
| **2** | 0.06* | 0.4* | 20† | 0.2* | 40† | 0.2* |
| **3** | 0.2* | 4* | 80† | 0.2* | 40† | 0.6* |
| **4** | 4* | 0 | n.d. | 0.1* | 10† | 0.2* |
| **7** | 0.01* | 0.1* | 10† | 0.02* | 1.25† | 0.1* |
| **8** | 0.1* | 1* | 40† | 0.2* | 20† | 2* |
| **9** | 2* | 0 | n.d. | 2* | n.d. | 2* |
| **10** | 0.2* | 0.7* | 40† | 0.04* | 1.25† | 0.1* |
| **11** | 0.6* | 10* | n.d. | 1* | n.d. | 2* |
| Propiconazole | 0.1* | | | | | |
| Amphotericin | 1* | 1* | 1† | | | |
| Chloramphenicol | | | | 0.01* | 1† | 0.1* |

*Minimal amount ($\mu$g) of compound to inhibit growth on a silica gel TLC plate.

†Minimal inhibition concentration MIC ($\mu$g/ml) of compound in an agar-dilution assay.

n.d. = MIC of compound not determined.

stage quadrupole instrument. Purity of the compounds was checked by HPLC; the column (Macherey–Nagel) was packed with Nucleosil RP-18 (5 $\mu$m, 125 × 4 mm i.d.).

*Plant material.* Roots of *N. laevis* were collected in 1994 in Seredou, province of Macenta, Guinea. A voucher specimen (No. 94084) is deposited at the Institut de Pharmacognosie et Phytochimie, Lausanne, Switzerland.

*Extraction and isolation.* Powdered roots (958 g) were extracted at room temp. successively with $CH_2Cl_2$ and MeOH to afford 6.84 and 107.58 g of extract, respectively.

A portion of the $CH_2Cl_2$ extract (6.5 g) was subjected to CC on silica gel, using mixts of petrol, EtOAc, $CHCl_3$ and MeOH of increasing polarity, giving frs 1–12. Compound **1** (35.4 mg) was isolated from frs 2 and 3 by MPLC on RP-18 with MeOH–$H_2O$ (13:7) and 3:2 respectively. Compound **2** (8, 9 mg) was isolated from fr. 3 by MPLC with MeOH–$H_2O$ (3:2). MPLC of fr. 4 (MeOH–$H_2O$, 11:9) and fr. 5 (MeOH–$H_2O$, 1:1) yielded **11** (33.1 mg). Compound **5** was isolated from fr. 6 by MPLC with MeOH–$H_2O$ (9:11). Fr. 7 was subjected to MPLC with MeOH–$H_2O$ (14:11); further gel filtration on Sephadex LH-20 with $CHCl_3$–MeOH yielded **4** (6.0 mg), **6** (1.5 mg), **7** (24.6 mg) and **8** (9.1 mg). Compounds **3** (6.5 mg) and **9** (4.2 mg) were isolated from fr. 8 by MPLC with MeOH–$H_2O$ (3:2).

To obtain 3,8-dimethoxydehydroiso-$\alpha$-lapachone, 5.0 mg **7** was dissolved in $Me_2CO$ (3 ml). To this soln, $Me_2SO_4$ (9.8 mg) and dry $K_2CO_3$ (46.8 mg) were added. The mixt. was refluxed for 24 hr. The $K_2CO_3$ was filtered off. The residue was dried, then dissolved in MeOH–$CHCl_3$ (1:1). From this soln, **10** (2.7 mg) crystallized as orange needles.

*7-Hydroxydehydroiso-α-lapachone* (**4**). Red plates, mp 225–231°, UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 207 (3.96), 222 (3.90), 265 (4.13), 302 (3.72), 350 (3.48). $^1$H NMR (200 MHz, $CD_3OD$): 7.87 (1H, *d*, *J* = 8.6 Hz, H-5), 7.35 (1H, *d*, *J* = 2.4 Hz, H-8), 7.07 (1H, *dd*, *J* = 8.5, 2.4 Hz, H-6), 5.44 (1H, *dd*, *J* = 10.8, 8.8 Hz, H-2), 5.13 (1H, *s*, H-11, *cis* to Me), 4.99 (1H, *s*, H-11, *trans* to Me), 3.32 (1H, *dd*, *J* = 17.1, 11.0 Hz, H-3), 2.93 (1H, *dd*, *J* = 17.3, 8.6 Hz, H-3), 1.80 (3H, *s*, H-12). EI/MS *m/z* (rel. int.): 256 $[M]^+$ (28), 254 (34), 243 (25), 241 (24), 228 (82), 227 (26), 213 (100), 185 (18), 157 (19), 149 (20), 121 (47), 120 (60), 92 (46). $^{13}$C NMR: see Table 1.

*6-Hydroxydehydroiso-α-lapachone* (**5**). Amorphous violet powder, mp >300°, UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 207 (4.01), 227 (3.96), 265 (4.05), 293 (3.78), 348 (3.35). $^1$H NMR (200 MHz, $CD_3OD$): 7.71 (1H, *d*, *J* = 8.6 Hz, H-8), 7.13 (1H, *d*, *J* = 2.7 Hz, H-5), 6.75 (1H, *dd*, *J* = 8.6, 2.6 Hz, H-7), 5.39 (1H, *dd*, *J* = 10.5, 9.0 Hz, H-2), 5.11 (1H, *s*, H-11, *cis* to Me), 4.97 (1H, *s*, H-11, *trans* to Me), 3.28 (1H, *dd*, *J* = 17.4, 10.8 Hz, H-3), 2.89 (1H, *dd*, *J* = 17.1, 8.7 Hz, H-3), 1.79 (3H, *s*, H-12). EI/MS *m/z* (rel. int.): 256 $[M]^+$ (13), 254 (13), 228 (27), 227 (13), 213 (100), 199 (19), 185 (24), 157 (29), 149 (33), 121 (67), 120 (94), 92 (60). $^{13}$C NMR: see Table 1.

*5,7-Dihydroxydehydroiso-α-lapachone* (**6**). Brown powder, mp >300°, UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 217 (3.96), 264 (3.85), 323 (3.49). $^1$H NMR (200 MHz, $CD_3OD$): 7.10 (1H, *d*, *J* = 2.5 Hz, H-8), 6.56 (1H, *d*, *J* = 2.2 Hz, H-6), 5.42 (1H, *dd*, *J* = 10.3, 9.0 Hz, H-2), 5.13 (1H, *s*, H-11, *cis* to Me), 5.01 (1H, *s*, H-11, *trans* to Me), 3.33 (1H, *dd*, *J* = 17.3, 10.8 Hz, H-3), 2.99 (1H, *dd*, *J* = 17.5, 8.7 Hz, H-3), 1.81 (3H, *s*, H-12). EI/MS *m/z* (rel. int.): 273 (19), 272 $[M]^+$ (100), 257 (17), 244 (25), 243 (19), 229 (94), 215 (14), 201 (15), 165 (17), 143 (33), 137 (40), 136 (42), 108 (23). $^{13}$C NMR not measured (lack of material).

*3-Hydroxy-5-methoxydehydroiso-α-lapachone* (**9**). Yellow needles, mp 121–122°, UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 210 (4.15), 218 (4.12), 273 (3.84), 394 (3.25). $^1$H NMR (200 MHz, $CDCl_3$): 7.75 (1H, *dd*, *J* = 7.7, 1.7 Hz, H-8), 7.68 (1H, *dd*, *J* = 7.8, 7.5 Hz, H-7), 7.28 (1H, *dd*, *J* = 7.8, 1.7 Hz, H-6), 5.35 (1H, *d*, *J* = 3.9 Hz, H-3), 5.13 (1H, *s*, H-11, *cis* to Me), 5.11 (1H, *d*, *J* = 4.4 Hz, H-2), 4.99 (1H, *s*, H-11, *trans* to Me), 4.02 (3H, *s*, OMe), 1.77 (3H, *s*, H-12). EI/MS *m/z* (rel. int.): 287 (16), 286 $[M]^+$ (100), 257 (14), 242 (11), 229 (9), 214 (11), 203 (16), 193 (21), 163 (23), 135 (7), 104 (10), 98 (15). $^{13}$C NMR: see Table 1.

*Crystallographic data for compound* **4**. $C_{15}H_{12}O_4$, orthorhombic, space group $P2_12_12_1$, $a$ = 5.168(1), $b$ = 6.247(1), $c$ = 39.104(5) Å, $Z$ = 4, 2900 reflections measured, 1359 independent reflections, 473 observed reflections $[I > 2\sigma(I)]$, final $R_1$ = 0.0511, $R_{w2}$ = 0.0657, Goodness of fit 0.81, residual density max. $min^{-1}$ 0.150/−0.175 e Å$^{-3}$. Absorption coefficient $\mu$ = 0.098 $mm^{-1}$; no correction for absorption was applied. Suitable crystals of **4** were grown from MeOH–$H_2O$ as orange plates.

Intensity data were collected at room temp. on a Stoe AED2 4-circle diffractometer using Mo$K_\alpha$ graphite monochromated radiation ($\lambda$ = 0.71073 Å) with $\omega/\Theta$ scans in the 2$\Theta$ range 3–50°. The structure was solved by direct methods using the program SHELXS-86 [23]. The refinement and all further calculations were carried out using SHELXL-93 [24]. All the H atoms were included in calculated positions. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on $F^2$.

No attempt was made to determine absolute configuration of the molecule. Bond lengths and angles are normal within experimental error. The three fused rings of the molecule lie in a plane, with the C-2 substituent inclined by 86° to the best plane through the 5-membered ring. In the crystal, molecules are linked by a possible intermolecular H bond involving hydroxyl HO7 and the carbonyl O-atom, O4, of a symmetry related molecule (operation: $1 + x$, $y - 1$, $z$).

Full tables of atomic parameters and bond lengths and angles may be obtained from the Cambridge Crystallographic Data Centre, U.K., on quoting the full journal citation. The molecular structure and crystallographic numbering scheme of **4** is illustrated in the

PLUTON [25] drawing (Fig. 1). Further details may be obtained from the author H. S.-E.

*Acknowledgement*—The authors would like to thank the Swiss National Science Foundation for the financial support of this work.

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