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# Quinones and Related Compounds in Higher Plants. II.[1] On the Naphthoquinones and Related Compounds from *Catalpa* Wood[2]

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As the naphthoquinones and a derivative relating to the already reported catalpalactone (**1**), the following seven substances have been isolated from *Catalpa ovata* wood: α-lapachone (**3**), α-dihydrocaryopterone (=9-hydroxy-α-lapachone) (**4**), 9-methoxy-α-lapachone (**5**), 4-hydroxy-α-lapachone (**6**), 4,9-dihydroxy-α-lapachone (**7**), 4-oxo-α-lapachone (**8**) and catalponol (**9**). Their structures, except catalponol (**9**) have been clarified.

Previously, we reported on the structure elucidation of catalpalactone (**1**) isolated from the wood of *Catalpa ovata* (Japanese name "Kisasage"). The structure of this substance suggests that it could be formed in the plant organism from prenylnaphthoquinone derivatives such as lapachol (**2**) by way of the cleavage between C-3 and C-4 as shown in Chart 1. This suggestion seems to be rationalized by the occurrence of lapachol (**2**) in the plant of the family Bignoniaceae such as *Streptospermum suaveolens*,[4b] *Tabebuia flavescens* and several plants of this genus.[4b]

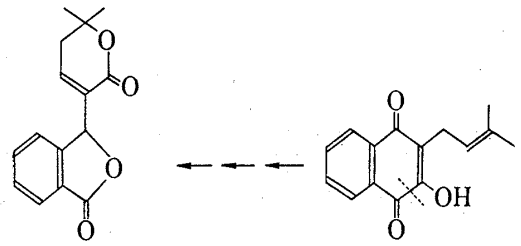

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Chart 1

In advance of the biosynthetic studies on catalpalactone aiming at establishing this postulation, we have precisely examined the naphthoquinones in *Catalpa ovata* wood.

Fractionantion of the methanolic extract of the *Catalpa* wood as shown in Chart 2 gave six naphthoquinones, α-lapachone (**3**), α-dihydrocaryopterone (=9-hydroxy-α-lapachone) (**4**), 9-methoxy-α-lapachone (**5**), 4-hydroxy-α-lapachone(**6**), 4,9-dihydroxy-α-lapachone (**7**) and an artefact 4-oxo-α-lapachone (**8**) along with a hydronaphthoquinone derivative designated as catalponol (**9**) besides catalpalactone (**1**), β-sitosterol and some other unidentified compounds. Among these, **5**, **6**, **7**, **8**, and **9** are new compounds.

On isolation of these substances, separation of the mixture of **3** and **4** as well as **6** and **7** was difficult and we barely succeeded to separate each component by column chromatography on acetylated polyamide. During the course of the separation of a mixture of **6** and **7** in this way, a small amount of 4-oxo-α-lapachone (**8**) was also obtained. The formation of this substance (**8**), which must be an artefact formed by the oxidation of **6**, made it more difficult to obtain intact **6**. However, these quinones were able to be separated easily through their

1) The paper entitled "Structure of Catalpalactone, a New Phthalide from Catalpa Wood," H. Inouye, T. Okuda, Y. Hirata, N. Nagakura and M. Yoshizaki, *Chem. Pharm. Bull.* (Tokyo), **15**, 786 (1967), is regarded as Part 1 of this series. *cf. Idem*, *Tetrahedron Letters*, **1965**, 1261.

2) A part of this work has been published in a preliminary form. H. Inouye, T. Okuda and T. Hayashi, *Tetrahedron Letters*, **1971**, 3615.

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4) *a*) K.V. Rao, T.J. McBride and J.J. Oleson, *Cancer Research*, **28**, 1952 (1968); *b*) A.K. Burnett and R.H. Thomson, *J. Chem. Soc.*, **1967**, 2100; *idem*, *ibid.*, **1968**, 850.

Table I. Compositions and Physical Properties of Quinones isolated from the Wood of *Catalpa ovata*

| Compounds | Compositions | mp (°C) | $[\alpha]_D$ in MeOH | IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$ | UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$) |
|---|---|---|---|---|---|
| **3** | $C_{15}H_{14}O_3$ | 117—118 | — | 1675, 1645, 1615, 1575 | 252(4.36), 283(4.13), 334(3.45) |
| **4** | $C_{15}H_{14}O_4$ | 120—122 | — | 1675, (sh.), 1645, 1610 | 247(4.16), 287(4.06), 410(3.57) |
| **5** | $C_{16}H_{16}O_4$ | 168—170 | — | 1675, 1645, 1625, 1585 | 247(4.21), 280(4.10), 380(3.70) |
| **6** | $C_{15}H_{14}O_4$[a] | — | +27.4° | 1700, 1635, 1605, 1575 (sh.) | 227(4.38), 248.5 (sh.) (4.19), 255 (sh.) (4.12), 287(4.20), 415 (3.47) |
| **7** | $C_{15}H_{14}O_5$ | 130—131 | +17.9° | 1685, 1645, 1615, 1585 | 246(4.01), 289(3.99), 415(3.47) |
| **8** | $C_{15}H_{12}O_4$ | 163—165 | — | 1705, 1685, 1650 (sh.), 1590, 1560 | 253(4.13), 259(4.16), 283(4.09), 334(3.40) |

a) assigned by mass spectrum

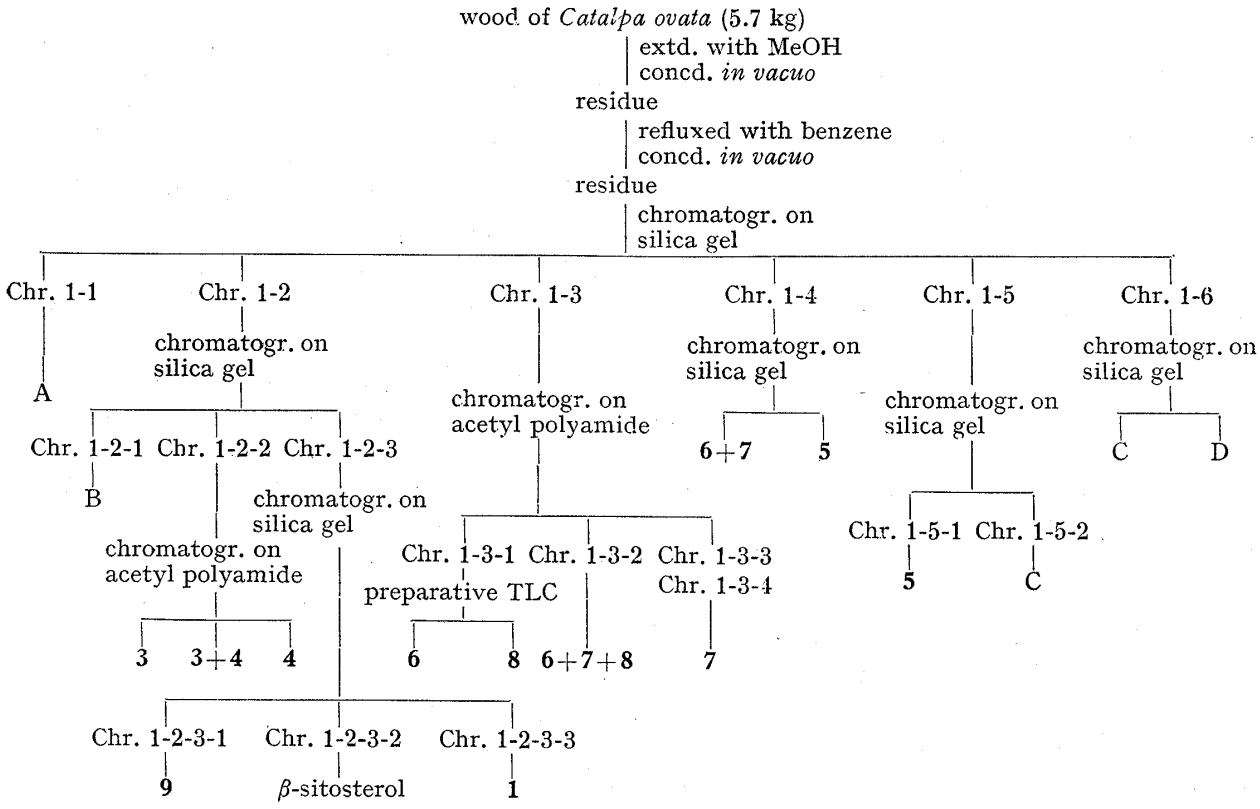
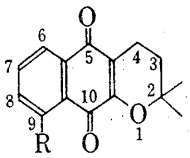



Chart 2. Separation of the Constituents of *Catalpa ovata*

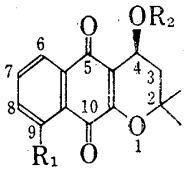
derivatives. For example, the methylation product of the mixture of **3** and **4** with diazomethane was separated clearly through chromatography on a silica gel column to the quinone (**3**) and the methyl ether (**5**) of compound **4**. On the other hand, acetylation of the mixture of **6** and **7** followed by column chromatography on silica gel resulted in the facile separation of monoacetate (**10**) of **6** and diacetate (**11**) of **7**.

We describe below the structure elucidation of compound **3** and so forth in due order, while that of **9** will be reported in the accompanying paper.

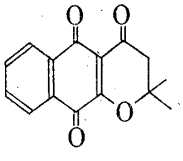
Compositions, melting points, specific rotations (if any) and principal infrared spectrum (IR) and ultraviolet spectrum (UV) absorptions of compounds (**3**)—(**8**) are shown



3 : R=H
4 : R=OH
5 : R=$OCH_3$
12 : R=OAc



6 : $R_1$=$R_2$=H
7 : $R_1$=OH, $R_2$=H
10 : $R_1$=H, $R_2$=Ac
11 : $R_1$=OAc, $R_2$=Ac
14 : $R_1$=H, $R_2$=Bz
16 : $R_1$=OBz, $R_2$=Bz



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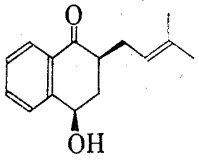

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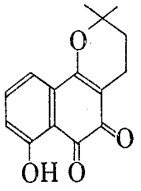

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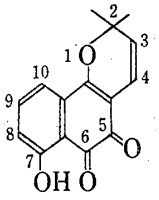

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Bż=benzoyl

Chart 3

in Table I, which indicates that they have physical properties characteristic of 1,4-naphthoquinone.

**α-Lapachone (3)**

The NMR spectrum of **3** shows an AB type four aromatic proton signals at δ 8.15—7.56 and a *gem*-dimethyl signal at δ 1.44. Signals due to the methylene protons appear as triplets at δ 2.63 and 1.81 ($J$=6 and 6 Hz). The nuclear magnetic resonance (NMR) data coupled with the properties shown in Table I indicate that **3** should be α-lapachone.[5] Indeed, the substance was identified with an authentic sample of α-lapachone.

**9-Hydroxy-α-lapachone (α-Dihydrocaryopterone) (4)**

Although the NMR spectrum of **4** resembles that of **3** suggesting the similarity between the structure of both compounds, the former has only three aromatic proton signals and instead an extra phenolic hydroxy proton signal at δ 11.76. In IR spectrum, compound **4** shows weak carbonyl absorption at 1675 $cm^{-1}$ along with a strong one at 1645 $cm^{-1}$, These spectral data suggest that **4** might have a hydrogen bonding between phenolic hydroxy and the carbonyl group. Its acetate (**12**), $C_{17}H_{16}O_5$, mp 176—180°, yellow needles, shows conversely strong absorption at 1670 $cm^{-1}$ and a relatively weak one at 1640 $cm^{-1}$. This phenomenon also supports the assumption described above. The ABC type signals of three aromatic protons of the acetate (**12**) at δ 8.13—7.18 further indicate that these protons locate adjacently on the benzene ring. On the other hand, work up of compound **4** with concentrated sulfuric acid gave red needles (**13**), $C_{15}H_{14}O_4$, mp 184—185°, whose spectral data differ greatly from those of **4**. For example, **13** shows UV absorption maxima at 254, 285 and 415 nm and strong IR carbonyl absorption at 1640 $cm^{-1}$ with a shoulder at 1660 $cm^{-1}$. Further, the NMR signal pattern of the aromatic protons of this compound (**13**) is remarkably different from that of **4**. These data indicate that **13** could be a β-naphthoquinone derivative. The persisting hydrogen bonded phenolic proton signal at δ 11.97 in its NMR spectrum shows, however, the survival of the carbonyl group of **4** at the peri position to the hydroxy group. These results led to the conclusion that substance **4** could be 9-hydroxy-α-lapachone. In fact, this substance was identified with α-dihydrocaryopterone,[6] the dihydro derivative of α-caryopterone occurring in *Caryopteris clandonensis* (Verbenaceae).

**9-Methoxy-α-lapachone (5)**

The NMR spectrum of compound **5** is very similar to that of **4** except the appearance of an extra signal at δ 3.96 due to a methoxy group on an aromatic ring and absence of any

5) A.R. Burnett and R.H. Thomson, *J. Chem. Soc.* (*C*), **1967**, 1261; M. Gates and D.L. Maesta, *J. Am. Chem. Soc.*, **70**, 614 (1948).
6) T. Matsumoto, C. Mayer and C.H. Eugster, *Helv. Chim. Acta*, **52**, 808 (1969).

hydroxy signal in the lower region below $\delta$ 7.80. Accordingly, **5** is supposed to be the methyl ether of compound **4**. This assumption was verified by the formation of **5** on methylation of **4**.

**4-Hydroxy-α-lapachone (6)**

As mentioned above, among several substances described in this paper, **6** was most difficult to isolate. We barely obtained a few mg of a thin-layer chromatographically homogeneous yellow syrup. Mass spectrum of this compound shows the molecular ion *m/e* 258. Acetylation product of this substance was also identical with **10**, $C_{17}H_{16}O_5$, mp 139.5—140.5°, $[\alpha]_D^{27}$ −14.2° (MeOH), which was obtained in a fairly good yield from the mixture of **6** and **7** through acetylation. Substance **10** shows UV absorption maxima at 245, 251, 284 and 336 nm and IR bands at 1740, 1685, 1650, 1620, 1600 (sh.) and 1580 $cm^{-1}$. Its NMR spectrum shows AB type signals at $\delta$ 8.22—7.58 due to four aromatic protons, a double doublet at $\delta$ 6.10 ($J$=3.5 and 4.5 Hz) due to a proton on an acetoxy bearing carbon atom and a doublet at $\delta$ 2.17 ($J$=3.5 Hz) assignable to methylene protons adjacent to this carbon atom, which is overlapped with a singlet at $\delta$ 2.09 of an alcoholic acetoxy group and finally two singlets at $\delta$ 1.57 as well as $\delta$ 1.53 of a *gem*-dimethyl group. Accordingly, **10** is the compound possessing an acetoxy group at C-4 of **3**, and hence the original quinone (**6**) is assumed to be 4-hydroxy-α-lapachone. The formation of **3** on hydrogenolysis of **10** is also compatible with this conclusion.

The absolute configuration of quinone (**6**) was determined by the application of the extended dibenzoate chirality rule[7] to the benzoate (**14**) derived from **6**. The circular dichroism (CD) spectrum (in MeOH) of the benzoate (**14**), $C_{22}H_{18}O_5$, mp 139—142° shows positive Cotton effect at 245 nm ($\Delta\varepsilon$=+13.4) and the negative one at 222 nm ($\Delta\varepsilon$=−7.7), while acetate (**10**) of quinone **6** does not show any Cotton effect in the region below 260 nm. Thus the Cotton effect of the benzoate (**14**) is considered to show the interaction of the two aromatic groups, that is, benzoyl and naphthoquinone moieties. As the sign of the first Cotton effect is positive, its chirality should be positive. Accordingly, the absolute configuration at C-4 might be assumed to be *S*.

This assumption was also supported by the Mills' rule.[8] Namely, as the difference $\Delta(M)_D$ in the molecular rotation between the benzoate (**14**) of **6** and **6** itself is (−62−71)=−133°, the C-4 of **6** could assume the configuration as those of L-glyceraldehyde series. Thus the conclusion described above was afreshly established.

**4,9-Dihydroxy-α-lapachone (7)**

The NMR spectrum of this compound shows a singlet at $\delta$ 11.73 due to a phenolic hydroxy group, signals due to three aromatic protons around $\delta$ 8.66—7.13, a triplet at $\delta$ 4.95 ($J$=6 and 6 Hz) due to a proton on a hydroxy bearing carbon atom, an alcoholic hydroxy multiplet at $\delta$ 3.75, a doublet at $\delta$ 2.08 ($J$=6 Hz) due to a methylene adjacent to the hydroxy bearing carbon and two singlets at $\delta$ 1.57 and 1.46 of a *gem*-dimethyl group. The IR spectrum of **7** shows a weak carbonyl absorption at 1685 $cm^{-1}$ and a strong one at 1645 $cm^{-1}$, while that of its acetate (**11**), $C_{19}H_{18}O_7$, mp 175—176°, shows conversely a strong absorption at 1680 $cm^{-1}$ and a relatively weak band at 1655 $cm^{-1}$. Therefore, a carbonyl group of compound **7** is supposed to be hydrogen bonded with the phenolic hydroxy group. The NMR spectrum of **11** shows an ABC type signal assignable to three aromatic protons at $\delta$ 8.15—7.23, a phenolic and an alcoholic acetoxy singlets at $\delta$ 2.45 and 2.08, a double doublet ($J$=3.5 and 4.5 Hz) at $\delta$ 6.08 due to a proton on an acetoxy bearing carbon and finally a doublet ($J$=3.5 Hz) at $\delta$ 2.12 due to methylene protons adjacent to this carbon. Work up of **7** with concentrated sulfuric acid gave red needles, $C_{15}H_{12}O_4$ (**15**), mp 155—158°. As in the case of **13**, both UV

7) N. Harada and K. Nakanishi, *Acc. Chem. Res.*, **5**, 257 (1972).
8) J.A. Mills, *J. Chem. Soc.*, **1952**, 4976.

and IR spectra revealed that this substance could be an *o*-quinone. Since a phenolic hydroxy proton singlet of this compound is still observed at $\delta$ 11.87, it is considered that the phenolic hydroxy group locates at the peri position to a carbonyl group, with which it is hydrogen bonded. The AB quartet at $\delta$ 6.63 and 5.73 assignable to two olefinic protons of **15** reveals that the conversion of **7** into *o*-quinone is accompanied with dehydration of the heterocyclic ring to give **15**. On the basis of these facts, it is concluded that **7** should be the 4-hydroxy derivative (4,9-dihydroxy-$\alpha$-lapachone) of **4**. Hydrogenolysis of **11** over palladised charcoal in ethanol yielding the acetate (**12**) of compound **4** and also the conversion of **15** into **4** under the same reaction condition verified this conclusion.

The CD spectrum (in MeOH) of the dibenzoate (**16**), $C_{29}H_{22}O_7$, mp 216—218°, shows positive Cotton effect at 240 nm ($\Delta\varepsilon=+2.7$) and negative one at 222 nm ($\Delta\varepsilon=-5.1$) indicating approximately the same CD spectral pattern as that of the benzoate of **6**, although the $\Delta\varepsilon$ values are small. The CD spectrum of **7** shows no Cotton effect in the region below 280 nm. Further, as the $\Delta(M)_D$ value is $(-296-49)=-345°$, the absolute configuration at C-4 of **7** is supposed to be *S* as in the case of **6**.

### 4-Oxo-$\alpha$-lapachone (8)

This substance **8** was found neither in the original extract nor in the fraction consisting of the mixture of **6** and **7**, while, it was first detected in an eluate of the acetylated polyamide column chromatography. It was also observed that 8 was gradually formed when powdery acetylpolyamide and the mixture of **6** and **7** were stirred in water. The NMR spectrum of **8** shows multiplets of four aromatic protons at $\delta$ 8.27—7.66, a singlet of methylene protons adjacent to a carbonyl group at $\delta$ 2.77 and another singlet of a *gem*-dimethyl group at $\delta$ 1.61. These data coupled with the features shown in Table I suggest that **8** would be formed by the oxidation of the alcoholic function of **6**.

## Experimental[9]

**Extraction of the Catalpa wood and Isolation of the Constituents**——*Catalpa* wood (5.7 kg) collected in Kyoto in October was cut into pieces and extracted with three 23-liters portions of hot MeOH. The extract was filtered and concentrated *in vacuo*. The residue was extracted eight times with 250 ml each of benzene under reflux for 1 hr. The combined benzene extracts were concentrated *in vacuo* yielding 40 g of dark brown residue. It was dissolved in $CH_2Cl_2$ and chromatographed on a silica gel column (750 g, 6.4×41 cm) eluting with the same solvent and 150 ml fractions were collected. Fractions (fr.) No. 1—6 (abbreviated as Chr. 1-1), fr. No. 7—25 (Chr. 1-2), fr. No. 26—54 (Chr. 1-3), fr. No. 55—61 (Chr. 1-4), fr. No. 62—68 (Chr. 1-5) and fr. No. 69—72 (Chr. 1-6) were combined and concentrated *in vacuo*, respectively, and residues of each combined fractions were further fractionated as follows:

Chr. 1-1: This fraction gave a colorless gelatinous residue A, 2.5 g, which has not been examined precisely.

Chr. 1-2: The residue of this fraction was rechromatographed on a silica gel column (500 g, 6.4×25 cm) eluting with benzene and 100 ml each of fraction was collected. Fractions No. 1—10 (Chr. 1-2-1) were combined and concentrated. The resulting residue was recrystallized from benzene affording 1.4 g of colorless granules, B, mp 72—73°, which has not been examined further. Fr. No. 11—17 (Chr. 1-2-2), indicating a yellow spot (*Rf* 0.57) on TLC, were combined and concentrated. The resulting residue was recrystallized from MeOH yielding 2.4 g of yellow needles, which were found to be a mixture of substance **3** and **4**. The mixture (75 mg) dissolved in a small amount of MeOH was poured into a column of acetylated

9) Melting points were determined on a Yanagimoto micro melting point apparatus and were uncorrected. NMR spectra were measured on a Varian A-60 spectrometer in $CDCl_3$ with trimethylsilane (TMS) as an internal standard. CD spectra were measured on a JASCO ORD-UV-6 spectrometer and optical rotations were determined on a Perkin-Elmer 141 polarimeter. Column chromatography was carried out on silica gel (Mallinckrodt) or acetylated polyamide prepared by standing a mixture of polyamide (Wako C-200, 100 g), pyridine and $Ac_2O$ (400 ml each) at room temperature overnight followed by the usual work up. Silica gel G acc. to Stahl (Merck) was used as the adsorbent for thin-layer chromatography (TLC). Unless otherwise specified, the developing solvent system was a mixture of benzene and AcOEt (8:2) and spots were detected by the yellow color of the naphthoquinone or by exposure to iodine vapor. The ratio of solvents used for chromatography was expressed in volume.

polyamide (20 g, 2.1×30 cm) and developed with water collecting 100 ml fractions. Fr. No. 11—15 indicating maximum absorption at 334 nm were combined and extracted with $(C_2H_5)_2O$, dried over anhyd. $MgSO_4$ and the solvent was removed. The residue was recrystallied from MeOH affording 8 mg of yellow needles (3), mp 117—118°, which were identified with an authentic sample of synthesized α-lapachone by mixed melting point and comparisons of IR and NMR spectra. *Anal.* Calcd. for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.15; H, 5.88. Fr. No. 16—24 indicating UV absorption at 334 nm together with visible absorption maximum at 410 nm were combined and worked up as mentioned above giving a mixture of **3** and **4**, yield 20 mg. Fr. No. 25—39 lacking UV absorption at 334 nm while indicating visible absorption at 410 nm were combined and worked up in the same way as mentioned above. Recrystallization of the residue from MeOH gave yellowish orange needles of α-dihydrocaryopterone (**4**), mp 120—122°. Yield 16 mg. This substance was identified with an authentic sample of α-dihydrocaryopterone by mixed melting point and comparison of IR and UV spectra. *Anal.* Calcd. for $C_{15}H_{14}O_4$: C, 69.76; H, 5.46. Found: C, 69.99; H, 5.47. NMR δ: 11.76 (s, phenol. OH), 7.63—7.08 (m, three arom. protons), 2.61 (t, $J$=6 and 6 Hz, 4-$H_2$), 1.81 (t, $J$=6 and 6 Hz, 3-$H_2$), 1.43 (s, $-\overset{-O}{\underset{|}{C}}(CH_3)_2$).

To another 100 mg aliquot of the above-described mixture of **3** and **4** was added an ethereal solution of $CH_2N_2$ and the mixture was left standing at room temperature overnight. After removal of the solvent, the residue was chromatographed on silica gel (15 g, 1.9×14 cm) eluting with benzene. Fractions indicating a spot of *Rf* 0.57 on TLC were combined and concentrated. Recrystallization of the residue from MeOH gave 36 mg yellow needles of α-lapachone (**3**), mp 117—118°. Fractions eluted successively after **3** showing a single spot of *Rf* 0.33 on TLC were combined and concentrated. The residue was recrystallized from MeOH to give 20 mg yellow needles of 9-methoxy-α-lapachone (**5**), mp 168—170°. Methylation of **4**, obtained in a pure state, with $CH_2N_2$ also gave **5** quantitatively. *Anal.* Calcd. for $C_{16}H_{16}O_4$: C, 70.58; H, 5.92. Found: C, 70.71; H, 5.78. NMR δ: 7.80—7.11 (m, three arom. protons), 3.96 (s, arom. $OCH_3$), 2.56 (t, $J$=6 and 6 Hz, 4-$H_2$), 1.78 (t, $J$=6 and 6 Hz, 3-$H_2$), 1.40 (s, $-\overset{-O}{\underset{|}{C}}(CH_3)_2$).

Fr. No. 18—31 (Chr. 1-2-3) were combined and concentrated. The residue was rechromatographed on silica gel (100 g, 3.5×27 cm) with a mixture of benzene and AcOEt (8:2) as eluent collecting 100 ml each fraction. Fr. No. 5—20 (Chr. 1-2-3-1) indicating a single spot of *Rf* 0.49 on TLC were combined and concentrated *in vacuo*. Distillation of the brownish oily residue under reduced pressure (0.13 mmHg) at 133—137° (bath temperature) gave catalponol (**9**) as a colorless oil. Yield 4.72 g. *Anal.* Calcd. for $C_{15}H_{18}O_2$: C, 78.23; H, 7.88. Found: C, 78.75; H, 7.66. $[\alpha]_D^{34}=+11°$ ($c$=1.2, MeOH), IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3450, 1680, 1600. UV $\lambda_{max}^{EtOH}$ nm(log ε): 248 (4.18), 291 (3.41). NMR δ: 8.11—7.28 (m, four arom. protons), 5.36—4.81 (m, $-\underset{H}{C}H(OH)-$, $>C=C<$), 1.71, 1.65 (s, $=C(CH_3)_2$).

Fr. No. 21—28 (Chr. 1-2-3-2) indicating a spot of *Rf* 0.38 on TLC were combined and concentrated. The residue was recrystallized from MeOH yielding 0.9 g of β-sitosterol as colorless scales, mp 139—140°. This substance was identified with an authentic sample of β-sitosterol by mixed melting point and the comparison of IR spectra. *Anal.* Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 83.76; H, 12.09. Conventional acetylation of this substance gave the acetate, mp 124—125°, which was identified with an authentic sample of β-sitosteryl acetate. *Anal.* Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found: C, 81.67; H, 11.58.

Fr. No. 32—58 (Chr. 1-2-3-3) showing a spot of *Rf* 0.36 on TLC were combined and concentrated. The residue was recrystallized from MeOH giving 8.3 g of catalpalactone (**1**) as colorless pillars, mp 111—112°, which were identified with the already reported catalpalactone in all respects. *Anal.* Calcd. for $C_{15}H_{14}O_4$: C, 69.76; H, 5.46. Found: C, 69.76; H, 5.65.

Chr. 1-3: This fraction was concentrated and the residue was recrystallized from MeOH giving 2.5 g of orange yellow needles, which were found to be a mixture of **6** and **7**. A 100 mg aliquot of the mixture was subjected to column chromatography on acetylpolyamide (20 g, 2.1×29 cm), in a similar manner as in the case of the separation of a mixture of **3** and **4**, with water as eluent collecting 50 ml fractions. Fr. No. 17—21 (Chr. 1-3-1) indicating UV absorption maxima at 247, 253, 264 and 288 nm were combined and extracted with benzene. Removal of the solvent gave 22 mg of the residue, which showed two spots of *Rf* 0.38 and 0.23 on TLC. The residue was subjected to preparative TLC and the band of *Rf* 0.38 was scratched and extracted with $CHCl_3$. Removal of the solvent gave 4 mg of 4-hydroxy-α-lapachone (**6**) as a yellow syrup. Mass spectrum *m/e*: 258 ($M^+$). The band of *Rf* 0.23 on the preparative TLC was also scratched and extracted with MeOH. Removal of the solvent gave 8 mg of the residue, which was recrystallized from MeOH giving 4-oxo-α-lapachone (**8**) as yellow granules, mp 163—165°.[10] *Anal.* Calcd. for $C_{15}H_{12}O_4$: C,

10) The formation of **8** on the chromatography was also verified in the following way. To a mixture of **6** and **7** (50 mg) in $H_2O$ (20 ml) was added acetylated polyamide (2 g) and the whole was stirred for 3 hr. After standing overnight, the mixture was extracted with benzene and the benzene extract was subjected to chromatography on silica gel (5 g, 1.3×10 cm) with the same solvent as eluent yielding 6 mg of **8** in pure state along with a mixture of **6** and **7**.

70.30; H, 4.72. Found: C, 70.20; H, 4.98. NMR $\delta$: 8.27—7.66 (m, 4×arom. protons), 2.77 (s, 3-$H_2$), 1.61 (s, $-\overset{|}{O}-C(CH_3)_2$).

A similar treatment of the combined fr. No. 22—23 (Chr. 1-3-2) of the acetylpolyamide column chromatography gave 5 mg of the residue, whose UV spectrum showed approximately the same absorption maxima as those of fr. No. 17—21 (Chr. 1-3-1), while that at 264 nm was relatively weak. Accordingly, this fraction is considered to be a mixture of **6**, **7** and **8**. The combined fr. No. 24—35 (Chr. 1-3-3) of the same chromatography was worked up in the same way giving 22 mg of the residue. Finally, elution of the acetylpolyamide column with MeOH gave fr. No. 36—39 (Chr. 1-3-4), which were combined and concentrated *in vacuo* yielding 22 mg of the residue. The UV spectra of these fractions (Chr. 1-3-3 and 1-3-4) did not show the absorption maxima at 264 nm, while those at 247, 252 and 290 nm were observed. Both residues were combined and recrystallized from MeOH giving orange yellow needles of 4,9-dihydroxy-α-lapachone (**7**), mp 130—131°. $[\alpha]_D^{25.5}$ +17.9° ($c$=1.01, MeOH). *Anal.* Calcd. for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15. Found: C, 65.88; H, 5.26.

On the other hand, the mixture consisting of **6** and **7** (200 mg) was acetylated by the usual method and the reaction product was extracted with $(C_2H_5)_2O$. The ethereal solution was washed with 5% HCl and $H_2O$, dried over anhyd. $MgSO_4$ and the solvent was removed. The residue was chromatographed on silica gel (40 g, 2.2×27.5 cm) eluting with a mixture of benzene and AcOEt (8: 2). Compound **10** and **11** whose *Rf* values on TLC were 0.51 and 0.45, respectively, were eluted in that order. Recrystallization of each substance from MeOH gave 77 mg of orange pillars (**10**), mp 139.5—140.5°, and 122 mg of yellow pillars (**11**), mp 175—176°, respectively. 4-Acetoxy-α-lapachone (**10**), $[\alpha]_D^{27}$ −14.2° ($c$=1.03, MeOH). *Anal.* Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 67.89; H, 5.51. UV $\lambda_{max}^{EtOH}$ nm(log $\varepsilon$): 245 (4.85), 251 (4.87), 284 (4.71), 336 (3.99). Acetylation of pure compound **6** also yielded this compound (**10**). 4,9-Diacetoxy-α-lapachone (**11**), $[\alpha]_D^{27}$ −14.0° ($c$=0.52, MeOH). *Anal.* Calcd. for $C_{19}H_{18}O_7$: C, 63.68; H, 5.06. Found: C, 63.77; H, 5.17. IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 1765, 1735, 1680, 1655, 1625, 1595. UV $\lambda_{max}^{EtOH}$ nm(log $\varepsilon$): 245 (sh.) (4.64), 250 (4.66), 280 (4.50), 346 (3.95). NMR $\delta$: 8.08 (q, $J$=7.5 and 1.5 Hz, 6-H), 7.74 (t, $J$=7.5 and 7.5 Hz, 7-H), 7.32 (q, $J$=7.5 and 1.5 Hz, 8-H), 1.53 and 1.50 (each s, $-\underset{-O}{C}(CH_3)_2$).

Chr. 1-4: This fraction was chromatographed on silica gel (60 g, 2.5×32 cm) eluted with a mixture of benzene and AcOEt (8: 2) and 50 ml fractions were collected. A mixture of **6** and **7** (fr. No. 11—23) (0.3 g) was eluted at first, and then fractions (No. 24—36) showing the main spot of *Rf* 0.33 followed. The combined residue of the latter fractions was recrystallized from MeOH to give 1.25 g of yellowish orange needles. mp 168—170°, which were identical with 9-methoxy-α-lapachone (**5**) in all respects.

Chr. 1-5: This fraction was rechromatographed on a silica gel column (100 g, 3.5×23.5 cm) with benzene as eluent collecting 100 ml each of fractions. Fr. No. 65—85 (Chr. 1-5-1) were combined and concentrated. The residue was further chromatographed on silica gel (20 g, 1.8×17 cm) eluting with $CHCl_3$ and 20 ml fractions were collected. Fr. No. 6—9 were combined and concentrated. The residue was recrystallized from MeOH giving yellowish orange needles, **5**. Yield 0.61 g. After evaporation of the combined fractions No. 103—135 (Chr. 1-5-2), the residue was recrystallized from benzene affording 0.25 g of substance C as colorless plates, mp 94—95°, which showed a spot of *Rf* 0.34 on TLC ($CHCl_3$–MeOH 98: 2). This substance was left unexamined.

Chr. 1-6: This fraction was concentrated. The residue was dissolved in $CHCl_3$ and the insoluble material was filtered off. The filtrate was concentrated to dryness and recrystallized from benzene giving another 0.06 g of substance C. The mother liquor was concentrated and the residue was chromatographed on silica gel (40 g, 2.5×20 cm) with $CHCl_3$ as eluent collecting 25 ml fractions. Combined fr. No. 41—46 and the mother liquor of substance C from Chr. 1-5-2 were rechromatographed on silica gel (30 g, 2.1×24.5 cm) eluted with $CHCl_3$ and 20 ml each of the fraction was collected. Fr. No. 9—21 indicating a yellow spot of *Rf* 0.65 on TLC ($CHCl_3$–MeOH 98: 2) were combined and concentrated to give 1.08 g of a dark brown residue D, which was also left unexamined.

**9-Acetoxy-α-lapachone (12)**——Substance **4** (30 mg) dissolved in 0.3 ml each of $Ac_2O$ and pyridine was left standing overnight. The reaction mixture was poured into ice water and extracted with $CH_2Cl_2$. The $CH_2Cl_2$ layer was washed with 5% HCl and $H_2O$ successively and the solvent was removed *in vacuo*. The residue was recrystallized from MeOH giving substance **12** as yellow needles, mp 176—180°. Yield 25 mg. *Anal.* Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 68.07; H, 5.65. IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 1765, 1670, 1640, 1615, 1590. UV $\lambda_{max}^{EtOH}$ nm(log $\varepsilon$): 250 (4.32), 280 (4.09), 344 (3.43). NMR $\delta$: 8.05 (q, $J$=7.0 and 1.5 Hz, 6-H), 7.69 (t, $J$=7.0 and 7.0 Hz, 7-H), 7.28 (q, $J$=7.0 and 1.5 Hz, 8-H), 2.61 (t, $J$=6.0 and 6.0 Hz, 4-$H_2$), 2.45 (s, arom. OAc), 1.80 (t, $J$=6.0 and 6.0 Hz, 3-$H_2$), 1.41 (s, $-\underset{-O}{C}(CH_3)_2$).

**7-Hydroxy-β-lapachone (13)**——Conc. $H_2SO_4$ (2 ml) was added dropwise to **4** (80 mg). After 3 min ice water was added to the mixture and the resulting precipitates were subjected to chromatography on silica gel (15 g, 1.6×15 cm) eluting with $CH_2Cl_2$. Fractions giving an orange red spot of *Rf* 0.42 on TLC were combined and concentrated. The residue was recrystallized from MeOH giving rise to red needles, **13**, mp 184—185°. Yield 62 mg. *Anal.* Calcd. for $C_{15}H_{14}O_4$: C, 69.76; H, 5.46. Found: C, 70.01; H, 5.57.

IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1660 (sh.), 1640, 1590. UV $\lambda_{max}^{(C_2H_5)_2O}$ (log $\varepsilon$): 254 (4.33), 285 (3.83), 415 (3.69). NMR $\delta$: 11.97 (s, phenol. OH), 7.68—6.95 (m, 3×arom. protons), 3.58 (t, $J$=6.0 and 6.0 Hz, 4-$H_2$), 1.84 (t, $J$=6.0 and 6.0 Hz, 3-$H_2$), 1.40 (s, $-\underset{\underset{-O}{|}}{C}(CH_3)_2$).

**Hydrogenolysis of 4-Acetoxy-α-lapachone (10)**——**10** (20 mg) was dissolved in EtOH (3 ml) and stirred for 20 min under $H_2$ atmosphere over Pd–C prepared from 10% $PdCl_2$ (0.2 ml) and activated charcoal (Darco, 20 mg). The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was recrystallized from MeOH to give 8.5 mg of yellow needles, mp 117—118°, which were identified with an authentic sample of α-lapachone (**3**) by mixed melting point and comparisons of IR and NMR spectra. *Anal.* Calcd. for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.09; H, 5.76.

**7-Hydroxy-3,4-dehydro-β-lapachone (15)**——Conc. $H_2SO_4$ (0.5 ml) was added dropwise onto **7** (20 mg). After 3 min ice water was added to the mixture, the resulting precipitate being collected by filtration and subjected to column chromatography on silica gel (20 g, 1.6×21 cm) eluted with a mixture of benzene and AcOEt (8: 2). Fractions indicating an orange red spot of *Rf* 0.66 on TLC were combined and concentrated. The residue was recrystallized from MeOH giving red needles, **15**, mp 155—158°. Yield 10 mg. *Anal.* Calcd. for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72. Found: C, 70.30; H, 4.82. IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1675 (sh.), 1645, 1625, 1580. UV $\lambda_{max}^{EtOH}$ nm(log $\varepsilon$): 245 (4.05), 283 (3.95), 440 (3.46). NMR $\delta$: 11.87 (s, phenol. OH), 8.11—7.10 (m, 3×arom. protons), 6.63 (d, $J$=10 Hz, 4-H), 5.73 (d, $J$=10 Hz, 3-H), 1.57 (s, $-\underset{\underset{-O}{|}}{C}(CH_3)_2$).

**Hydrogenolysis of 4,9-Diacetoxy-α-lapachone (11)**——**11** (30 mg) was dissolved in EtOH (5 ml) and stirred under $H_2$ atmosphere for 30 min over Pd–C prepared from 10% $PdCl_2$ (0.3 ml) and activated charcoal (Darco, 30 mg). After removal of the catalyst by filtration, the filtrate was concentrated *in vacuo*. The residue was recrystallized from MeOH giving yellow needles, mp 176—180°, yield 15 mg, which were identified with an authentic sample of 9-acetoxy-α-lapachone (**12**) by mixed melting point and comparisons of IR and NMR spectra. *Anal.* Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 67.83; H, 5.34.

**Catalytic Reduction of 7-Hydroxy-3,4-dehydro-β-lapachone (15)**——**15** (18 mg) was dissolved in abs. EtOH (5 ml) and stirred under $H_2$ atmosphere for 15 min over Pd–C prepared from 10% $PdCl_2$ (0.3 ml) and activated charcoal (Darco, 30 mg). The catalyst was filtered off and the filtrate was concentrated *in vacuo* yielding 17 mg of crude crystals, which were recrystallized from MeOH to give orange yellow needles, mp 120—122°. This substance was identified with an authentic sample of 9-hydroxy-α-lapachone (**4**) by mixed melting point and comparisons of IR and NMR spectra. *Anal.* Calcd. for $C_{15}H_{14}O_4$: C, 69.76; H, 5.46. Found: C, 70.03; H, 5.62.

**Benzoate of 6 and 7, 4-Benzoyloxy-α-lapachone (14) and 4,9-Dibenzoyloxy-α-lapachone (16)**——To a solution of the mixture consisting of **6** and **7** (25 mg) in pyridine (0.5 ml) was added benzoyl chloride (50 mg) and the whole was heated until the resulting crystals were dissolved. To the reaction mixture, in which crystals separated again on cooling, was added $H_2O$ (2 ml) in order to decompose excess benzoyl chloride and the resulting yellow precipitates were collected by filtration and recrystallized from MeOH giving 32 mg of yellow needles. They were subjected to preparative TLC giving two kinds of benzoates, 4-benzoyloxy-α-lapachone (**14**) (2 mg) and 4,9-dibenzoyloxy-α-lapachone (**16**) (19 mg). Recrystallization of both substances from MeOH gave yellow needles, respectively. **14**, mp 139—142°. Mass spectrum *m/e*: 362 ($M^+$). $[\alpha]_D^{23}$ −17.2° ($c$=0.57, MeOH). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1715, 1682, 1650, 1615, 1600 (sh.). UV $\lambda_{max}^{EtOH}$ nm(log $\varepsilon$): 234 (4.41), 241 (sh.) (4.40), 251 (4.36), 279 (sh.) (4.20), 283 (4.21), 334 (3.58). NMR $\delta$: 8.23—7.18 (m, 9 arom. protons), 6.40 (dd, $J$=3.5 and 4.5 Hz, $-\underline{CH}-OCOC_6H_5$), 2.27 (m, 3-$H_2$), 1.58 (s, $-O-\underset{\underset{(CH_3)_2}{|}}{C}-$). **16**, mp 216—218°. *Anal.* Calcd. for $C_{29}H_{22}O_7$: C, 72.19; H, 4.60. Found: C, 72.26; H, 4.57. $[\alpha]_D^{23}$ −61° ($c$=0.11, MeOH). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1730, 1715, 1680, 1650, 1590. UV $\lambda_{max}^{EtOH}$ nm(log $\varepsilon$): 233 (4.62), 248 (sh.) (4.42), 277 (4.18), 344 (3.46). NMR $\delta$: 8.62—7.32 (m, 13×arom. protons), 6.37 (dd, $J$=3.5 and 4.5 Hz, 4-H), 2.25 (m, 3-$H_2$), 1.53 (s, $-\underset{\underset{-O}{|}}{C}(CH_3)_2$). **16** was also prepared from pure compound **7** in the same way as described above.

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