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Inhibitory Effects of Lapachol Derivatives on Epstein-Barr Virus Activation

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Abstract—Sixteen derivatives (**2**–**17**) synthesized from the naphthoquinone lapachol (**1**), were tested for their inhibitory effects on Epstein–Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), as a test for potential cancer chemopreventive agents. They exhibited a variety of inhibitory activities from very high to moderate, which allow us to suggest structure–activity relationships. Ten of these derivatives are reported for the first time, their structures being thoroughly determined by spectroscopic methods.

Introduction

The quinone structure is common to numerous natural products, and it is associated with anticancer, antibacterial, antimalarial, and fungicide activities.[1] The biological activity is frequently related to the ability of quinones to accept one or two electrons to form the corresponding radical anion or dianion species. Sometimes the activity is due to the acid-base properties of the compounds. The cytotoxicity of quinones is often attributed to DNA modification.[1] Recent works point out that some naphthoquinone derivatives are inactivators of human cytomegalovirus protease,[2] whereas other derivatives show HRV3C-protease inhibitory activity.[3] Cancer chemoprevention is regarded as a promising avenue for cancer control, and has become increasingly important in recent years.[4] In this sense, inhibitory effects on Epstein–Barr virus early antigen (EBV-EA) activation have already been reported for more than 50 quinones,[5–10] including some naphthoquinones such as vitamin K.

All these biological data, along with their structural characteristics, point towards 1,4 and 1,2- naphthoquinones being privileged structures.[11]

In the present paper, we evaluate 16 naphthoquinones for their anti-tumor-promoting activity, in an in vitro assay on EBV-EA activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as the promotor.

The compounds (**2**–**17**) were synthesized from lapachol (**1**), a prenylnaphthoquinone isolated from the wood of *Tabebuia impetiginosa* (Bignoniaceae).[12] Species that contain lapachol and several biogenetically related naphthoquinones (e.g., *tahaebo, pau d'arco* and *lapacho roxo*) are widely used in American folk medicine for the treatment of cancer, lupus, infections, wounds, and many other diseases.[13] The results of the present investigation indicate that some of the lapachol analogues might be valuable as potent cancer chemopreventive agents.

Results and Discussion

Most of the derivatives used in this work were obtained by reactions of acylation, cyclization or modifications on the side chain of lapachol (**1**) (see Scheme 1).

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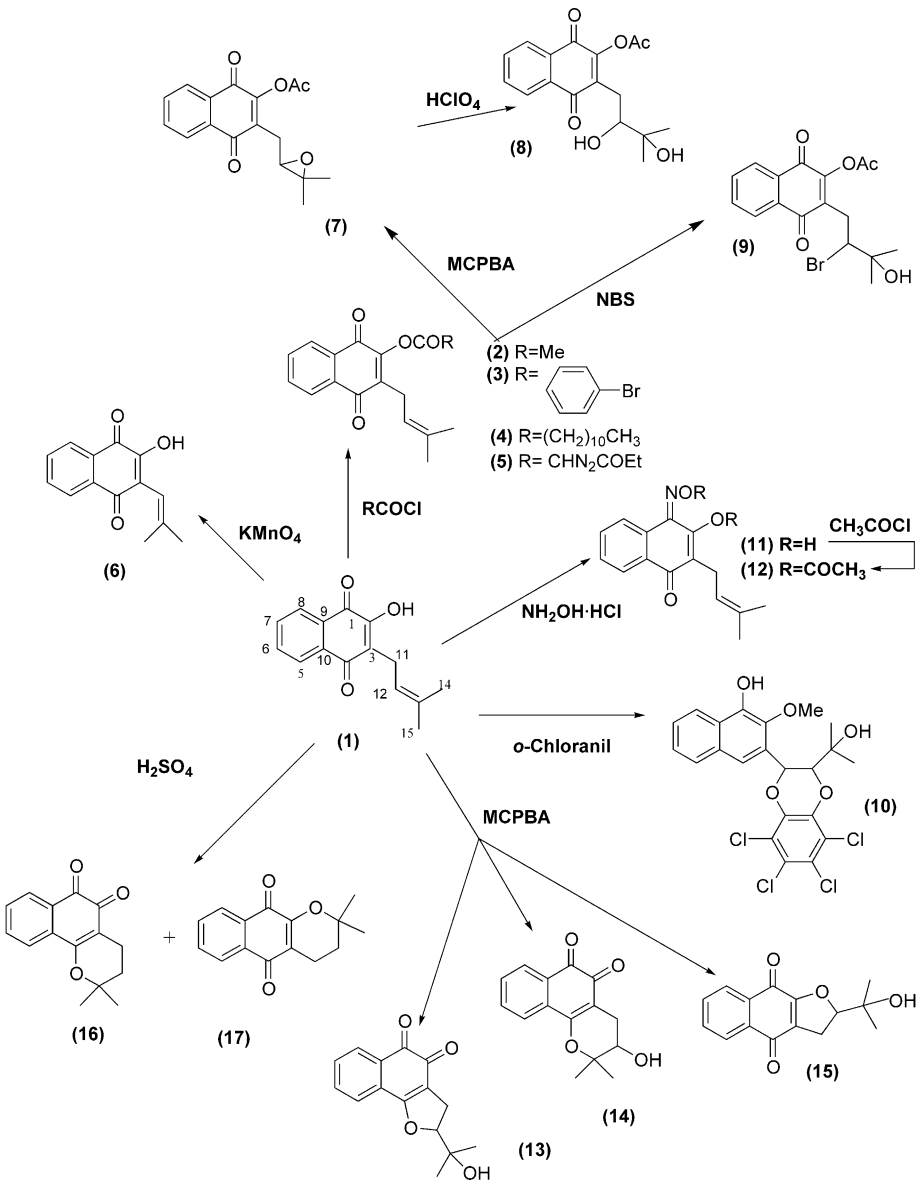


Scheme 1.

Modifications of the C-2 hydroxy group: introduction of –COR

The derivatives **2**, **3**, **4** and **5** were obtained by treating compound (**1**) with a variety of acylating agents of different nature and lipophilic character (acetyl chloride, *p*-bromo-benzoyl chloride, lauroyl chloride and diazo-malonyl chloride[14]) using a small excess of acylating agents, dry CH_2Cl_2 and lutidine as base. Spectroscopic data of the new compounds (**4** and **5**), whose structures were rigorously elucidated and have not been previously reported in the literature, are showed in the Experimental.

Derivatives **3**, **4** and **5** showed activities similar to those of lapachol, while the acetyl derivative **2** turned out to be more active than **1**. It is even more effective than β-carotene, a vitamin A precursor that has been widely studied in cancer prevention using animals[15] (see Table 1). Compound **2** has strong anti-tumor promoting activity, even at 10 mol ratio/TPA (100% inhibitory activity at 1000 mol ratio/TPA, and more than 75 and 40% even at 500 and 100 mol ratio/TPA, respectively), and preserved high viability of Raji cells (more than 70% at 10–1000 mol ratio/TPA).

These data suggest that replacing a hydrogen-bond donating substituent on C-2 with an hydrogen-bond acceptor substituent increases the activity when the acyl group does not have more than two carbons. There is always the chance that compound **2** could be a more potent lipophilic prodrug of **1** instead of being active in its own right. But we do not have enough arguments to support this possibility.

Modifications on the side chain

Compound **6** was obtained by Hooker's oxidation.[16] This compound showed an important inhibitory activity (10.7% inhibition of induction at 10 mol ratio/TPA).

Table 1. Percentage of Epstein–Barr virus early antigen (EBV-EA) induction in the presence of compounds **1**–**17**

Compd	Concentration (mol ratio/TPA)[a]			
	1000	500	100	10
1	17.5[b] (70)	44.6	63.6	97.8
2	0 (70)	22.7	56.0	82.1
3	21.3 (70)	48.0	71.9	100
4	17.3 (60)	49.5	76.9	100
5	16.9 (60)	46.7	72.5	100
6	2.5 (70)	33.8	61.3	89.3
7	4.7 (70)	35.2	62.4	90.2
8	5.2 (70)	38.5	63.7	91.7
9	14.7 (60)	45.9	71.6	100
10	23.6 (60)	50.3	73.9	100
11	19.9 (70)	47.8	68.5	100
12	20.7 (70)	49.7	73.7	100
13	10.5 (60)	42.2	66.2	94.8
14	12.3 (60)	40.6	63.9	92.9
15	7.7 (60)	40.8	65.0	92.5
16	15.7 (60)	43.1	68.2	96.3
17	14.6 (60)	41.7	64.0	94.7
β-Carotene	8.6 (70)	34.2	82.1	100

Values in parantheses represent viability percentages of Raji cells; unless otherwise stated, the viability percentages of Raji cells were more than 80%.
[a]Mol ratio/TPA (32 pmol = 20 ng/mL), 1000 mol ratio = 32 nmol, 500 mol ratio = 16 nmol, 100 mol ratio = 3.2 nmol, and 10 mol ratio = 0.32 nmol.
[b]Values represent percentages of EBV-EA induction in the presence of the test compound relative to the positive control (100%).

The shortening of the side chain produces a structure flatter than that of lapachol **(1)**, and this effect could explain the observed increase in activity.

We also modified the double bond of the lateral chain of the derivative **2**, which is already more potent than lapachol. **(2)** was treated with MCPBA to obtain the corresponding (±) epoxy derivative **7** in 60% yield, which under treatment with $HClO_4$ in catalytic amounts afforded the compound **8** in 96% yield.

In addition, the reaction of **2** with NBS yielded the hydroxy-halogenated compound **9** in 94% yield. All modifications of the side chain resulting in the formation of **7**, **8** and **9** increase the inhibitory activity with respect to lapachol **1**, but not with respect to the acetylated compound **2**. The substitution of the double bond on the isoprenyl side chain for different functionalities resulted in a modest decrease in inhibitory activities, which indicates that the presence of the double bond in 1,4-naphthoquinones derivatives is not essential to achieve inhibitory activity.

Spectroscopic data of the new compounds **7**, **8** and **9** are shown in the Experimental.

Compound **10** was formed by treating **1** with chloranil. This compound has a naphthol-type structure whose lateral chain has been possibly produced by a hetero Diels–Alder reaction between the *o*-dicarbonyl system of chloranil and the double bond C_{12}–C_{13}. At present, we are not able to suggest a mechanism to explain the formation of this compound in detail. The structure of **10** has been rigorously determined by NMR homo and heteronuclear experiments (COSY, ROESY, HSQC and HMBC).

Modifications on the C-1 carbonyl

The reaction of compound **1** with hydroxylamine hydrochloride produced compound **11** in a regioselective form. This fact can be explained analyzing the different resonance structures (Scheme 2) where the carbonyl group on C-4 can 'pull' electrons from the oxygen atom located on C-2.

The structure of **11** was confirmed by analysis of the HMBC spectrum, which showed three bond couplings between the hydrogens H-11 and the carbonyl group at δ 184.2.

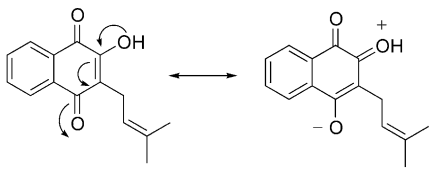
Compound **11**, under acetylating conditions, yielded the corresponding acetyl derivative **12**. **11** and **12** proved to be less active than compound **1**, which indicates that the carbonyl group plays an important role for the activity.

Tricyclic derivatives

Treatment of **1** with MCPBA via an epoxide intermediate yielded several orthoquinones: the angular o-dihydrofuran derivative **13**, the angular pyran derivative **14**, and the linear dihydrofuran 1,4-naphthoquinone **15**. Derivative β-lapachone **16**, and its α-isomer derivative **17** were obtained under treatment with diluted H_2SO_4 via the corresponding cationic intermediate. Table 1 shows the results obtained when the derivatives **13**–**17** were biologically evaluated. All tricyclic compounds were more active than lapachol, being the compounds that present five-membered rings more effective that the corresponding derivatives with six-membered rings. With respect to the series of six-membered rings, the most active was the one with an hydroxy group (**14**). The compounds **13**, **14** and **16** have a chromophoric *ortho*-quinonic system structurally different from that of the lapachol. In these compounds, the degrees of freedom of the side chain are limited and these molecules are practically planar. These planar molecules turned out to be slightly more active than lapachol.

The best result in the tricyclic series was obtained with the derivative **15**, which presents the 1,4-naphthoquinone system.

In short, from the 16 derivatives, compounds **2**, **6**, **7**, **8** and **15** were more active than the precursor lapachol **1**, being also more effective than the product of reference in these type of studies, β-carotene. All these compounds conserve the original 1,4-naphthoquinone system, which seems to be important to produce high



Scheme 2.

inhibitory activity. Derivatives **4**, **5**, **9**, **13**, **14**, **16** and **17** turned out to be more active than lapachol but less active than β-carotene. Lower activities were obtained with the derivatives **11** and **12,** which possess an oxime group on C-1 instead of the corresponding carbonyl function. The voluminous benzoate **3** and the naphthol **10** also rendered poor results. In this case the increase of size of the molecule may be affecting the inhibitory activity.

Based on all these results, the preparation of new derivatives as valuable chemopreventive agents is in progress.

Experimental

General

IR spectra were recorded in $CDCl_3$ on a Bruker IFS 55 spectrophotometer. ^1H and ^{13}CNMR spectra were recorded on a Bruker at 300 and 75 MHz, respectively, with TMS as internal reference. The 2D NMR experiments were run on a Bruker at 400 MHz. EI/MS and HR-EI/MS were recorded on a Micromass Autospec. TLC 1500/LS 25 Schleicher and Schuell foils were used for thin-layer chromatography, while silica gel (0.2–0.63 mm) and Sephadex LH-20 were used for column chromatography. Lapachol was isolated from *Tabebuia wood* (Bignoniaceae).[12]

Bioassays

Chemicals. The cell culture reagent and *n*-butyric acid were purchased from Nacali Tesque, Inc. (Kyoto, Japan). TPA was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

In vitro EBV-EA induction effect. The EBV genome-carrying lymphoblastoid cells, Raji cells, derived from Burkitt's lymphoma, were cultivated in RPMI-1640 medium. The Raji cells were incubated for 48 h at 37 °C in a medium containing *n*-butyric acid (4 mmol), TPA (32 pmol), and various amounts of test compounds. Smears were made from the cell suspension, and the EBV-EA-inducing cells were stained by means of an indirect inmunofluorescence technique. The details of the in vitro assay have been reported previously.[17,18]

Acetate of lapachol (2). 182 mg of **(1)** were treated with acetyl chloride (1.8 equiv), lutidine (2 equiv) in CH_2Cl_2 at 0 °C for 5 min. The crude product was treated with diluted HCl, the organic layer was washed with H_2O and separated. The aqueous layer was extracted several times with CH_2Cl_2. The combined organic extracts were washed with brine and dried over $MgSO_4$. After solvent removal the residue was purified by flash chromatography (silica gel, 9:1, hexanes/EtOAc) to yield 213 mg of compound **2** (99.7%), which showed identical spectroscopic data to those reported in the literature.[19]

***p*-Bromobenzoate of lapachol (3).** Following the procedure described above, 36 mg of **(1)** (0.14 mmol), were treated with 1.8 equiv of 4-bromo benzoyl chloride and 2 equiv of lutidine. After purification by flash chromatography (silica gel, 9:1, hexanes/EtOAc), 57 mg of compound **(3)** (95.7%) was obtained. **(3)** showed identical spectroscopic data to those reported in the literature.[19]

Lauroate of lapachol (4). Following the procedure described above, 70 mg (0.28 mmol) were treated with 1.5 equiv of lauroyl chloride and 2.5 equiv of lutidine in 5 mL of dry CH_2Cl_2 at 0 °C for 5 min. The product was purified by Sephadex LH-20 column (2:1:1 hexanes/MeOH/$CHCl_3$), yielding 93 mg (100%) of compound **(4).** ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.08 (m, 2H), 7.71 (m, 2H), 5.07 (t, $J=7.2$ Hz, 1H), 3.27 (d, $J=7.2$ Hz, 2H), 2.66 (t, $J=7.4$ Hz, 2H), 1.8–1.7 (m, 2H), 1.76 (s, 3H), 1.68 (s, 3H), 1.54 (bs, 14H), 1.45 (m, 2H), 0.88 (m, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 184.4 (s), 178.4 (s), 170.8 (s), 150.9 (s), 138.2 (s), 134.6 (s), 134.0 (d), 133.7 (d), 132.1 (s), 130.9 (s), 126.6 (d), 126.5 (d), 118.5 (d), 33.8 (t), 31.9 (t), 29.6 (t), 29.4 (t), 29.2 (×2) (t), 29.0 (t), 25.7 (q), 24.8 (t), 24.2 (t), 23.6 (t), 22.6 (t), 17.9 (q), 14.1 (q). EI HR-MS *m/z*: 424.2633 [calcd for $C_{27}H_{36}O_4$ (M^+) 424.2614]. IR ($CHCl_3$) ν_{max}: 2922, 2853, 2360, 1774, 1678, 1637, 1596, 1463, 1377, 1330, 1295, 1178, 1143, 1096, 949, 720 cm^{-1}.

Diazomalonate of lapachol (5). Following the procedure described for compounds **2**–**4**, 169 mg (0.69 mmol) of **1** were treated with 1.5 equiv of diazomalonyl chloride[14] and 3.5 equiv of lutidine, in dry CH_2Cl_2 for 30 min. The product was purified by flash chromatography (silica gel, 7:3, hexanes/EtOAc) to yield 136 mg (51%) of compound **5**. ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.07 (m, 2H), 7.71 (m, 2H), 5.07 (t, $J=7.2$ Hz, 1H), 4.34 (q, $J=7.1$ Hz, 2H), 3.29 (d, $J=7.2$ Hz, 2H), 1.73 (s, 3H), 1.66 (s, 3H), 1.33 (t, $J=7.1$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): 184.1 (s), 178.1 (s), 160.1 (s), 157.8 (s), 156.9 (s), 150.1 (s), 138.9 (s), 135.0 (s), 134.2 (d), 133.9 (d), 132.0 (s), 130.7 (s), 126.8 (d), 126.6 (d), 118.2 (d), 63.1 (t), 25.6 (q), 23.7 (t), 17.8 (q), 14.1 (q). EI–MS (*m/z*): 382 (M^+, 6.4), 339 (M^+-CO_2, 10.5), 241 (lapachol, 100). EI HR-MS *m/z* 382.1185 [calcd for $C_{20}H_{18}O_6N_2$ (M^+) 382.1165]. IR ($CHCl_3$) ν_{max} 2982, 2933, 2148, 1770, 1676, 1640, 1594, 1447, 1373, 1321, 1259, 1181, 1080. 1052, 978, 948, 851, 754, 715 cm^{-1}.

2-Hydroxy-3(1′-isobutenyl)-1,4-naphthoquinone (6). 50 mg of **(1)** in 2 mL of NaOH (1%) solution were treated with 0.5 mL of $KMnO_4$ (10%) for 24 h. The reaction mixture was filtered to separate the MnO_2 formed, and the filtrate was acidified with diluted HCl (10%), and an orange solid appeared. This solid was separated by filtration and purified by flash chromatography (silica gel, 7:3, hexanes/EtOAc) to yield 16 mg (35%) of compound **(6)**, recovering 22 mg (44%) of unreacted compound **(1)**. ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.09 (m, 2H), 7.72 (m, 2H), 7.51 (s, 1H), 6.00 (s, 1H), 1.99 (s, 3H), 1.68 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 184.7 (s), 181.6 (s), 151.1 (s), 143.6 (s), 134.9 (d), 133.0 (d), 129.5 (s), 126.9 (d), 126.1 (d), 120.9 (s), 113.6 (d), 26.5 (q), 21.7 (q). EI HR-MS *m/z* 228.0788 (calcd for $C_{14}H_{12}O_3$ (M^+) 228.0786). IR ν_{max}: 3364, 2928, 1661, 1645, 1593, 1377, 1343, 1325, 1300, 1277, 1214, 1043, 891, 795, 729 cm^{-1}.

12,13-Epoxy lapachol acetate (7). 32 mg (0.11 mmol) of compound **(2)** was treated with 33.3 mg (1.2 equiv) of MCPBA at 0 °C for 26 h. The solvent was removed under vacuum and the corresponding residue was purified by Sephadex LH-20 column ($CHCl_3$/MeOH/hexanes, 1:1:2) to yield 20 mg (60%) of compound **7**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.10 (m, 2H), 7.74 (m, 2H), 2.90 (m, 2H), 2.75 (m, 1H), 2.39 (s, 3H), 1.39 (s, 3H), 1.28 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 184.4 (s), 177.9 (s), 167.8 (s), 152.5 (s), 135.6 (s), 134.2 (d), 134.0 (d), 131.9 (s), 130.9 (s), 126.8 (d), 126.7 (d), 61.9 (d), 59.0 (s), 24.5 (t), 24.5 (q), 20.5 (q), 18.9 (q). EI HR-MS m/z 300.1025 [calcd for $C_{17}H_{16}O_5$ (M^+) 300.0998]. IR ($CHCl_3$) ν_{max} 2958, 2930, 1778, 1680, 1639, 1597, 1451, 1424, 1379, 1340, 1333, 1305, 1174, 1153, 1090, 1055, 1014, 944, 861, 729 cm^{-1}.

12,13-Dihydroxy lapachol acetate (8). 54 mg of compound **(7)** were treated with 0.2 mL of diluted $HClO_4$ (5%) in a mixture of THF/H_2O at 0 °C for 21 h. The reaction was quenched with diluted $NaHCO_3$. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with brine and dried over $MgSO_4$. After solvent removal the residue was purified by flash chromatography (silica gel, 7:3, hexanes/EtOAc) to yield 55 mg (96%) of compound **(8)**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.09 (m, 2H), 7.73 (m, 2H), 3.56 (dd, J = 9.9 and 2.3 Hz, 1H), 2.83 (dd, J = 13.0 and 2.3 Hz, 1H), 2.69 (dd, J = 13.0 and 9.9 Hz, 1H), 2.39 (s, 3H), 1.29 (s, 3H), 1.27 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 185.4 (s), 177.8 (s), 168.4 (s), 152.2 (s), 137.3 (s), 134.2 (d), 134.0 (d), 131.9 (s), 130.8 (s), 126.8 (d), 126.7 (d), 72.9 (d), 29.1 (s), 27.3 (t), 25.9 (q), 24.0 (q), 20.4 (q). EI HRMS m/z 318.1122 [calcd for $C_{17}H_{18}O_6$ (M^+) 318.1103]. IR ($CHCl_3$) ν_{max} 3521, 2977, 1776, 1675, 1639, 1595, 1371, 1340, 1297, 1176, 1072, 1014, 946, 880, 756, 733 cm^{-1}.

12-Bromo, 13-hydroxy lapachol acetate (9). 262 mg of (**2**) were treated with 180 mg of NBS in 10 mL of tBuOH/H_2O 1:1. The reaction mixture was stirred for 2 h, then the solvent was removed to half of its volume and the mixture of the reaction was extracted with ether. The combined organic extracts were washed with brine and dried over $MgSO_4$. The product was purified by flash chromatography (silica gel, 7:3, hexanes/EtOAc) to yield 330 mg (94 mg) of compound (**9**). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.10 (m, 2H), 7.75 (m, 2H), 4.36 (dd, J = 10.7, 3.3 Hz, 1H), 3.28 (dd, J = 13.7, 3.3 Hz, 1H), 3.16 (dd, J = 13.7, 10.8 Hz, 1H), 2.41 (s, 3H), 1.47 (s, 3H), 1.46 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 184.5 (s), 177.8 (s), 167.7 (s), 158.4 (s), 136.0 (s), 134.2 (d), 134.0 (d), 131.9 (s), 130.9 (s), 126.8 (d), 126.7 (d), 72.7 (s), 64.2 (d), 28.5 (t), 27.0 (q), 25.2 (q), 20.5 (q). EI HR-MS m/z 380.0263 [calcd for $C_{17}H_{17}O_5Br$ (M^+) 380.0259]. IR ($CHCl_3$) ν_{max}: 3525, 2980, 1778, 1678, 1640, 1595, 1460, 1427, 1371, 1329, 1298, 1173, 1036, 1016, 948, 732, 701 cm^{-1}.

Preparation of compound 10. 69 mg (0.28 mmol) of chloranil in 3 mL of CH_3CN were treated with 3.3 equiv of lapachol **(1)** at rt for 14 h. The solvent was eliminated under vacuum and the corresponding residue was purified by flash chromatography (silica gel, 7:3, hexanes/EtOAc) to yield 12 mg (9%) of compound **10**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.15 (m, 2H), 7.49 (m, 2H), 6.80 (s, 1H), 5.73 (d, J = 2.9 Hz, 1H), 4.39 (d, J = 2.9 Hz, 1H), 3.92 (s, 3H), 1.80 (s, 3H), 1.47 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 149.8 (s), 142.6 (s), 139.8 (s), 137.1 (s), 126.7 (s), 126.4 (d), 126.2 (d), 125.4 (s), 125.1 (s), 124.0 (s), 122.1 (d), 121.7 (d), 120.5 (s), 120.2 (s), 107.9 (s), 100.0 (d), 76.7 (s), 72.3 (d), 68.2 (d), 55.5 (q), 24.3 (q), 23.3 (q). EI HR-MS m/z 503.9880 [calcd for $C_{22}H_{18}O_5Cl_4$ (M^+) 504.1912].

Preparation of compound 11. 57 mg of **1** in 10 mL of NaOH solution (5%) were treated with 22 mg of NH_2OH·HCl for 2 h. The reaction mixture was acidified with AcOH, and the resultant solid was filtered to yield 54 mg of **(9)** (91%). ^{1}H NMR (MeOD, 300 MHz) δ 9.01 (d, J = 7.5 Hz, 1H), 8.11 (d, J = 7.2 Hz, 1H), 7.63 (m, 2H), 5.21 (m, 1H), 3.23 (d, J = 7.0 Hz, 2H), 1.76 (s, 3H), 1.65 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 184.8 (s), 158.6 (s), 139.5 (s), 132.0 (d), 131.3 (s), 130.9 (s), 130.2 (d), 129.5 (d), 125.9 (d), 121.4 (d), 117.0 (s), 24.5 (t), 21.6 (q), 16.6 (q). EI HR-MS m/z 257.1052 [calcd for $C_{15}H_{15}O_3N$ (M^+) 257.1037]. IR ($CHCl_3$) ν_{max}: 2922, 2852, 2360, 2341, 1809, 1723, 1616, 1591, 1378, 1292, 1237, 1204, 951, 771 cm^{-1}.

Preparation of compound 12. 32 mg of **11** in 3 mL of CH_2Cl_2 at 0°C were treated with 2 equiv of lutidine and acetyl chloride (1.8 equiv) for 5 min. The crude was treated with diluted HCl, the organic layer was washed with H_2O and separated. The aqueous layer was extracted several times with CH_2Cl_2. The combined organic extracts were washed with brine and dried over $MgSO_4$. After solvent removal the residue was purified by chromatography on Sephadex LH-20 to yield 40 mg of compound (12) (97%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: (d, J = 7.5 Hz, 1H), 8.26 (d, J = 7.4 Hz, 1H), 7.67 (m, 2H), 5.08 (t, J = 7.2 Hz, 1H), 3.25 (d, J = 7.1 Hz, 2H), 2.38 (s, 6H), 1.75 (s, 3H), 1.66 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 183.8 (s), 168.5 (s), 167.4 (s), 152.1 (s), 144.4 (s), 133.9 (s), 133.4 (s), 133.2 (d), 131.9 (d), 131.4 (s), 130.9 (d), 127.7 (d), 126.1 (s), 119.2 (d), 25.7 (q), 23.5 (t), 20.6 (q), 19.7 (q), 17.9 (q). IR ($CHCl_3$) ν_{max}: 2915, 2360, 1778, 1633, 1591, 1371, 1290, 1177, 1005, 954, 928, 777 cm^{-1}.

Preparation of derivatives 13, 14 and 15. 355 mg of **1** were treated with 432 mg of MCPBA (1.2 equiv) in 10 mL of dry CH_2Cl_2 at rt for 24 h. The evolution of the reaction was followed by TLC, when compound **1** disappeared the mixture of reaction was treated with $NaHCO_3$ (5%), the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with brine and dried over $MgSO_4$. The product was purified by flash chromatography (silica gel, 4:1, hexanes/EtOAc) to yield 102 mg (27%) of compound **13**, 214 mg (57%) of compound **14** as a dark yellow solid, and 54 mg (14%) of compound **15**. The spectroscopic data were identical to the reported for the same compounds obtained from natural source.[20,21]

Preparation of compounds 16 and 17. 940 mg of **1** in 30 mL of water were treated with 3 mL of concd H_2SO_4 for 3 h. After this time it appeared as an orange solid, which was separated by filtration and washed with H_2O at 0 °C. The solid was purified by flash chromatography (silica gel, 9:1, hexanes/EtOAc) to yield 370 mg (94 mg) of compound **16** (39%), 318 mg (34%) of **17** and 246 mg of lapachol were recovered. The spectroscopic data were identical to those reported for the same compounds obtained from natural sources.[22,23]

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