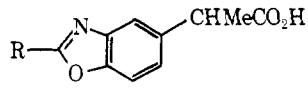
Table I. Activity of Compounds 1-8, Phenylbutazone, and Benoxaprofen in the Carrageenan-Induced Rat Paw Edema Test



Compd no.	R	Act. of test compd: Dose,[b] mg/kg × 2	Act. of test compd: % redn[c]	Act. of phenylbutazone[a]: Dose,[b] mg/kg × 2	Act. of phenylbutazone[a]: % redn[c]
1	2-Pyridyl	100	47	50	43
		50	36	50	32
2	3-Pyridyl	50	37	50	46
3	4-Pyridyl	50	15*	50	48
4	2-Thienyl	100	40	50	38
		50	24	50	32
5	5-Chloro-2-thienyl	50	34	50	43
6	2-Furyl	50	23	50	23
7	5-Chloro-2-furyl	100	46	50	39
		50	27	50	32
8	1-Methyl-2-pyrrolyl	50	17	50	39
Benoxaprofen	4-ClC_6H_4	50	78	50	61

[a]When tested at the same time as the test compound. [b]Doses were given orally at 3 and 0.5 hr prior to carrageenan. [c]The edema was measured 2.5 hr after the carrageenan injection. All of the results except the one marked * were significant ($p > 0.02$) on Student's t test.

tate (5.5 g, 0.026 mol) and 5-chloro-2-thiophenecarboxaldehyde (5.0 g, 0.031 mol) in toluene (75 ml) was heated under reflux and the H_2O which formed was removed in a Dean-Stark apparatus. The solvent was evaporated and the residue was dissolved in AcOH (100 ml). Lead tetraacetate (17 g, 0.043 mol) was added and the reaction mixture was stirred overnight at room temperature. H_2O (10 ml) was added and the solvent was removed under reduced pressure. The residue was extracted with CH_2Cl_2 and the organic solution was dried (Na_2SO_4) and treated with C. The solvent was evaporated and the residue was dissolved in warm EtOH (50 ml). A solution of NaOH (1.0 g, 0.026 mol) in H_2O (5 ml) was added and the resulting solution was stirred for 1 hr at room temperature. H_2O (150 ml) was added and, after a further 2 hr, the solution was concentrated to a small volume. The aqueous solution was extracted with $CHCl_3$ and acidified (pH 1) with concentrated HCl. The required product was extracted with $CHCl_3$ and the organic solution was dried (Na_2SO_4) and evaporated. The residue was recrystallized from toluene and EtOH–H_2O to give pure **5** (1.7 g, 19%): mp 185°. Anal. ($C_{14}H_{10}ClNO_3S$) C, H, Cl, N.

2-(2-Furyl)-α-methyl-5-benzoxazoleacetic Acid (6). This was made in a similar manner to compound 4. The acid was obtained as light cream crystals (19%), mp 160–162°, after recrystallization from Me_2CO and EtOH–H_2O. Anal. ($C_{14}H_{11}NO_4$) C, H, N.

2-(5-Chloro-2-furyl)-α-methyl-5-benzoxazoleacetic Acid (7). A mixture of ethyl 3-amino-4-hydroxy-α-methylbenzeneacetate (6.0 g, 0.029 mol), 5-chloro-2-thiophenecarboxaldehyde (5.5 g, 0.038 mol), sodium sulfate (20 g, 0.14 mol), and toluene was stirred for 2 hr at room temperature. The filtered solution was evaporated and the residue was oxidized and hydrolyzed as described for the preparation of **5**. This gave, after recrystallization from H_2O, pure **7** (15%): mp 161°. Anal. ($C_{14}H_{10}ClNO_4$) C, H, Cl, N.

α-Methyl-2-(1-methyl-2-pyrrolyl)-5-benzoxazoleacetic Acid (8). 3-Amino-4-hydroxy-α-methylbenzeneacetonitrile[1] (4.0 g, 0.025 mol) was condensed with 1-methyl-2-pyrrolecarboxaldehyde (2.5 g, 0.02 mol) and the product was oxidized using the conditions described for the preparation of **5**. The resulting α-methyl-2-(1-methyl-2-pyrrolyl)-5-benzoxazoleacetonitrile (4.4 g, purity approximately 80% by NMR) and concentrated HCl (50 ml) were stirred at 100° for 1.5 hr, treated with C, and filtered. The solution was evaporated under reduced pressure and the residue was dissolved in 2 *N* NaOH and extracted with $CHCl_3$. The aqueous solution was then acidified (pH 2) with concentrated HCl and extracted with $CHCl_3$. The organic extracts were dried ($NaSO_4$) and evaporated to yield a solid which was recrystallized from EtOH–H_2O. This gave pure **8** (1.0 g, 29%): mp 123°. Anal. ($C_{14}H_{14}N_2O_3$) C, H, N.

Acknowledgment. We wish to thank our colleagues in the Pharmacology Department of our Company for the biological results.

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A Lapachol Derivative Active against Mouse Lymphocytic Leukemia P-388

M. da Consolação F. Linardi, M. M. de Oliveira,* and M. R. P. Sampaio

Section of Pharmacology, Instituto Biológico, São Paulo, Brazil. *Received March 13, 1975*

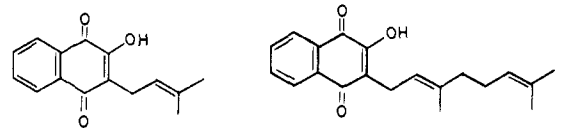
Lapachol [2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone] and its analogs [2-(3,7-dimethyl-2,6-octadienyl)-3-hydroxy-1,4-naphthoquinone and 2-(3,3-dibromo-2-propenyl)-3-hydroxy-1,4-naphthoquinone] have been described, among almost a hundred synthesized analogs, as active against rat tumor Walker 256 carcinosarcoma. The acetylglucosylation of lapachol results in a compound which extends lapachol activity becoming effective against mouse lymphocytic leukemia P-388. When mice inoculated with 10^6 leukemic cells were treated with the drug during 9 days, their life span increased 80% over the control animals. Identification spectral data (uv, ir, ^1H NMR, and MS) of the compound obtained by synthesis are given.

It is well known that activity of a drug depends upon its reaching the site of action and that derivatives, referred to sometimes as "prodrugs",[1] which reach this site more efficiently and are there degraded to the active compound, may be therapeutically more effective. In a recent publication, Segal and coworkers[2] showed that the activity of saponin is due to the aglycone. The hemolytic effect of a particular saponin will depend on its degree of adsorption by the cell and on the presence of specific glycosidases on the cell wall. The present paper describes a modification of lapachol by glycosidation which extends and enhances its antitumor activity.

Lapachol, 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (1), occurs in the wood of several species of the family Bignoniaceae[3] and is presently commercialized in Brazil as an antitumor drug. Experimentally, lapachol is

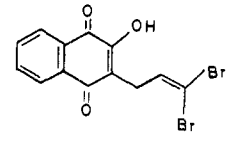
active against two types of solid tumors, Walker 256 carcinosarcoma and Murphy–Sturm lymphosarcoma. It is interesting that before Walker 256 was introduced as an experimental tumor, lapachol was considered inactive. It is, in fact, inactive in mice neoplasias, Sarcoma 180, Adenocarcinoma 755, Lewis lung carcinoma, lymphocytic leukemia P-388, and leukemia L1210.

As its chemical structure is a simple one, several derivatives have been obtained by synthesis; Hartwell and Abbott reported[4] that of 68 synthetic analogs only one, 2-(3,7-dimethyl-2,6-octadienyl)-3-hydroxy-1,4-naphthoquinone (2), was active against Walker 256. A more recent study of Herman[5] indicated a high activity against Walker 256 for 2-(3,3-dibromo-2-propenyl)-3-hydroxy-1,4-naphthoquinone (3).



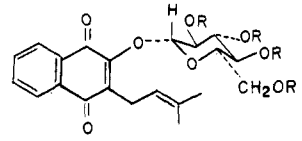
1

2

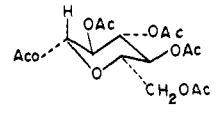


3

The present study describes the activity of two new compounds, the 2-β-*O*-glucoside of lapachol (4) and its tetraacetyl derivative (5).



4, R = H
5, R = CH_3CO



6

Lapachol was isolated from *Tabebuia avellanedae* and treated with α-bromotetraacetylglucose in the presence of silver carbonate to give 5 which was deacetylated to 4.

As lethal action of sugar in ascitic tumor cells in vitro is reported in the literature,[7] glucose derivative 1,2,3,4,6-pentaacetyl-α-D-glucopyranose (6) was also tested in mouse leukemia P-388.

Lapachol (1), the glucoside 4, and pentaacetylglucose (6) were inactive but the 2-(3-methyl-2-butenyl)-3-(tetraacetyl-β-D-glucopyranosyloxy)-1,4-naphthoquinone (5) showed activity at 110 mg/kg × 9 (drug applied daily in 1.2% Tween 80) and gave a 100% increase in life span at a dose of 130 mg/kg × 9. Probably the right degree of liposolubility was reached and the "prodrug" could be absorbed by the P-388 tumor cells while the glucosyloxy bond was labile to enzymes present in the cells. The glucolapachol owing to its less hydrophobicity was not active.

In contrast lapachol itself, as mentioned above, exhibits marked activity in the solid tumor Walker 256 with a therapeutic index of 6 and a reduction of tumor growth of 92% when administered orally at 150 mg/kg daily, whereas both lapachol glucoside (4) and its tetraacetyl derivative (5) were inactive in this tumor. They were not absorbed or, in the case of compound 5, the required glucosidase may not have been available to liberate lapachol, the bioactive compound.

Experimental Section

Melting points were determined on a Monoscop IV apparatus and are uncorrected. The uv spectra were recorded on a Perkin-Elmer Model 127, ir on a Perkin-Elmer Model 521, and ^1H NMR at 60 and 100 MHz on a Varian T-60 and a Varian XR 100, respectively. Mass spectra (70 eV) were determined on a Varian-Atlas CH_5 at Federal University, Rio, and on a RMU-7MG Hitachi at IPT, São Paulo.

Table I. Action of Lapachol (1), Its Glucoside 4 and Tetraacetyl Glucoside 5, and β-Pentaacetylglucose 6 on Lymphocytic Leukemia P-388 in BDF_1 Mice

Substance	Dose (mg/kg) × time[a]	ILS, %[b]
Lapachol (1)	150 × 9	10
Lapachol glucoside (4)	130 × 9	3
Lapachol glucoside tetraacetate (5)	110 × 9	27
	130 × 9	80
Pentaacetylglucose (6)	130 × 9	0

[a] ip daily in 1.2% Tween 80. [b] Increase in life span; ILS ≥ 25% signifies positive activity.

Table II. Action of Lapachol (1), Its Glucoside 4, and Tetraacetyl Glucoside 5 on Walker Carcinosarcoma 256 (im) in Rats

Substance	Dose (mg/kg) × time[a]	% of tumor inhibn[b]
Lapachol (1)	150 × 4	87.5
Lapachol glucoside (4)	130 × 4	19.2
Lapachol glucoside tetraacetate (5)	150 × 4	23.2
	170 × 4	11.0

[a] ip daily in 1.2% Tween 80. [b] Values ≥ 58% signifies positive activity.

Lapachol (1). Finely powdered wood (500 g) of *Tabebuia avellanedae* was extracted at room temperature with petroleum ether (bp 30–60°). Lapachol crystallized on concentration of the extract and recrystallized from benzene–petroleum ether. After further extraction of the powdered wood with methanol (1 l.) and evaporation of the methanol, the residue was suspended in benzene and filtered giving, after concentration and addition of petroleum ether, a further batch of lapachol: mp 139–140°; total yield 15.9 g (3.19%); ν_{max} (KBr) 3360 (OH), 1625 and 1650 cm^{-1} (CO); λ_{max} (EtOH) 250, 275, 325, and 395 nm (log ε 4.37, 4.33, 3.71, 3.23); ^1H NMR (60 MHz in $CDCl_3$) δ 1.63 (3 H, s, CH_3), 1.80 (3 H, s, CH_3), 3.32 (2 H, d, J = 7 Hz, $-CH_2-$), 5.28 (1 H, t, J = 7 Hz, vinyl H), 7.35 (1 H, s, OH), 7.76 (2 H, m, 6, 7-H), 8.10 (2 H, m, 5, 8-H); mass spectrum (RMU-7MG Hitachi) *m/e* 242 (30.55, M^+).

2-(3-Methyl-2-butenyl)-3-(tetraacetyl-β-D-glucopyranosyloxy)-1,4-naphthoquinone (5). To a solution of lapachol (0.005 mol) and 2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide (0.007 mol) in dry pyridine (20 ml) was added 0.0014 mol of freshly prepared silver carbonate. After stirring for 3 hr the silver salts were filtered and washed with methanol, and the combined filtrates were treated with 5% aqueous acetic acid (300 ml). The resulting brown precipitate was washed with water and dissolved in acetone, the residual salts were filtered, and the solution was evaporated. The solids obtained were dissolved in chloroform and purified by chromatography over a silica gel column, giving 5 (0.0008 mol, 16%): mp 62–65° dec from ethyl acetate–methanol; ν_{max} (KBr) 1750 (acetate), 1630 and 1660 (quinone), 1220 cm^{-1} (CO); λ_{max} (EtOH) 245.0, 250.0, 274.0, and 332.0 nm (log ε 4.15, 4.16, 4.04, 3.42); ^1H NMR (100 MHz in $CDCl_3$) δ 1.67 (3 H, s, CH_3), 1.80 (3 H, s, CH_3), 1.99 (3 H, s, acetyl CH_3), 2.05 (6 H, s, 2 acetyl CH_3), 2.13 (3 H, s, acetyl CH_3), 3.34 (2 H, d, J = 6 Hz, $ArCH_2C=$), 3.7 (1 H, m, 5′-H), 4.15 (2 H, m, 6′-H_2), 5.0–5.2 (4 H, m, vinyl H and 2′,3′,4′-H), 5.85 (1 H, m, 1′-H), 7.65–7.80 (2 H, m, 6, 7-H), 7.90–8.15 (2 H, m, 5, 8-H); mass spectrum (Varian-Atlas CH5) *m/e* 331 (1, tetraacetylglucose fragment), 242 (14, lapachol fragment), 227 (27, *m/e* 331 − HOAc − CH_3CO − H). Anal. Calcd for $C_{29}H_{32}O_{12}\cdot 0.5H_2O$: C, 59.83; H, 5.70. Found: C, 59.64; H, 5.66.

2-(β-D-Glucopyranosyloxy)-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (4). A suspension of the foregoing tetraacetate (0.0007 mol) in dry methanol (5 ml) was treated with 2 *N* sodium methoxide (0.5 ml), agitated until complete solution occurred, and kept a further 12 hr in the refrigerator. Water was added; the product was extracted with ethyl acetate and chromatographed over silica gel with EtOAc–MeOH, giving 2-(β-D-glucopyranosyloxy)-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (4) (0.0006 mol, 85.71%): ν_{max} (KBr) 3400 (hydroxy), 1630 and 1660 cm^{-1} (quinone); mass spectrum (RMU-7MG Hitachi) *m/e* 242 (17, lapachol fragment), 163 (3.1, glucose fragment). Anal. Calcd for $C_{21}H_{24}O_8 \cdot H_2O$: C, 59.65; H, 6.20. Found: C, 59.22; H, 5.84.

This glycoside gave lapachol and β-lapachone[6] on acid (2 *N* H_2SO_4–MeOH, 1:1) hydrolysis.

β-D-Glucopyranose Pentaacetate (6). This compound was obtained from β-D-glucopyranose by the usual way: crystallized from ethanol; mp 130–131.5°.

Biological Assay. (a) Leukemia P-388. The procedure of the Cancer Chemotherapy National Service Center[8] was used. BDF_1 mice from the cross of female C57BL/6 and male DBA/2 were inoculated with 10^6 viable P-388 lymphocytic leukemia cells. The control groups received physiological saline containing 1.2% Tween 80 and the test groups received the drug under investigation emulsified with 1.2% Tween 80. The increase of survival time of the treated group over controls was determined, a value equal to or exceeding 25% being considered demonstrative of activity. The results are shown in Table I.

(b) Walker 256. The National Cancer Institute procedure[9] was used. Wistar rats were inoculated intramuscularly with Walker 256 tumor cells. Treatment of animals with the drugs began the third day after implantation. Activity was measured as tumor weight. The degree of inhibition was calculated as 100 − 100 T/C, where T and C are the mean tumor weight from treated and control animals, respectively.

A value of 58% or above is statistically significant antitumor activity. The results are summarized in Table II.

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Nonclassical Nicotine Antagonists[1]

Charles F. Barfknecht,* Clinton A. Taylor, Jr.,

Division of Medicinal Chemistry and Natural Products, College of Pharmacy

Lawrence A. Peterson, Fouad M. Sharabi, and John P. Long

Department of Pharmacology, College of Medicine, The University of Iowa, Iowa City, Iowa 52242. *Received May 27, 1975*

A series of "nonclassical" nicotine antagonists was synthesized and compared to the "classical" nicotine antagonist, hexamethonium, by means of the isolated guinea pig atria preparation. **2** was found to be the most potent, followed by hexamethonium and the other antagonists. With the exception of **5**, the bisquaternary compounds **1–3** and **7–9** were found to be more potent than the monoquaternary compounds **4**, **6**, and **10–12**. Within a series of compounds (**1–6** or **7–12**), those compounds possessing two phenyl rings proved to be more potent than those possessing one or three phenyl rings. These and other aspects of the structure–activity relationship of this class of compounds are discussed.

In light of nicotine's role[2] in elevating the blood pressure and increasing the heart rate of smokers, a drug which could block this pressor effect of nicotine may safeguard smokers from cardiovascular damage. Unfortunately, "classical" nicotine antagonists, such as hexamethonium, tetraethylammonium, and triethylcholine, also inhibit normal ganglionic transmission at doses which block nicotine. A "nonclassical" nicotine antagonist was discovered by Wong and Long[3] in 1967 and has been studied extensively. This compound, 4,4′-bis[*N*-(2,2-diethoxyethyl)-*N*,*N*-dimethylammonioacetyl]biphenyl dibromide (**2**), given the trivial name DMAE in the pharmacological literature, was found to have the ability to completely antagonize the pressor effects of nicotine at dose levels too low to affect ganglionic transmission.[4,5] Because **2** did exhibit neuromuscular blockade as well as catecholamine potentiation,[6] its structure was altered to form 4,4′-bis[*N*,*N*-bis(2-ethoxyethyl)-*N*-methylammonioacetyl]biphenyl dibromide (**8**), given the trivial name DEO in the pharmacological literature. **8** was found to be devoid of catecholamine potentiation, but it did exhibit some neuromuscular and ganglionic blockade as well as a drastically reduced duration of action.[7] Because **8** and two other congeners of **2** (dibenzofuran and *p*-terphenyl) exhibited different potencies and spectra of activity from **2**, it was felt that a detailed structure–activity relationship study of this class of compounds was in order.[8]

The structure–activity relationship study has two potential goals: (1) the determination of the molecular parameters needed to maximize the "nonclassical" antagonism of nicotine and (2) the further elucidation of the site of action of nicotine in the adrenergic nervous system.

Chemistry. As shown in Schemes I and II, all the compounds **1–12** were prepared by a Hofmann alkylation procedure involving the addition of the desired amine (**19** or