Effects of Quinone Derivatives on an Experimental Tumor

M. M. de OLIVEIRA ˣ, M. C. F. LINARDI, and M. R. P. SAMPAIO

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Abstract □ In continuation of studies on the activity of known solid tumor inhibitors, four acetylated glycosyl derivatives of 1,4-quinones were prepared and tested against Ehrlich ascitic tumor. All four compounds significantly inhibited growth of this neoplasm. UV, IR, and mass spectra are given for the three new synthetic quinone derivatives.

Keyphrases □ Quinone derivatives, acetylglycosyl—synthesized, evaluated for antitumor activity in mice □ Antitumor activity—various acetylglycosyl quinone derivatives evaluated for antitumor activity in mice □ Structure-activity relationships—various acetylglycosyl quinone derivatives evaluated for antitumor activity in mice

In the search for broader spectrum antitumor activity similar to that previously found with a lapachol derivative against leukemia P-388 (1), acetylated glycosides of natural quinones were prepared and tested against Ehrlich carcinoma.

Acetylated glucosides or galactosides of lawsone (2-hydroxy-1,4-naphthoquinone) (I), juglone (5-hydroxy-1,4-naphthoquinone) (II), and lapachol [2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone] (III) were synthesized as derivatives. Juglone, found in *Juglans regia* L. (walnut), has been used as a fungicide in dermatosis (2); lawsone, from *Lawsonia alba* Lam. (Lythraceae) (henna), was known in ancient Egypt (2) as a component of cosmetics; several species of *Biognoneaceae* produce lapachol, a drug that has been studied for anticancer activity in its natural form or as derivatives (1, 3).

Normalization of glucose metabolism in tumor brain sections by natural glycosides differs according to the sugar moiety (4); for this reason, both galactose and glucose derivatives of lawsone were synthesized. Previous work (3, 5, 6) showed that both juglone and lapachol inhibit solid tumors (Walker carcinosarcoma 256 and Ehrlich solid carcinoma), while juglone and lawsone are effective against sarcoma 180. The purpose of this study was to investigate the action of derivatives of these quinones on the Ehrlich ascitic tumor.

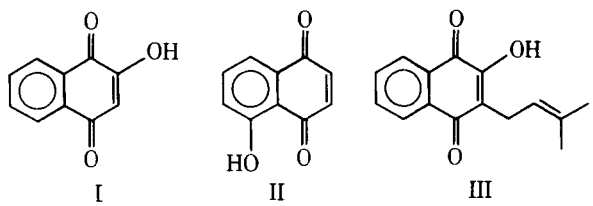
EXPERIMENTAL[1]

2-(Tetraacetyl-β-D-galactopyranosyloxy)-1,4-naphthoquinone (IV)—To a solution of lawsone (870 mg, 5 mmoles) and 2,3,4,6-tetra-acetyl-α-galactopyranosyl bromide (2.877 g, 7 mmoles) in dry pyridine (20 ml) was added freshly prepared silver carbonate (966 mg, 3.5 mmoles). The mixture was stirred for 3 hr, the silver salts were filtered and washed with methanol, and the combined filtrates were treated with 5% aqueous acetic acid (200 ml). The resulting brown precipitate was washed with distilled water and dissolved in acetone; the residual salts were filtered, and the solution was then evaporated under vacuum.

The obtained solid was dissolved in chloroform and purified by silica gel column chromatography with elution using ethyl acetate. Compound IV was crystallized from ethyl acetate–methanol in a 756-mg yield (1.5 mmoles, 30%), mp 212–213°; IR (ν_{max}): 1740 (acetate), 1660 and 1680 (quinone), and 1240 (CO) cm^{-1}; UV (λ_{max}): 330 (log ϵ 3.83), 273 (4.31), 249 (4.36), and 243 (4.37) nm; mass spectrum: *m/e* 504 (0.1, M^+) and 331 (39, tetraacetylgalactose fragment).

Anal.—Calc. for $C_{24}H_{24}O_{12}$: C, 57.14; H, 4.76. Found: C, 57.11; H, 4.69.

2-(Tetraacetyl-β-D-glucopyranosyloxy)-1,4-naphthoquinone (V)—This compound was prepared by the same procedure as IV, using



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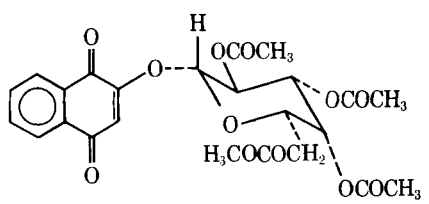
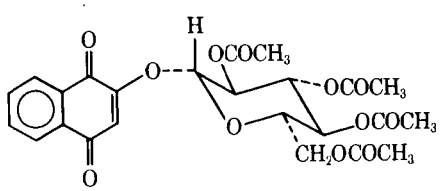
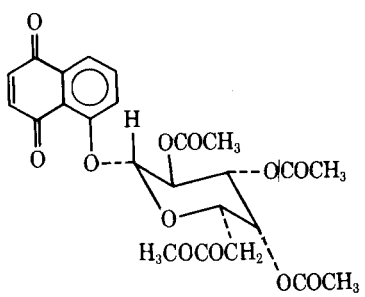


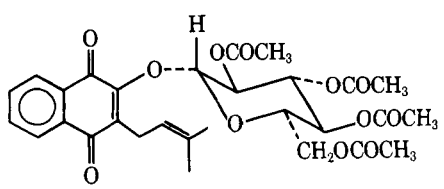
IV



V



VI



VII

[1] Melting points were measured on a modified Köfler block and are uncorrected. Elemental analyses were performed on a Hitachi 026 apparatus at the Instituto de Pesquisas Technológicas, São Paulo, Brazil. IR spectra were recorded in potassium bromide pellets on a Perkin-Elmer 124 spectrophotometer. UV spectra were recorded in methanol solution on a Perkin-Elmer 521 spectrophotometer. Mass spectra (70 ev) were run on a Hitachi RMU-7MG spectrometer at the Instituto de Pesquisas Technológicas. Lawsone and juglone were purchased from Kock-Light (England).

Table I—Activity of 1,4-Naphthoquinones and Their Glycosyl Derivatives against Ehrlich Ascites Carcinoma

Compound	Dose, mg/kg	Tumor Inhibition, %	Number of Survivors at Day 12	Weight Difference at Day 5
II	2.0	100.0	5/6	−2.5
	2.0	100.0	5/6	−2.7
	1.5	76.5	6/6	−1.1
	1.5	57.0	6/6	−1.7
VI	6.5	100.0	6/6	−1.4
	6.5	98.0	6/6	−2.5
	4.0	99.5	6/6	−2.1
	4.0	80.4	6/6	−2.2
I	10.0	0.0	6/6	−0.8
	10.0	0.0	5/6	−0.8
	8.0	0.0	6/6	−0.8
	8.0	0.0	6/6	+1.0
V	14.0	99.0	6/6	−1.6
	14.0	100.0	6/6	−2.3
	9.0	98.8	6/6	−1.6
	9.0	69.7	5/6	−1.7
IV	18.0	100.0	4/6	−6.0
	18.0	100.0	6/6	−3.2
	14.0	100.0	6/6	−2.9
	14.0	100.0	5/6	−3.9
	9.0	86.0	5/6	−3.5
	9.0	99.7	6/6	−2.5
III	150.0	12.0	6/6	−1.3
	150.0	0.0	4/6	−1.4
VII	150.0	95.4	6/6	−0.4
	150.0[a]	99.6	6/6	−5.6

[a] Not injected on 7th day.

lawsone (870 mg, 5 mmoles), 2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide (2.877 g, 7 mmoles), and active silver carbonate (966 mg, 3.5 mmoles) in dry pyridine (20 ml). The yield of glucosyl derivative was 697 mg (1.38 mmoles, 27.6%), mp 136–138° (ethyl acetate–methanol); IR (ν_{max}): 1740 (acetate), 1660 and 1680 (quinone), and 1230 (CO) cm^{-1}; UV (λ_{max}): 330 (log ϵ 3.80), 270 (4.31), 248 (4.35), and 243 (4.36) nm; mass spectrum: *m/e* 504 (0.2, M^+) and 331 (44, tetraacetylglucose fragment).

Anal.—Calc. for $C_{24}H_{24}O_{12}\cdot 0.25H_2O$: C, 56.64; H, 4.81. Found: C, 56.70; H, 4.75.

5-(Tetraacetyl-β-D-galactopyranosyloxy)-1,4-naphthoquinone (VI)—This compound was prepared by the same procedure as IV, using juglone (435 mg, 2.5 mmoles), 2,3,4,6-tetraacetyl-α-D-galactopyranosyl bromide (1.562 g, 3.8 mmoles), and active silver carbonate (469 mg, 1.7 mmoles) in dry pyridine (10 ml). The yield of galactosyl derivative was 302 mg (0.6 mmole, 24%), mp 80–82° (ethyl acetate–methanol); IR (ν_{max}): 1740 (acetate), 1650 and 1670 (quinone), and 1230 (CO) cm^{-1}; UV (λ_{max}) 250 nm (sh); mass spectrum: *m/e* 504 (0.1, M^+).

Anal.—Calc. for $C_{24}H_{24}O_{12}\cdot 0.25H_2O$: C, 56.64; H, 4.81. Found: C, 56.50; H, 5.06.

Lapachol (III)—Lapachol was extracted from *Tabebuia avellanedae* as described previously (1), mp 140°. The IR spectrum was superimposable on that of an authentic sample.

2-(3-Methyl-2-butenyl)-3-(tetraacetyl-β-D-glucopyranosyloxy)-1,4-naphthoquinone (VII)—The derivative was obtained from a solution of lapachol (1.210 g, 5 mmoles) and 2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide (2.877 g, 7 mmoles) by the same methods as the other derivatives. The yield was 458 mg (0.8 mmole, 18%), mp 62–65° dec.; the IR spectrum was superimposable on that of the previously prepared sample (1).

Biological Tests—The procedure of the National Cancer Institute was used (7). Swiss mice were inoculated with 5×10^6 viable cells of Ehrlich ascitic tumor from a donor 7 days after the cell passage. The drug was homogenized in a mortar with acetone (2%) and polysorbate 80 detergent (two drops). Controls received water, detergent, and acetone in the same proportion as used in the drug homogenate.

Drug treatment was given from the 1st to the 7th day. The animals were sacrificed on the 12th day, and the ascitic liquid was withdrawn and weighed. An experiment, to be valid, had to show more than 65% survivors on the day of evaluation.

Inhibition values equal to or higher than 58% were considered demonstrative of drug activity.

RESULTS AND DISCUSSION

All synthesized compounds showed activity on Ehrlich ascitic tumor (Table I). Of the parent quinones, only juglone inhibited tumor development; lapachol and lawsone were inactive. There seemed to be no modification in the anticancer effect when glucose or galactose derivatives were used; the activities of the two lawsone derivatives were very similar (Table I).

Improper homogenization probably accounted for the weight variations observed in experiments with the lapachol derivative (Table I). No better results could be obtained, however, in experiments with most of the other permitted vehicles (7).

In some experiments, 100% inhibition was obtained, and a pronounced reduction of ascites was found with all doses used. Although the growth of Ehrlich ascites cell tumor is fairly sensitive to many foreign materials, the protective effect of the glycosyl derivatives and the inactivity of the parent dihydroxyquinones (excepting juglone) point to a striking improvement over the capacity of the original compounds for being absorbed by the tumor cell.

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