PLANT ANTICANCER AGENTS. XII.[1] ISOLATION AND STRUCTURE ELUCIDATION OF NEW CYTOTOXIC QUINONES FROM *TABEBUIA CASSINOIDES*

M. MADHUSUDANA RAO and DAVID G. I. KINGSTON*

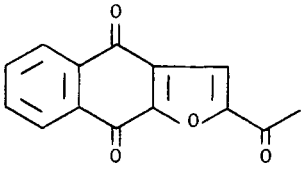
Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

ABSTRACT.—Isolation of the three new cytotoxic furonaphthaquinones **1**, **2**, and **3** (or **4**) from *T. cassinoides* is described. The compounds were characterized by ^1H nmr, uv, ir, and ms. The synthesis of quinone **1** is also reported.

As part of a systematic survey of botanical sources for anticancer activity, an alcoholic extract of the stem bark of *Tabebuia cassinoides* (Lam.) DC. (Bignoniaceae) was examined. This extract showed slight but reproducible activity in the P-388 *in vivo* bioassay (T/C 127 and 125 at 200 mg/kg in two separate tests), and a full-scale investigation of it was thus initiated in an attempt to isolate the antileukemic constituent.

Partitioning of the ethanol extract (54g) between chloroform and water gave a chloroform fraction (6.8 g) showing T/C 132 at 50 mg/kg in the P-388 assay. Partitioning of the chloroform fraction between 90% aqueous methanol and hexane yielded an inactive hexane fraction and a methanol-soluble fraction (3.6 g) with T/C 122 at 50 mg/kg and an ED_{50} of 27 μg/ml in the KB cell culture assay.

Further purification of the methanol fraction by chromatography on silica gel resulted in a loss of *in vivo* activity, and subsequent re-extractions of the original stem bark sample yielded extracts that were uniformly inactive in the P-388 *in vivo* assay. The first fraction eluted from the column showed significant cytotoxicity in the KB cell culture assay (ED_{50} 1.9 μg/ml), however, and purification of this fraction by chromatography and recrystallization yielded the three new cytotoxic naphthaquinones **1**–**3** (or **4**).



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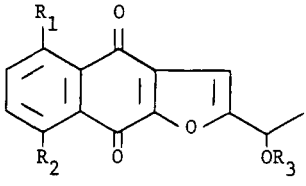
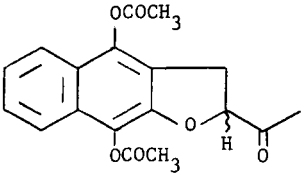


2 $R_1=R_2=R_3=H$
3 $R_1=R_3=H$ $R_2=OH$
4 $R_1=OH$ $R_2=R_3=H$
5 $R_1=R_2=H$ $R_3=COCH_3$
6 $R_1=R_2=H$ $R_3=COC(CF_3)(OCH_3)C_6H_5$



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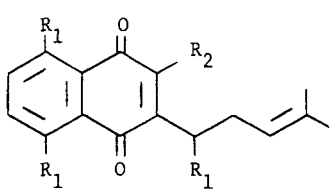
Compound **1**, $C_{14}H_8O_4$, was an orange crystalline substance with an ED_{50} of 1.0 μg/ml in the KB cell culture assay. Its ^1H nmr spectrum in $CDCl_3$ was rather simple with two two-proton multiplets centered at δ 8.2 and 7.8 ppm, a one-proton singlet at 7.60 ppm, and a three-proton singlet at 2.70 ppm. Its ir spectrum showed absorptions at 1690 and 1670 cm^{-1} and its uv spectrum was characteristic of napthaquinones.

[1]For previous paper see reference 1.

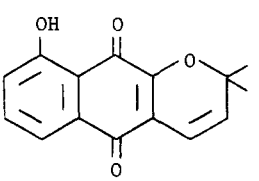
The quinonoid nature of compound **1** was shown both by its ir and its uv spectral data, which correspond with the spectra for known napththaquinones (with the exception that compound **1** does not show the uv absorption at about 440 nm given by some naphthaquinones (2)), and by its reductive acetylation to the tetrahydrodiacetate **7**. These data are only consistent with the formulation of the quinone as 2-acetyl-4H,9H-naphtho[2,3-b]furan-4,9-dione (**1**). The only reasonable alternate structure would be the 3-acetyl derivative, but the proton in the 2-position of this compound would be expected to have a chemical shift around 8.5–9 ppm based on shifts in model compounds (3). Thus applying the chemical shift differences calculated for 2- and 3-acetylfuran (3) to the chemical shifts of the 2- and 3-protons of 4H,9H-naphtho[2,3-b]furan-4,9-dione (4) yielded a predicted value of 7.96 ppm for the 3-proton of the 2-acetyl derivative, and 8.65 ppm for the 2-proton of the 3-acetyl derivative. Clearly the observed shift of 7.60 ppm is most consistent with the first alternative.

The second compound isolated was a yellow crystalline substance, $C_{14}H_{10}O_4$, with an ED_{50} of 2.0 μg/ml in the KB cell culture assay. Its ^1H nmr spectrum in $CDCl_3$ showed absorption at δ 8.2 (2H, m), 7.7 (2H, m), 6.84 (1H, s), 5.06 (1H, q), 2.9 (1H, bd s) and 1.68 (3H, d) ppm, and its uv spectrum was typical of a naphthaquinone. These data indicate that the compound has the structure 2-(1-hydroxyethyl)-4H,9H-naphtho[2,3-b]furan-4,9-dione (**2**). Confirmation of the secondary nature of the hydroxyl function was obtained by acetylation to the monoacetate **5**, which gave a ^1H nmr spectrum showing a quartet at 6.04 ppm, indicating a characteristic acylation shift of about 1 ppm for the α proton of a secondary alcohol.

The remaining structural problem concerned the nature of the chiral center in the side-chain of quinone **2**. Measurement of the specific rotation gave a value of 0° within experimental error, but the small amount of sample available made it possible that a small rotation would have gone undetected. The (+)α-methoxy-α-trifluoromethylphenylacetate derivative (**6**) of the quinone was thus prepared (5), and its ^1H nmr spectrum showed two doublets of intensity three halves each for the methyl signal at 1.87 ppm, and two singlets for the furan proton at 6.90 and 6.78 ppm. These data indicated that the quinone **2** was indeed racemic. This conclusion does not preclude its status as a natural product since other natural products such as the napthaquinone shikalkin (**8**) are known to occur as racemates (2).



8 R_1=OH R_2=H
9 R_1=H R_2=OH



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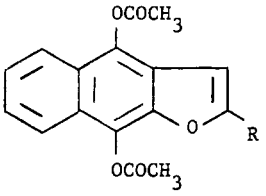
The third compound was obtained as orange needles, m.p. 156.5–157.5°, and it showed an activity of 1.0 μg/ml in the KB cell culture assay. Its mass spectrum indicated the presence of an additional oxygen atom as compared to compound **2**, and its ^1H nmr spectrum in $CDCl_3$ showed absorptions at δ 12.18 (1H, s), 7.75 (1H, dd, J=2Hz, 8Hz), 7.60 (1H, dd, J=8, 8Hz), 7.26 (1H, dd, J=2Hz, 8Hz), 6.83 (1H, s), 5.04 (1H, q, J=6Hz), 2.4 (1H, bd), and 1.64 (3H, d, J=6Hz) ppm. Its uv spectrum was similar to that of the other naphthaquinones examined. Treatment with aqueous sodium hydroxide gave a violet color, as observed with juglone.

The data given above indicate that this third compound is a naphthaquinone

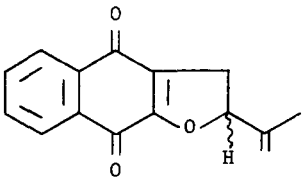
containing a *peri*-hydroxyl group and an aromatic ABX proton system, together with the same substituted furan system as in quinone **2**. The compound is optically inactive, and thus the side-chain chiral center is racemic, as with quinone **2**. The two structures possible for the quinone are then restricted to **3** and **4**, in which the additional hydroxyl group occupies either the 5- or the 8-position. A choice between these two structures cannot be made on the basis of the available data since the furan oxygen atom can interact with the quinone system both directly and by conjugation through the furan double bond; comparison of spectral data with those of simple model compounds thus gives ambiguous results. An attempt to prepare the 2,3-dihydro derivative by hydrogenation was unsuccessful, yielding a mixture of products including unreacted starting material. The lack of sufficient sample precluded any further investigation along these lines. The structure **3** is preferred on biogenetic grounds and by analogy with compounds such as lambertellin and α-caryopterone (**10**) (6).

The structure of quinone **1** was confirmed by its synthesis by the route described below. Diacetate **11**, prepared by reductive acetylation of the corresponding quinone (7), was acetylated with excess acetyl chloride and aluminum chloride in methylene chloride (no other conditions were found satisfactory). The resulting acetyl diacetate **12** was hydrolyzed (Zemplen conditions) and allowed to oxidize in air to yield a material identical (^{1}H nmr, hplc, mp) with the original quinone **1**.



11 R=H

12 R=$COCH_3$



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The observation that quinones **1** and **2** both show significant activity in the KB cell culture may be significant since the related compound lapachol (**9**), which has an ED_{50} value of 4.4 μg/ml in KB cell culture (8), showed sufficient *in vivo* activity to reach clinical trial at the National Cancer Institute. The statement has been made that in the quinone series, activity against KB cells at levels less than 1 μg/ml appears to correlate with activity in the L-1210 lymphoid leukemia system *in vivo* (8). The limited amount of compound **1** available for *in vivo* testing did not enable a complete determination of its activity to be made; it was found to be non-toxic but inactive in the P-388 lymphocytic leukemia assay at doses up to 35 mg/kg. It is quite likely, however, that various derivatives of **1** could be prepared with improved cytotoxicity, and it is possible that these derivatives would show increased *in vivo* activity.

The occurrence of naphthaquinones in various members of the genus *Tabebuia* is well known, and lapachol (**9**) is one of the major constituents of several *Tabebuia* species (2). The biosynthesis of quinone **1** thus may occur *via* cyclization of lapachol to dehydro-iso-α-lapachone (**13**) (9), followed by oxidative cleavage of the exocyclic methylene group and dehydrogenation of the dihydrofuran ring. Quinones **2** and **3** (or **4**) could then be derived by simple reductive and oxidative transformation of quinone **1**. Although the three quinones are all relatively simple compounds, they have not previously been reported in the literature.

EXPERIMENTAL

MATERIALS AND METHODS.—Materials and methods were generally the same as those previously described (10). Mass spectra were recorded on a Varian-MAT 112 gas chromatograph-mass spectrometer, and ^{1}H nmr spectra on a Varian EM-390 nmr spectrometer with

tetramethyl silane as internal standard. The extracts, fractions, and compounds were tested under the auspices of the Drug Research and Development Program of the National Cancer Institute (11). An isolate is considered active if it shows an $ED_{50} \leqslant 4$ μg/ml in the KB cell culture and/or a T/C value ⩾125 in the P-388 *in vivo* assay. Plant material, PR 47208, was collected by the Economic Botany Laboratory, Science and Education Administration, BARC-East, U.S.D.A., Beltsville, Maryland. A voucher specimen is on deposit in the Herbarium of the National Arboretum, Agricultural Research Service, U.S.D.A., Washington, D.C.

Isolation and properties of quinones

Isolation.—Dried *Tabebuia cassinoides* (0.5 kg) was ground in a hammermill and extracted with 95% ethanol at room temperature and then at 50°. The combined ethanol extracts were evaporated and partitioned as described in the text to yield a methanol-soluble fraction (3.6 g). Repetition of the extraction on a larger scale (5 kg of plant material) yielded 50 g of crude methanol fraction, which was subjected to chromatography on silica gel with elution by chloroform-methanol, 9:1. The first major fraction eluted (5.4 g) was cytotoxic (ED_{50} 1.9 μg/ml in the KB cell culture). Purification of a portion of this fraction (0.58 g) by treatment with ether and recrystallization of the residue from chloroform-methanol yielded quinone **1**. Purification of a second portion of this fraction (0.5 g) was effected by chromatography on silica gel, preparative tlc (ethyl acetate-hexane, 80:20), and final separation of the resulting mixture of quinones **2** and **3** (or **4**) by preparative hplc [EM Labs RP-8 packing, CH_3CN–H_2O (40:60)].

Quinone **1**.—Quinone **1** crystallized from methanol as orange needles, mp 224–5° (25 mg). (Found: C, 69.99; H, 3.14. Calc. for $C_{14}H_8O_4$: C, 70.00, H, 3.36%); uv spectrum λ max (EtOH) nm (log ε), 253(4.36), 272(4.28), 341(3.28); ms, *m/z* (relative intensity) 240(M^+, 89), 225(100), 157(20), 141(12), 129(11), 113(28), 76(12), 53(11), and 43(19).

Tetrahydrodiacetate **7**.—Quinone **1** (25 mg), was heated with acetic anhydride (2 ml), sodium acetate (100 mg), and Zn dust (50 mg) at 140° for 2 hr. The reaction mixture was filtered into ice-water, and the filtrate worked up in the usual way to yield crude product. Purification of this material by ptlc [chloroform-methanol (99:1)], and hplc [Partisil M9 column, chloroform-hexane (1:2)] yielded pure tetrahydrodiacetate **7** as the major reaction product. (Found: M^+ 328. $C_{18}H_{16}O_6$ requires 328); ir spectrum ($CHCl_3$) 1760–1770 cm^{-1} (vs), 1720 cm^{-1} (m); ^1H nmr spectrum ($CDCl_3$): δ=7.8 (2H,m), 7.45 (2H,m), 5.18 (1H,dd), 3.45 (2H,m), 2.47 (3H,s), 2.44 (3H,s), and 2.32 (3H,s) ppm; ms, *m/z* (relative intensity) 328 (M^+, 9), 286 (34), 244 (100), 202 (44), 201 (16), 115 (10), and 43 (94).

Quinone **2**.—Quinone **2** crystallized from methanol as yellow needles, mp 140–142° (10 mg). (Found: M^+ 242.09. $C_{14}H_{10}O_4$ requires 242.06); $[\alpha]_D$=0 ($CHCl_3$); uv spectrum λ max (EtOH) nm (log ε) 250 (4.44), 275 inf. (4.10), 340 (3.27); ms, *m/z* (relative intensity) 242 (M^+, 42), 240 (29), 227 (100), 225 (51), 224 (23), 200 (56), 199 (43), 171 (25), 115 (35), 113 (21), 105 (38), and 104 (18).

Acetate **5**.—Quinone **2** was acetylated (Ac_2O/pyr), and the product purified by preparative tlc [ethyl acetate-hexane (40:60)] to yield acetate **5**. ^1H nmr spectrum ($CDCl_3$) δ=8.2 (2H,m), 7.7 (2H,m), 6.87 (1H,s), 6.04 (1H, q, *J*=7 Hz), 2.13 (3H,s), and 1.68 (3H, d, *J*=7 Hz) ppm.

(+)α-Methoxy-α-trifluoromethylphenylacetate **6**.—(+)-α-Methoxy-α-trifluoromethylphenylacetic acid (1.0 g) was heated under reflux with thionyl chloride (5 ml) for 24 hr. The thionyl chloride was removed (rotary evaporator) and the acid chloride distilled in a Kugelrohr apparatus at 70° and 1 mm. Quinone **2** (10 mg), dissolved in chloroform (1 ml), was then treated with the acid chloride (8 drops) and pyridine (8 drops). The reaction mixture was allowed to stand at room temperature for 24 hr and worked up in the usual way. The crude product was purified by preparative tlc [silica gel, chloroform-methanol (98:2)]. The isolated product had a ^1H nmr spectrum showing δ=8.2 (2H,m), 7.8 (2H,m), 7.7 (5H,c), 6.90 (1/2H,s), 6.78 (1/2H,s), 6.27 (1H,q), 3.54 (3H, two s), 1.9 (3/2H, d), 1.84 (3/2H, d) ppm.

Quinone **3** (or **4**).—Quinone **3** (or **4**) crystallized from methanol as orange-yellow needles, mp 156.5–157.5° (10 mg). (Found: M^+ 258.02. $C_{14}H_{10}O_3$ requires 258.05); $[\alpha]_D$=0 ($CHCl_3$); uv spectrum λ max (EtOH) nm (log ε) 235 (inf., 4.28), 247 (4.41), 300 (3.81), 398 (3.71); ir spectrum ($CHCl_3$) 3450, 1672(s), 1640(vs) cm^{-1}; ms, *m/z* (relative intensity) 258 (M^+, 74), 243 (100), 216 (37), 215 (39), 187 (17), 123 (32), and 121 (24).

Synthesis of quinone (1)

Acetyl diacetate (**12**).—Diacetate **11** (200 mg), prepared by reductive acetylation of the corresponding quinone (7), was treated with aluminum chloride (8 g) and acetyl chloride (5 ml) in methylene chloride (50 ml) under reflux conditions for a total of 7 hr. The product was decomposed with dilute HCl, and the organic portion was extracted with methylene chloride and subjected to chromatography on silica gel. Elution with chloroform-ethyl acetate yielded a crude fraction which crystallized from chloroform-hexane to yield the acetyl diacetate **12** (10 mg), mp 176–7°; (Found: C, 65.88; H, 4.09. Calc. for $C_{18}H_{14}O_6$: C, 66.28; H, 4.29); ^1H nmr spectrum: δ 7.98 (2H,m), 7.53 (2H,m), 7.47 (1H, s), 2.63, 2.60, and 2.57 ppm (3H each, s); ms, *m/z* 326 (M^+), 284, 242, 225, 199, 171, 105; uv spectrum λ max (EtOH) nm (log ε) 220 (4.48), 265 (4.53), and 332 (4.26); ir spectrum ($CHCl_3$): 1780, 1692, 1565, 1370, 1190, 1170, and 1045 cm^{-1}.

Quinone (**1**).—Acetyl diacetate **12** (5 mg) was dissolved in methanol (5 ml) and treated with a few drops of a dilute solution of sodium methoxide in methanol. The mixture was allowed to stand for 3 hours and then worked up by neutralization, dilution with water, and

extraction with chloroform. Purification of the crude product by hplc (acetonitrile-water (40:60) on LiChrosorb RP-8, ¼″ x 25 cm) yielded the quinone **1** as the only major product, mp 220°. The product had an identical retention time on hplc as the natural material, and its ^{1}H nmr spectrum showed the same absorptions as the natural product.

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LITERATURE CITED

1. Part XI, E. Jones, O. Ekundayo, and D. G. I. Kingston, *J. Nat. Prod.*, **44**, 443 (1981).
2. R. H. Thomson, *Naturally Occurring Quinones*, Academic Press, London and New York (1971).
3. S. Gronowitz, I. Johnson and A.-B. Hornfeldt, *Chem. Scr.*, **7**, 211 (1975).
4. J. W. Mathieson and R. H. Thomson, *J. Chem. Soc.* (C), 153 (1971).
5. J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, **95**, 512 (1973).
6. T. Matsumoto, C. Mayer, and C. H. Eugster, *Helv. Chim. Acta*, **52**, 808 (1969).
7. C. Rivalle, E. Gisagni, and J. Andre-Louisfert, *Tetrahedron*, **30**, 2192 (1974).
8. J. S. Driscoll, G. F. Hazard, Jr., H. B. Wood, Jr., and A. Goldin, *Cancer Chemother. Rep. Pt. 2*, **4**, (2), 1 (1974).
9. W. Sandermann, M. H. Simatupang, and W. Wendelborn, *Naturwissenschaften*, **55**, 38 (1969).
10. D. G. I. Kingston, B. T. Li, and F. Ionescu, *J. Pharm. Sci.*, **66**, 1135 (1977).
11. R. L. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.*, **3** (2), 1 (1972).