Studies on the Structure and Stereochemistry of Cytotoxic Furanonaphthoquinones from *Tabebuia impetiginosa*: 5- and 8-Hydroxy-2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-diones

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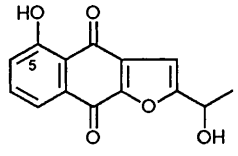
Chemical investigation of the bark of *Tabebuia impetiginosa* (Bignoniaceae) afforded cytotoxic furanonaphthoquinones, including 5-hydroxy-2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione and its 8-hydroxy isomer. The position of the phenolic group in these two compounds was established by X-ray crystallography. The furanonaphthoquinones were found to be mixtures of both enantiomers in different proportions, *i.e.*, (for the 5-hydroxy isomer) *R*:*S* 1:23 and (for the 8-hydroxy isomer) *R*:*S* 3:1. The synthesis of the racemic naphthofurans, their optical resolution into enantiomerically pure forms, and the determination of their absolute stereochemistry are described. The structure of kigelinone was also established to be that of the 5-hydroxy isomer, not the 8-hydroxy one, through this study.

Tabebuia impetiginosa (Bignoniaceae) (syn. *T. avellanedae* Lorenz, *Ipê roxo* in the native language) is a national flower of Brazil and is widespread in Brazil and other South American countries. The plant has been used as a diuretic and astringent, and also as a folk remedy for the prevention and treatment of cancer. The isolation of lapachol from the bark of this plant has been reported.[1] Bignoniaceous plants are known to contain furanonaphthoquinones: Inoue and his colleagues reported the isolation of kigelinone, which was tentatively assigned as 8-hydroxy-2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione, from *Kigelia pinnata*[2] and 2-isopropenylnaphtho[2,3-*b*]furan-4,9-dione from *Radermachera sinica* Hemsl.;[3] Rao and Kingston reported the isolation of cytotoxic 2-acetyl- and 2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-diones[4] and a compound which exhibited the same spectral data as kigelinone from *T. cassinoies* Lam. DC.[5] In the course of our screening for antitumour-active substances from plant sources, it was found that an alcoholic extract of the bark of *T. impetiginosa* exhibited antitumour effects against sarcoma 180 (ascites form) and increased the lifespan of tumour-bearing ICR mice through intraperitoneal administration.

Solvent partition followed by chromatographic separation of the active principle provided 5-hydroxy- and 8-hydroxy-2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-diones **1** and **2** as a mixture, along with the known 2-acetyl- and 2-(1-hydroxyethyl)-naphtho[2,3-*b*]furan-4,9-diones.[4]

In this paper we describe the unambiguous assignment of the structure of compounds **1** and **2** with respect to the position of the hydroxy group and the enantiomeric purities of compounds **1** and **2**. Preparation of the optically active furanonaphthoquinones **1** and **2** and the determination of their absolute stereochemistry are also described.[6] During the progress of our investigation Wagner and his colleagues have recently reported the isolation of compounds **1** and **2** from the same plant and the characterization of **1** and **2** on the basis of selective INEPT NMR experiments.[7]

A purified material containing compounds **1** and **2**, which were inseparable on silica gel chromatography, was suggested by their ^1H and ^{13}C NMR spectra (see Table 1) to be a *ca.* 1:1 mixture. The H–H COSY spectrum of the mixture helped to classify the two sets of signals. One set of proton signals,



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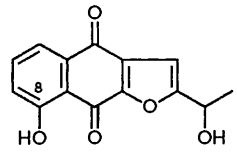

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including a characteristic phenolic proton signal at δ 12.16 (this isomer is designated as **1** in this text), was essentially identical with that published for kigelinone.[2] The other set of proton signals, which exhibited a phenolic proton signal at δ 12.0 (this isomer is designated as **2**, was attributed to the positional isomer of kigelinone. The mass spectra of the mixture and its acetylated product exhibited molecular ion peaks at *m*/*z* 258 and 342, respectively, which supported the structural formulation. Final proof of the structure of the components came from the spectral comparison (^1H and ^{13}C NMR, and HPLC) of the natural and synthetic materials (*vide infra*).

The previous assignment of the phenolic group to the C-8 position of kigelinone was based on the chemical shift of the phenolic proton.[2,8] However, the observed difference in the chemical shift of the phenolic protons between species **1** and **2** was very small ($\Delta\delta$ 0.16 ppm). Further, an empirical rule[8] that the phenolic proton of 8-hydroxy compounds is observed at higher field than that of their 5-hydroxy counterparts suggested the possibility that kigelinone is the 5-hydroxy isomer. Therefore, we decided to establish firmly the structure of compounds **1** and **2**. Unfortunately, in 2D-NMR (HMBC and COLOC) experiments on compounds **1** and **2** a cross-peak between C-4 and 3-H was not observed. Moreover, the C-4 signal was observed as a sharp singlet ($^3J_{C-H}$ ~0 Hz) in the LSPD experiments, which accounted for the results of the 2D-experiments. Therefore, we undertook the structure determination by X-ray crystallography.

An X-ray diffraction analysis was performed on a crystal of the synthetic sample of (*R*)-**2** (*vide infra*).[9] The molecular structure of compound **2** is illustrated in Fig. 1, which clearly indicates this isomer to be the 8-hydroxy one. Thus, it is now firmly established that the isomer whose phenolic hydrogen resonated at δ 12.00 (isomer **2**) is the 8-hydroxy compound, and

Table 1 NMR spectral data of 5-hydroxy- **1** and 8-hydroxy **2** furanonaphthoquinones ($CDCl_3$)

	1		**2**	
Atom	δ_H	δ_C	δ_H	δ_C
2		165.7		166.0
3	6.84 (d, 0.7)	103.3	6.85 (d, 0.7)	104.1
3a		131.1		132.0
4		186.4		178.4
4a		115.2		133.2
5		162.3	7.72 (d, 7.9)	120.1
6	7.27 (d, 7.7)	125.2	7.61 (t, 7.9)	136.2
7	7.62 (t, 7.7)	136.2	7.26 (d, 7.9)	125.2
8	7.76 (d, 7.7)	119.9		162.6
8a		132.6		114.7
9		172.6		179.6
9a		151.9		151.1
1′	5.04 (q, 6.7)	63.7	5.04 (br q, 6.7)	63.7
2′	1.66 (d, 6.7)	21.4	1.66 (d, 6.7)	21.4
2′-OH	2.39[a] (d, 6.0)		2.23[a] (d, 6.0)	
5/8-OH	12.16 (s)		12.00 (s)	

[a] The chemical shifts are variable within the range δ 2.2–2.4 depending on the sample preparation and concentration.

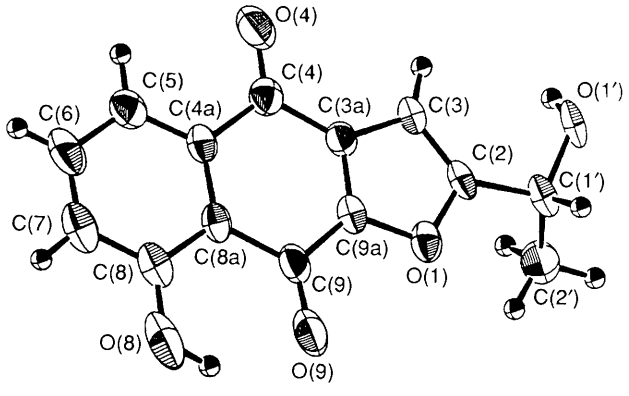


Fig. 1 X-Ray molecular structure of (*R*)-**2**

as a consequence the isomer whose phenolic proton is observed at δ 12.16 is the 5-hydroxy one. Therefore the structure of kigelinone needs to be revised to 5-hydroxy-2-(1-hydroxyethyl)-naphtho[2,3-*b*]furan-4,9-dione. Our conclusion is in accord with that of Wagner's group.[7]

In order to supply materials for stereochemical and biological studies, the naphthofuranoquinones **1** and **2** were synthesized according to Scheme 1.

The hydroxybenzofuran **3** was prepared in three steps starting from *o*-vanillin according to the published method.[10] Oxidation of compound **3** with Fremy's salt in acetone–water gave the furanobenzoquinone **4**, which was reduced with disodium dithionite and the hydroquinone was then protected as tetrahydropyran-2-yl (THP) ether. The three-step sequence afforded the benzofuran **5** in 79% yield.

Coupling of the lithiated derivative of compound **5**, generated from reaction with n-BuLi in tetrahydrofuran (THF) at −78 °C, with acetaldehyde afforded the alcohol **6** in 89% yield. Cleavage of the THP group of compound **6** under acidic conditions, followed by oxidation with Ag_2O, gave the quinone **7** in 75% yield. The quinone was subjected to Diels–Alder reaction with 1-(trimethylsiloxy)cyclohexa-1,3-diene at room temperature for one day to give the adducts **8** and **9** as a mixture. Enolization of the adducts with NaH in THF at 0 °C, deprotection of the silyl ether with aq. NH_4Cl, oxidation with Ag_2O (leading to the quinones **10** and **11**) and elimination of ethylene (retro-Diels–Alder reaction) by heating at 90 °C furnished compounds **1** and **2** as a mixture in 65% yield.

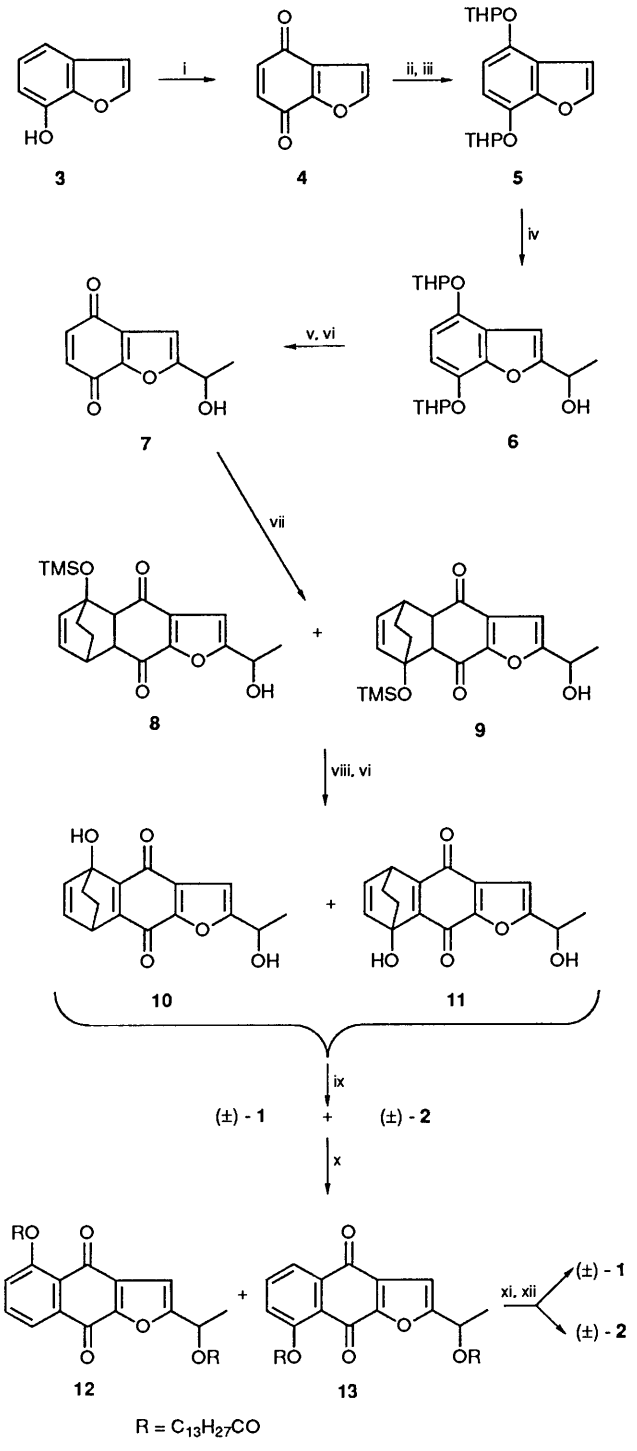
Although the mixture was not separable as described above, the corresponding diester of a long fatty acid was found to be suitable for the separation. Therefore, the mixture of phenols **1** and **2** was treated with myristoyl chloride in pyridine, and the obtained diester was separated by silica gel column chromatography to afford the less polar diester **12** and the more polar diester **13**. Alkaline hydrolysis of the separated diesters **12** and **13** completed the preparation of the racemic naphthoquinones **1** and **2** in 18 and 55% yield, respectively. The ^1H and ^{13}C NMR spectra of the synthetic isomers **1** and **2** were superposable on each set of the spectra of the natural mixture.

Optical resolution of the racemates **1** and **2** was then achieved by HPLC on a chiral column (CHIRALCEL OJ). Elution of compound **1** with hexane–ethanol (5:1) afforded the more mobile [(+)-**1**] and the less mobile [(−)-**1**] enantiomers. Under similar conditions the racemate **2** was separated into the more mobile [(+)-**2**] and the less mobile [(−)-**2**] enantiomers.

The absolute stereochemistry of the separated enantiomers was established by correlation to lactic acid. The more mobile (+)-enantiomer of compound **1** was converted into its bis-[(*R*)-MTPA] ester,* which was then ozonized. The product was treated with diazomethane and analysed by TLC in comparison with authentic samples of the (*R*)-MTPA esters of (*R*)- and (*S*)-methyl lactates. Formation of the (*R*)-MTPA ester of methyl (*R*)-lactate, but not that of the (*R*)-MTPA ester of methyl (*S*)-lactate, was detected. This was further verified by GLC. It is therefore established that the (+)-enantiomer of compound **1** has (*R*)-stereochemistry and the (−)-enantiomer therefore (*S*)-stereochemistry. Similarly, degradation of the less mobile (−)-enantiomer of compound **2** afforded the (*R*)-MTPA ester of (*S*)-methyl lactate. Thus, the absolute stereochemistry of (−)-**2** was determined to be (*S*). Physical properties of the racemic and optically active isomers **1** and **2** prepared in the present study, along with data for the literature materials, are summarized in Table 2.

Kigelinone from *Kigelia pinnata* was reported to have $[\alpha]_D$ −17.5°, whereas the substance from *Tabebuia cassinoies* Lam. DC. is not optically active (Table 2). The compounds **1** and **2**, recently isolated from the same plant, were claimed as the (−)- and (±)-forms, respectively. It is therefore interesting to examine the enantiomeric purity of our sample from *T. impetiginosa*. The mixture of compounds **1** and **2** was analysed by HPLC on a

* MTPA = α-methoxy-α-trifluoromethyl-α-phenylacetyl.



Scheme 1 *Reagents and conditions:* i, Fremy's salt, 0 °C; ii, 10% aq. $Na_2S_2O_4$; iii, DHP, PPTS; iv, BuLi, −78 °C; then MeCHO, −78 °C; v, PTSA; vi, Ag_2O; vii, 1-(trimethylsiloxy)cyclohexa-1,3-diene; viii, NaH, 0 °C; then saturated aq. NH_4Cl; ix, 90 °C; x, myristoyl chloride, pyridine; xi, chromatographic separation; xii, 0.1 mol dm^{-3} NaOH

chiral column (CHIRALCEL OJ). The HPLC profile is illustrated in Fig. 2, from which the ratio of (*R*)- to (*S*)-enantiomer of isomer **1** was calculated to be 1:23, whereas that of isomer **2** was 3:1. At present we are not sure whether the plant biosynthesizes isomers **1** and **2** in the observed proportions or whether one of the enantiomers of the once formed racemic mixture is more rapidly metabolized. A similar instance of this type was reported with some other furanonaphthoquinones.[3] The furanonaphthoquinones **1** and **2** (racemic mixture) showed

Table 2 Physical properties of the furanonaphthoquinones **1** and **2**

Compound	M.p. (°) (from AcOEt)	$[\alpha]_D$ (°) (in $CHCl_3$)
Racemic **1**	161–162	
(*R*)-**1**	171–173	+16.9 (*c* 0.12)
(*S*)-**1**	169–170	−16.5 (*c* 0.16)
Kigelinone **1** in ref. 2	180–181[a]	−17.5
1 in ref. 5	156.6–157.5[b]	0
(−)-**1** in ref. 7	156–158	−16.6 (*c* 0.1[c])
Racemic **2**	160	
(*R*)-**2**	184–186	+11.4 (*c* 0.18)
(*S*)-**2**	178–180	−11.3 (*c* 0.13)
Racemic **2** in ref. 7	145–147	0 (*c* 0.007[c])

[a] From ethanol. [b] From methanol. [c] In methanol.

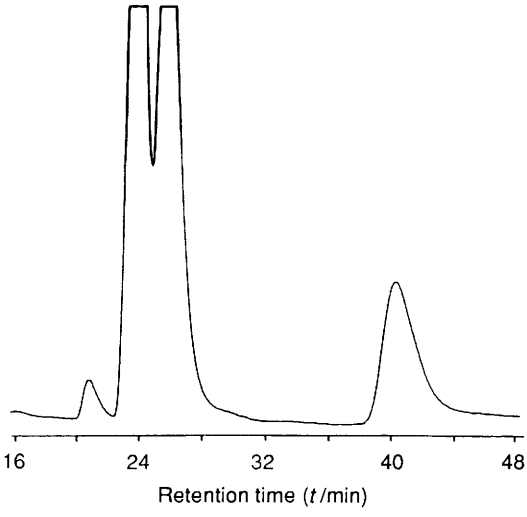


Fig. 2 HPLC chromatogram of the natural furanonaphthoquinones **1** and **2**. Authentic samples were eluted as follows: (*R*)-**1**, 20.7 min; (*S*)-**1**, 23.5 min; (*R*)-**2**, 25.5 min; (*S*)-**2**, 40.2 min. Conditions: column CHIRALCEL OJ 25 cm × 4.6 mm i.d., solvent hexane–ethanol (3:1), flow rate 0.5 cm^3 min^{-1}; UV detection at 240 nm. The calculated ratios (*R*)-**1**:(*S*)-**1** and (*R*)-**2**:(*S*)-**2** were based on computer-output values of the peak areas.

almost the same cytotoxic activity against the L-1210 cultured cell line in *vitro* (IC_{50}: **1**, 12 μg cm^{-3}; and **2**, 16 μg cm^{-3}; however, no significant activity was observed against mouse leukaemia P-388 i.p.–i.p. assay system. Further, there were no differences between the (*R*) and (*S*) enantiomers of compounds **1** and **2** in their *in vitro* cytotoxic activity against L-1210 cells.

Experimental

M.p.s were determined on a Yazawa BY-1 micromelting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained on a JEOL GX-270 or GSX-500 spectrometer for solutions in $CDCl_3$ or $(CD_3)_2SO$ with tetramethylsilane as internal reference. Chemical shifts in the ^{13}C NMR spectra were expressed in relation to $CDCl_3 = \delta_C$ 77.0 or $(CD_3)_2SO = \delta_C$ 39.5, and *J*-values are given in Hz. IR and UV spectra were obtained on a JASCO IR-810 and a Shimadzu UV-200 spectrometer, respectively. Cytotoxic and anti-tumour activities were estimated as reported previously.[11]

Isolation.—The powdered bark of *T. impetiginosa* (2.6 kg), collected at São Paulo, Brazil, was extracted with methanol (7.5 dm^3) at room temperature. The concentrated extract (450 g) was partitioned between butan-1-ol and water (pH 8.0). The butanol fraction was concentrated and the residue (201 g) was chromatographed on a silica gel column. The fraction (5 g)

eluted with chloroform–methanol (97:3) was then separated by silica gel preparative TLC (PLC) [chloroform–methanol (95:5) as a developing solvent]. The fraction eluted from the band having R_f ~0.7 was further separated on reversed-phase PLC [methanol–water (4:1)]. The fraction eluted from the yellow band having R_f ~0.3 was finally separated on a reversed-phase HPLC column [SHIMPAC CLC-ODS, 15 cm × 6 mm i.d.; solvent methanol–water (4:1), flow rate 0.5 cm^3 min^{-1}; detection at 254 nm]. Collection and concentration of the peak eluted at 19.4 min afforded a mixture of compounds **1** and **2** (15 mg) as a solid, m.p. 141–143 °C, ν_{max}(KBr)/cm^{-1} 3410, 1675, 1640, 1600, 1575 and 1530; λ_{max}(MeOH)/nm 247, 295 and 412; *m/z* 258 (M^+, 72%), 243 (M − Me, 100), 216 (M − CH_2CO, 46), 215 (M − CH_3CO, 61), 187 (M − CH_3CO − CO_3, 48) and 43 (90). For the ^1H and ^{13}C NMR spectra ($CDCl_3$), see the text. The yield of the mixture was ~0.04% of the bark.

From a slightly more mobile zone in the reversed-phase preparative HPLC separation, the known 2-ethylnaphtho-[2,3-*b*]furan-4,9-dione [*m/z* 226 (M^+)] and 2-acetylnaphtho-[2,3-*b*]furan-4,9-dione [*m/z* 240 (M^+)] were isolated. Their ^1H NMR spectral data were in good agreement with those reported.[3,4]

4,7-*Bis*-(*tetrahydropyran*-2-*yloxy*)*benzo*[b]*furan* **5**.—To a solution of KH_2PO_4 (9.6 g, 70.5 mmol) in water (2 dm^3)–acetone (423 cm^3) was added Fremy's salt (49.6 g, 185 mmol) and the resulting suspension was cooled to 0 °C. A solution of 7-hydroxybenzo[*b*]furan **3**[10] (10.0 g, 74.6 mmol) in acetone (100 cm^3) was added dropwise to the suspension and the mixture was stirred for 1 h at 0 °C before being extracted with ethyl acetate (500 cm^3 × 3), and the combined extracts were washed with 10% aq. $Na_2S_2O_4$ (1 dm^3) and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave crude 4,7-dihydroxybenzo[b]furan (10 g).

To a solution of crude hydroquinone in CH_2Cl_2 (100 cm^3) were added dihydropyran (DHP) (300 cm^3) and pyridinium toluene-*p*-sulphonate (PPTS) (catalytic amount) and the mixture was stirred for 12 h at room temperature, diluted with water (300 cm^3), and extracted with CH_2Cl_2 (200 cm^3 × 2). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on silica gel (CH_2Cl_2) to give the *benzofuran* **5** (18.7 g, 79%) as an oil, ν_{max}(neat)/cm^{-1} 1490 (C=C); δ_H($CDCl_3$) 3.55–3.65 (2 H, m, 6′-H^a of THP), 3.92–4.10 (2 H, m, 6′-H^b of THP), 5.45–5.48 (1 H, m, 2′-H of THP), 6.82 (1 H, d, *J* 8.8, benzene ring H), 6.88 (1 H, d, *J* 1.6, 3-H), 6.96 (1 H, d, *J* 8.8, benzene ring H) and 7.55 (1 H, d, *J* 1.6, 2-H).

1-{4,7-*Bis*(*tetrahydropyran*-2-*yloxy*)*benzo*[b]*furan*-2-*yl*}-*ethanol* **6**.—n-BuLi (1.5 mol dm^{-3} solution in hexane; 47.6 cm^3, 71.4 mmol) was added dropwise to a solution of the benzofuran **5** (18.5 g) in THF (86 cm^3) at −78 °C under nitrogen and the solution was stirred for 1 h at the same temperature. Acetaldehyde (50 cm^3) was added dropwise and the reaction mixture was stirred for 1 h at −78 °C before being quenched by saturated aq. NH_4Cl and extracted with CH_2Cl_2 (100 cm^3 × 3). The combined extracts were washed with water, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel [hexane–ethyl acetate (4:1)] to give the *alcohol* **6** (19.1 g, 89%) as an oil, ν_{max}(neat)/cm^{-1} 3300 (OH) and 1490 (C=C); δ_H($CDCl_3$) 1.55–2.15 (12 H, m, THP Hs and Me), 3.45 (1 H, br, OH), 3.55–3.65 (2 H, m, 6′-H^a of THP), 3.92–4.10 (2 H, m, 6′-H^b of THP), 5.10 (1 H, m, C*H*OH), 5.45–5.48 (1 H, m, 2′-H of THP), 5.55–5.60 (1 H, m, 2′-H of THP), 6.82 (1 H, d, *J* 8.8, benzene ring H), 6.90 (1 H, d, *J* 1.6, 3-H) and 6.95 (1 H, d, *J* 8.8, benzene ring H).

2-(1-*Hydroxyethyl*)*benzo*[b]*furan*-4,7-*dione* **7**.—To a solution of the alcohol **6** (25.0 g, 69.2 mmol) in methanol (50 cm^3) was added toluene-*p*-sulphonic acid (PTSA) monohydrate (1.08 g, 6.91 mmol) and the reaction mixture was stirred for 1 h at room temperature, diluted with water (100 cm^3), and extracted with ethyl acetate (50 cm^3 × 2). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated to dryness to give crude 2-(1-hydroxyethyl)benzo[*b*]furan-4,7-diol.

To a solution of the crude product in THF (100 cm^3) were added Ag_2O (16.0 g, 69.2 mmol) and $MgSO_4$ (16 g). The mixture was stirred for 1 h at room temperature and then filtered. Concentration of the filtrate, followed by recrystallization from CH_2Cl_2–hexane, gave the *quinone* **7** (9.97 g, 75.4%) as yellow crystals, m.p. 87–88 °C; ν_{max}(KBr)/cm^{-1} 3500 (OH) and 1670 (C=O); δ_H($CDCl_3$) 1.60 (3 H, d, *J* 6.5, Me), 3.45 (1 H, br, OH), 5.00 (1 H, m, C*H*OH) and 6.70 (3 H, br s, ArH) (Found: C, 62.3; H, 4.1. $C_{10}H_8O_4$ requires C, 62.50; H, 4.20%).

5-*Hydroxy*-2-(1-*hydroxyethyl*)*naphtho*[2,3-b]*furan*-4,9-*dione* **1** *and* 8-*Hydroxy*-2-(1-*hydroxyethyl*)*naphtho*[2,3-b]*furan*-4,9-*dione* **2**.—A solution of the quinone **7** (3.0 g, 23.4 mmol) and 1-(trimethylsiloxy)cyclohexa-1,3-diene (3.9 g, 23.4 mmol) in CH_2Cl_2 (20 cm^3) was stirred for 1 day at room temperature. Evaporation of the solution under reduced pressure gave the Diels–Alder adducts **8** and **9**.

A solution of the crude adducts in THF (10 cm^3) was added dropwise to a suspension of NaH (60% in Nujol; 2.5 g, 62.4 mmol) in THF (20 cm^3) at 0 °C and the mixture was stirred for 30 min at the same temperature before being quenched by the addition of saturated aq. NH_4Cl, acidified with dil. HCl, and then extracted with ethyl acetate (50 cm^3 × 3). The combined extracts were washed with water (50 cm^3), dried over $MgSO_4$, and concentrated to dryness. The residue was dissolved in THF (30 cm^3) and, after the addition of Ag_2O (3.62 g, 15.6 mmol) and anhydrous $MgSO_4$ (3.62 g), was stirred for 1 h at room temperature. The mixture was filtered and the filtrate was evaporated to give the quinones **10** and **11**.

The crude quinones were heated at 90 °C for 30 min to give the crude title compounds **1** and **2** and the product was chromatographed on silica gel [hexane–ethyl acetate (4:1)] to give a mixture of compounds **1** and **2** (2.62 g, 65%) as orange crystals, m.p. 141–143 °C (from CH_2Cl_2–hexane); ν_{max}(KBr)/cm^{-1} 3450 (OH) and 1670 (C=O).

Separation of Compounds **1** *and* **2**.—Myristoyl chloride (4.6 g, 18.7 mmol) was added to a solution of the mixture of compounds **1** and **2** (2.0 g, 7.75 mmol) in pyridine (24 cm^3) and the mixture was stirred for 30 min at room temperature before being concentrated under reduced pressure. Chromatography on silica gel [hexane–ethyl acetate (20:1)] gave the less polar diester **12** (1.14 g) and the more polar diester **13** (3.53 g).

To a stirred solution of compound **12** (1.14 g, 1.68 mmol) in THF (20 cm^3) at 0 °C was added dropwise 0.1 mol dm^{-3} aq. NaOH (40 cm^3) and the reaction mixture was warmed to room temperature. After 4 h the mixture was neutralized by the addition of dil. HCl and was then extracted with ethyl acetate (50 cm^3 × 3). The combined extracts were washed with brine, dried over $MgSO_4$, and evaporated to give a crude product. This was washed with hexane to afford the 5-*hydroxy compound* **1** (0.36 g, 18% from the mixture of **1** and **2** as orange crystals, λ_{max}(MeOH)/nm (log ε) 230sh, 247 (4.45), 295 (3.85) and 400 (3.75); δ_C[$(CD_3)_2SO$] 186.2 (C-4), 172.0 (C-9), 166.9 (C-2), 161.1 (C-5), 151.2 (C-9a), 136.5 (C-7), 132.6 (C-8a), 130.7 (C-3a), 124.7 (C-6), 119.2 (C-8), 115.1 (C-4a), 103.0 (C-3), 61.9 (C-1′) and 21.6 (C-2′) (Found: C, 64.9; H, 3.9. $C_{14}H_{10}O_5$ requires C, 65.12; H, 3.90%).

The 8-hydroxy compound **2** (1.11 g, 55.5% from the mixture of **1** and **2** was obtained as orange crystals from diester **13** in the same manner as described for compound **1**, λ_{max}(MeOH)/nm

(log ε) 235 (4.52), 250sh, 295, (3.84) and 420 (3.87); δ_C-[$(CD_3)_2SO$] 179.5 (C-4), 177.7 (C-9), 167.3 (C-2), 161.3 (C-8), 150.5 (C-9a), 136.4 (C-6), 133.3 (C-3a), 131.1 (C-4a), 124.7 (C-7), 119.4 (C-5), 114.7 (C-8a), 103.6 (C-3), 61.9 (C-1′) and 21.6 (C-2′) (Found: C, 64.9; H, 3.9).

The NMR (in $CDCl_3$) and physical data are listed in Tables 1 and 2. The ^{13}C assignments given in Table 1 were based on the INEPT, C–H COSY, COLOC, and HMBC experiments recorded in $(CD_3)_2SO$ as solvent.

Optical Resolution of Compounds **1** *and* **2**.—The resolution was achieved by preparative HPLC under the following conditions: column CHIRALCEL OJ 50 × mm i.d. (Daicel Co.); solvent hexane–ethanol (5:1); flow rate 60 cm^3 min^{-1}; UV detection at 240 nm.

Injection of the racemate **1** (200 mg) in THF (5 cm^3) afforded the more mobile enantiomer (80 mg, retention time 64 min) and the less mobile enantiomer (100 mg, retention time 76 min). The racemate **2** (200 mg) was similarly separated into the more mobile enantiomer (85 mg, retention time 56 min) and the less mobile enantiomer (100 mg, retention time 70 min).

These optically active samples were crystallized from ethyl acetate and their physical properties are included in Table 2.

Determination of Absolute Stereochemistry of Compounds **1** *and* **2**.—(*S*)-MTPA acid chloride (30 mm^3), prepared from (*R*)-MTPA acid, was added to a solution of compound (+)-**1** (10 mg) in dry pyridine (100 mm^3) and the mixture was stirred for 2 h at room temperature. Ice chips were added and the mixture was extracted with ethyl acetate. The organic layer was washed successively with dil. HCl, saturated aq. $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel [hexane–ethyl acetate (10:1)] to afford the bis-MTPA ester as an oil, δ_H 1.71 (3 H, d, *J* 6.3, 2′-H_3), 3.54 (3 H, d, *J* 1.4, OMe), 3.86 (3 H, d, *J* 1.0, OMe) and 6.26 (1 H, d, *J* 6.8, 1′-H).*

Ozone was bubbled through a solution of the MTPA diester (10 mg) in methanol at −78 °C until the starting material disappeared on TLC analysis. Argon was then bubbled through the solution to remove residual ozone and the solvent was then evaporated at reduced pressure. The residue was treated with an excess of ethereal diazomethane at room temperature for 30 min and then the solvent was evaporated off. The residue was analysed by TLC [hexane–ethyl acetate (7:1), developed twice]. Under UV light the MTPA ester of (*R*)-methyl lactate (R_f 0.67), but not the MTPA ester of (*S*)-methyl lactate (R_f 0.60), was detected. The band corresponding to the region of the MTPA esters of (*RS*)-methyl lactate was scraped off and eluted with AcOEt. The eluate was analysed by GC-MS [Shimadzu GC-MS DF-9020 attached to an OV-1 capillary column (Shimadzu CBP-1, 15 m), oven temperature 130 °C, injection temperature 190 °C]. The retention times for the authentic (*R*)- and (*S*)-ester were 12.0 and 12.8 min, respectively, whereas the above sample exhibited a peak only at 12.0 min. The mass spectra of the authentic (*R*)- and (*S*)-ester as well as that of the aforementioned sample were indistinguishable, *m/z* 320 (M^+), 300 (M − HF), 251 (M − CF_3), 216, 189 [CCF_3(OMe)Ph, base peak].

Compound (−)-**2** was transformed similarly into the (*S*)-ester, which was characterized by both TLC and GC-MS. The intermediate MTPA-ester showed δ_H 1.77 (3 H, d, *J* 6.6, 2′-H_3), 3.50 (3 H, d, *J* 1.2, OMe), 3.86 (3 H, d, *J* 1.0, OMe) and 6.25 (1 H, d, *J* 6.8, 1′-H).

Enantiomeric Purity of Natural Compounds **1** *and* **2**.—The natural sample mixture of compounds **1** and **2** (no crystallization was attempted) was analysed by HPLC by using a chiral column. For HPLC conditions see the legend to Fig. 2.

* Primed locants refer to the acyloxyethyl side-chain.

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