An Efficient and Stereoselective Dearylation of Asarinin and Sesamin Tetrahydrofurofuran Lignans to Acuminatolide by Methyltrioxorhenium/H_2O_2 and UHP Systems

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The synthesis of stereoisomers of acuminatolide is rare and requires complex and time-consuming multistep procedures. Asarinin (**1**) and sesamin (**2**), two diasteromeric tetrahydrofurofuran lignans, are efficiently mono-dearylated by methyltrioxorhenium (MTO, **I**) and hydrogen peroxide (H_2O_2) or urea hydrogen peroxide adduct (UHP) as primary oxidant to give (−)-(7*R*,8′*R*,8*R*)-acuminatolide (**3A**) and (+)-(7*S*,8*R*,8′*R*)-acuminatolide (**3B**), respectively, in high yield and diastereoselectivity (de >98%). The oxidation of **1** was also performed with novel heterogeneous catalysts based on the heterogenation of MTO on poly(4-vinylpyridine) and polystyrene resins. In these latter cases **3A** was obtained with a different yield and selectivity depending on the physical−chemical properties of the support. Cytotoxic effects of **3A** and **3B** in mammalian cell lines *in vitro* are also reported.

Tetrahydrofurofuran lignans are one of the largest groups of lignans[1] whose members are of special interest owing to their powerful antitumoral,[2] antiinflammatory,[3] antioxidant,[4] and insecticide properties,[5] including phosphodiesterase inhibition[6] and hypocholesterolemic activities in humans.[7] The biological activities of lignans can be modified[8] by modulating the degree of oxidation at specific benzylic and arylic positions,[9] as observed in the cytochrome P 450-mediated oxidations in mammalian cells, where stable metabolites with aliphatic and aromatic hydroxylation and increased pharmacological properties are produced.[10] Since general and selective methods for the oxidation of tetrahydrofurofuran lignans are still lacking,[11] novel synthetic strategies are needed to prepare highly oxidized derivatives for the evaluation of their biological activities. A catalyst useful for this purpose is methyltrioxorhenium ($MeReO_3$, MTO, compound **I**),[12] which has been used in several organic transformations including the oxidation of hydrocarbons[13] and the hydroxylation of arene derivatives[14] both in combination with H_2O_2 or urea hydrogen peroxide adduct (UHP). Moreover, MTO retains its high catalytic activity after heterogenation on readily available and low-cost polymers.[15] Irrespective of the applied experimental conditions, the activation of H_2O_2 or UHP by MTO proceeds through the formation of a monoperoxo [$MeRe(O)_2O_2$] and a bis-peroxo [$MeReO(O_2)_2$] η^2-rhenium complex.[16] The transfer of a single oxygen atom from these peroxo η^2-rhenium complexes to substrate is achieved by a butterfly-like transition state similar to that previously suggested for cyclic organic peroxides such as dimethyldioxirane (DMDO).[17] Recently, we reported an efficient procedure to prepare highly oxidized aryltetralin lignans by MTO/H_2O_2 systems,[18] in which the selective oxidation of podophyllotoxin at the C-4 benzylic position and the simultaneous ring-opening of the D-ring lactone moiety increased the anti-topoisomerase II activity while causing the disappearance of the undesired inhibition of tubulin. On the basis of this study, we report here the first general and selective procedure for the oxidative mono-dearylation of the tetrahydrofurofuran lignans asarinin (**1**)[19] and sesamin (**2**)[20] to (−)-(7*R*,8′*R*,8*R*)-acuminatolide (**3A**) and (+)-(7*S*,8*R*,8′*R*)-acuminatolide (**3B**), respectively (the numbering of carbon atoms in tetrahydrofurofuran lignan derivatives is as in ref 1b). These transformations proceeded in high yields and were diastereoselective (de >98%) using H_2O_2 or UHP as primary oxidants. Acuminatolide **3** is a natural lignan detected in low amounts in the aerial parts of Australian *Helichrysum acuminatum*. To date, only few syntheses of stereoisomers of acuminatolide have been reported. These syntheses require complex and time-consuming multistep procedures.[21] As a general application of this procedure, the oxidation of **1** was also performed with novel heterogeneous catalysts based on the heterogenation of MTO (**I**) on poly(4-vinylpyridine) (PVP-2/MTO, **II**) and polystyrene (PS-2/MTO, **III**) 2% cross-linked with divinylbenzene (Figure 1).[15a] The cytotoxic activities of **3A**, **3B**, and its parent compound **1** in mammalian cell lines *in vitro* are also reported.

Results and Discussion

For the oxidation of asarinin (**1**) and sesamin (**2**) with MTO (**I**) under homogeneous conditions, tetrahydrofurofuran lignans (1.0 mmol) were added to a solution of MTO (0.1 mmol) in CH_2Cl_2/CH_3CN or CH_2Cl_2/EtOH (5.0 mL). The primary oxidant, H_2O_2 or UHP (3.0 mmol), was added to the reaction mixture in several batches over a period of 24 h at room temperature.

The results of these oxidations are summarized in Table 1 and Scheme 1. In the absence of MTO less than 2% of conversion of substrate was observed. Treatment of **1** with the MTO/H_2O_2 system in CH_2Cl_2/CH_3CN afforded **3A** as the main reaction product in 60% yield (68% conversion of substrate) in addition to lignan **4**, a byproduct of ring-opening of the lactone moiety in **3A** (Scheme 1, Table 1, entry 1). The keto-lignan **5** and 3,4-methylenedioxyphenol **6** (not shown) were also detected in low yields by GC-MS analyses (<3%). The configuration of the chiral centers in **3A** were confirmed by the NOEs between H-8 and H-8′, as well as between H-7 and H-8. In agreement with the high reactivity of MTO in protic solvents,[12] the same reaction performed in CH_2Cl_2/EtOH gave **3A** in 79% yield in the presence of minor amounts of **4** (Table 1, entry 2). However, the best results were obtained when MTO was dissolved in CH_2Cl_2/CH_3CN with UHP as the primary oxidant. In this case **3A** was isolated as the only recovered product in 87% yield and 65% conversion of substrate (Table 1, entry 3).

Irrespective of the experimental conditions, **3A** was diastereoisomerically pure (de >98%), suggesting that the peroxo η^2-rhenium complexes of MTO were able to approach the tetrahydro furofuran skeleton at the benzylic C-7′ position in a highly regio- and

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Scheme 1

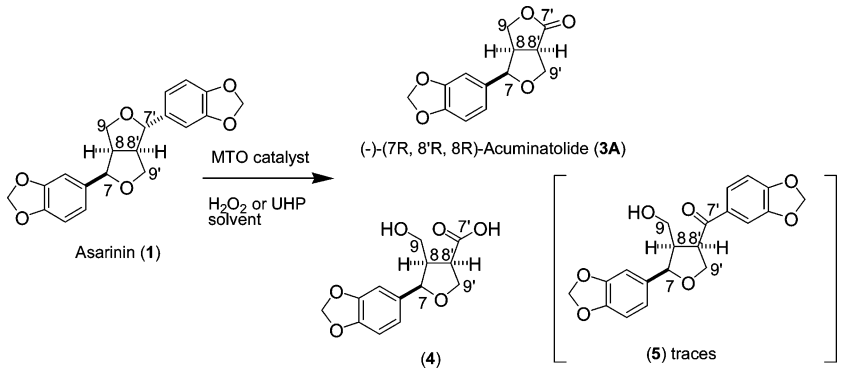


Table 1. Oxidation of Asaranin **1** and Sesamin **2** to (−)-Acuminatolide **3** with Homogeneous and Heterogeneous MTO/H_2O_2 or UHP Systems

entry	substrate	catalyst	oxidant	solvent	conversion (%)	product(s)	yield (%)
1	**1**	MTO	H_2O_2	CH_2Cl_2/CH_3CN	68	**3**, (**4**), [**5**]	68, (25), [<5]
2	**1**	MTO	H_2O_2	CH_2Cl_2/EtOH	88	**3**,(**4**)	79, (13)
3	**1**	MTO	UHP	CH_2Cl_2/CH_3CN	65	**3**	87
4	**2**	MTO	H_2O_2	CH_2Cl_2/CH_3CN	49	**3**	85
5	**2**	MTO	UHP	CH_2Cl_2/CH_3CN	41	**3**	82
6	**1**	PVP-2/MTO	H_2O_2	CH_2Cl_2/CH_3CN	51	**3**	85
7	**1**	PVP-2/MTO	H_2O_2	CH_2Cl_2/EtOH	60	**3**	89
8	**1**	PS-2/MTO	H_2O_2	CH_2Cl_2/CH_3CN	50	**3**, (**4**)	61, (20)
9	**1**	PS-2/MTO	H_2O_2	CH_2Cl_2/EtOH	55	**3**, (**4**)	56, (24)

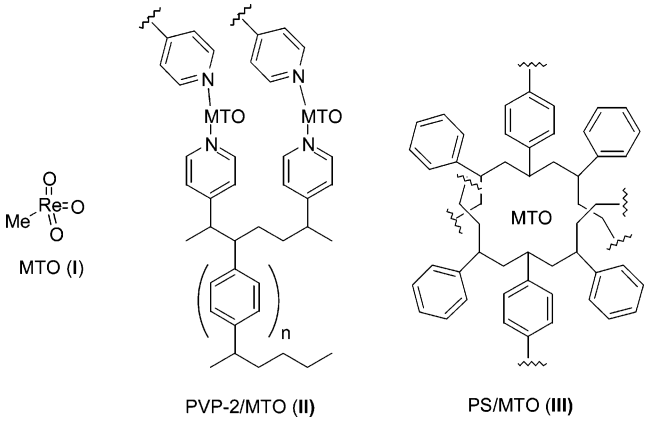
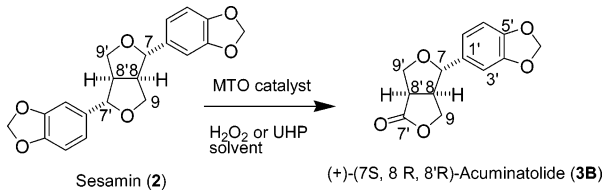


Figure 1. Structures of MTO (**I**) and poly(4-vinylpyridine) (PVP-2/MTO, **II**) and polystyrene (PS-2/MTO, **III**) 2% cross-linked (with divinylbenze) heterogeneous catalysts.

stereoselective process. The oxidation at the benzylic C-7 position to give the known stereoisomer **3B** did not operate under our experimental conditions. On the basis of the high reactivity of MTO toward a tertiary C−H bond, it is reasonable to suggest that the oxidation of **1** proceeded via an initial oxygen atom insertion into the C7′−H benzyl ethereal bond, followed by ring-opening of the hemiacetal moiety to give **5**.[22] Successive Bayer−Villiger rearrangement of **5** and lactonization then afford **3A**. This hypothesis is in accordance with the presence of traces of 3,4-methylenedioxyphenol **6** and with the known catalytic properties of MTO in Bayer−Villiger rearrangement and lactonization reactions.[14b,15a] It is significant to note that a similar reaction pathway is operative in native Australian *Helichrysum acuminatum*, where acuminatolide is effectively produced from its immediate biogenetic precursor, acuminatin, by regioselective oxidation of one selected benzylic carbon center, loss of the corresponding aryl group, and lactonization.[23] The selective formation of **3A**, and the absence of **3B**, produced by MTO attack from the less hindered endo face of the terahydrofurofuran skeleton, suggests that in this initial oxidation step parameters other than simple steric hindrance need to be considered. Detailed data on the stereoselectivity of oxidation of

Scheme 2



isomeric cycloalkanes with MTO have not previously been reported. In our case, a π-interaction between MTO and the aromatic moiety at the exo face of the tetrahydrofurofuran ring may be invoked. This may explain the observed selectivity for the oxidation at the axial C-7′ position despite the fact that a different coordinating ability of the two oxygens of the furofuran ring toward the rhenium atom cannot be completely ruled out. This hypothesis finds support at the different values for the ν(ReO) stretching vibration frequencies that have been observed for MTO when microencapsulated in polystyrene, compared to other polymeric resins due to specific π-interactions of the rhenium atom with the aromatic moieties of the support.[15a]

We also carried out the oxidation of sesamin (**2**), which is characterized by both the C-7 and C-7′ aryl groups in equatorial orientations, under similar experimental conditions. The oxidation of **2** with the MTO/H_2O_2 system in CH_2Cl_2/CH_3CN afforded **3B** as the only recovered product in 85% yield and 41% conversion of substrate (Scheme 2, panel b, Table 1, entry 4). Similar results were obtained with UHP as the primary oxidant (Table 1, entry 5). In the latter case, only compound **3B** was produced (de >98%) irrespective of whether the C-7 or C-7′ benzylic position is oxidized.

The oxidation of asarinin (**1**) with PVP-2/MTO (**II**) and PS-2/MTO (**III**) was then studied in an effort to evaluate the general applicability of this transformation under heterogeneous conditions.[15a] The heterogenation of MTO on polymeric supports is an important tool because it allows for an easier recovery of the catalyst, decreases the toxicity of the reaction byproducts and wastes, and sometimes tunes the selectivity depending on the physicochemical properties of the support. The oxidations were carried out by treating **1** (1.0 mmol) with the appropriate catalyst (125 mg; loading factor 1.0) and H_2O_2 (8.0 mmol) in CH_2Cl_2/CH_3CN or CH_2Cl_2/EtOH

Table 2. Cytotoxicity of Asaranin **1**, (7*R*,8′*R*,8*R*)-Acuminatolide (**3A**), (+)-(7*S*,8*R*,8′*R*)-Acuminatolide (**3B**), and Lignan **4** against the Murine Fibroblast Cell Line (3T3 cells)

entry	compound	IC_{50} (μM)[a]
1	**1**	0.2
2	**3A**	>20
3	**3B**	>20
4	**4**	3.0

(Table 1) at room temperature. As a general reaction pattern, the oxidation of **1** with catalysts **II** and **III** required more severe reaction conditions (large excess of H_2O_2, 60 °C, and 3 days) than MTO, thus suggesting the presence of a kinetic barrier for the approach of **1** to rhenium polymeric compounds (Table 1, entries 6 and 7 versus entries 1 and 2). Alternatively, the selectivity of catalyst **II** with H_2O_2 in both CH_2Cl_2/CH_3CN and CH_2Cl_2/EtOH mixtures was higher than that observed for MTO under similar experimental conditions, affording **3A** as the only recovered product in high yield (Table 1, entries 6 and 7 versus entries 1 and 2). It is likely that this high selectivity is due to the known buffering effect of the pyridinyl groups on the Lewis and Brönsted acidity of MTO. In accordance with this hypothesis, the oxidation of **1** with catalyst **III**, which lacks pyridinyl moieties, afforded appreciable amounts of **4** as a side-product (Table, entries 8 and 9).

Cytotoxicity Assay. With the exception of data on the incorporation of tritiated thymidine into human leukemia (HL-60) cells in the presence of asarinin and sesamin isolated from the northern prickly ash, *Zanthoxylum americanum*, limited data are available about the cytotoxicity of tetrahydrofurofuran lignans and of products of their oxidative modification.[24]

The cytotoxic effects of **3A**, **3B**, and of the ring-opened derivative **4** were evaluated using the murine fibroblast cell line (3T3 cells), the plasmocytoma murine cell line (NSO cells), normal human lymphocytes PHA-stimulated, and the human lymphoblastoid cell line (Daudi cells) and compared with that of the parent tetrahydrofurofuran lignan, asarinin (**1**). The IC_{50} values (μM) reported in Table 2 refer to the fibroblast cell line and are representative of all other cell lines studied.[25]

Acuminatolide stereoisomers **3A** and **3B** showed a lower cytotoxic effect ($IC_{50} > 20$ μM) than **1** (Table 2, entry 2 versus 1), thus suggesting a specific role of both aryl moieties of the tetrahydrofurofuran scaffold on the toxicity of lignan derivatives. It is worth noting that **4** was more cytotoxic than **3A** and **3B**, with an $IC_{50} < 4$ μM (Table 2, entry 4). These data are in agreement with data previously reported on the effect of the lactone moiety on the biological activity of aryltetralin lignans such as isopodophyllotoxone derivatives.[18]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 343plus polarimeter. All commercial products were of the highest grade available and were used as such. Asarinin (Aldrich) and sesamin (Sigma) were used without further purification. All oxidations were performed on enantiomerically pure asarinin and sesamin. The absolute configurations of products **3A** and **3B** were assigned as (7*R*,8′*R*,8*R*)-acuminatolide (**3A**) and (+)-(7*S*,8*R*,8′*R*)-acuminatolide (**3B**) by comparison with authentic samples, by NOE experiments (see Discussion), and by considering that the reaction does not modify the enantiomeric purity of previously existing stereocenters in parent substrates. NMR spectra were recorded on a Bruker (200 MHz) instrument. TLC was carried out using Merck Kieselgel 60 F_{254}. When necessary, chromatographic purifications were performed on columns packed with normal silica gel, 230−400 mesh, using the flash technique (petroleum ether/EtOAc, 7:3). The reaction mixtures were silylated using BSTFA (bis[trimethylsilyl]trifluoroacetamide) and TMCS (trimethylchlorosilane) in pyridine and analyzed by GC-MS using a Shimadzu QP5000 GC-MS system equipped with a 17A GC, an AOC-20i autoinjector, and the GC/MS solution software A10.02 under the following conditions: Alltech AT-20 column (0.25 mm, 30 m), temperature program 100−280 °C at 10 °C min^{-1}, injector temperature 250 °C; detector (FID) temperature 300 °C, carrier flow helium. Mass spectra were recorded with an electron beam of 70 eV. Melting points (mp) were determined on a Reichert Kofler apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Paragon 500 FT-IR spectrometer.

Preparation of Heterogeneous MTO Catalysts II and III. Poly-(4-vinylpyridine)/MTO 2% cross-linked with divinylbenzene (PVP-2%/MTO **II**) and polystyrene/MTO 2% cross-linked with divinylbenzene (PS-2%/MTO **III**) catalysts were prepared as previously reported.[15a] MTO (77 mg, 0.3 mmol) was added to a suspension of the appropriate resin (600 mg) in EtOH (4 mL), or THF in the case of polystyrene. The mixture was stirred for 1 h using a magnetic stirrer. The solvent was removed by filtration, and the solid residue was washed with EtOAc and finally dried under high vacuum. In every case, MTO was completely embedded within the polymer. This was confirmed by spectroscopic analyses of the residue obtained after evaporation of the organic layers. The catalysts were used without any additional purification.

Oxidation of Asarinin and Sesamin. General Procedures. (a) Homogeneous oxidation with MTO (I). Tetrahydrofurofuran lignans **1** and **2** (1.0 mmol) were added to a solution of MTO (**I**) (0.1 mmol) in CH_2Cl_2/CH_3CN or CH_2Cl_2/EtOH (5.0 mL). H_2O_2 or UHP (3.0 mmol) was added to the reaction mixture in several batches at room temperature over a period of 24 h. The stirred solution became yellow due to the formation of the peroxy species. The reaction mixture was stirred at room temperature until no more starting material was detected by TLC. After removal of the solvent under reduced pressure the crude reaction mixture was mixed with CH_2Cl_2. For oxidations performed with UHP, the urea was filtered off at the end of the reaction to afford the crude product as a pale yellow oil. The crude product was purified on columns packed with normal silica gel, 230−400 mesh, using the flash technique (petroleum ether/EtOAc, 7:3).

(b) Heterogeneous Oxidation with MTO Catalysts II and III. Tetrahydrofurofuran lignans **1** and **2** (1.0 mmol) were added to a suspension of the appropriate catalyst (125 mg; loading factor 1.0) and H_2O_2 (8.0 mmol) in CH_2Cl_2/CH_3CN or CH_2Cl_2/EtOH (5.0 mL). The reaction mixture was stirred at room temperature until no more starting material was detected by TLC. At the end of the reaction the catalyst was recovered by filtration and washed with CH_2Cl_2. After removal of the solvent, under reduced pressure, the crude reaction mixture was obtained as a pale yellow oil. The crude product was purified by flash chromatography.

(−)-(7*R*,8′*R*,8*R*)-Acuminatolide (3A): colorless needles (EtOAc/hexane); mp 118 °C (lit.[21a,24] mp 118−119 °C); $[\alpha]^{25}_D$ −95.1 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1775 (CO), 1611, 1510, 1499 (C═C−Ar), 1245 (C−O) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 6.81 (br s, 1H, Ar−H), 6.76 (br s, 2H, Ar−H), 5.94 (s, 2H, OCH_2O), 4.61−4.59 (d, 1H, $J_{7',8'} = 6.75$, H-7′), 4.51−4.46 (dd, 1H, $J_{9'eq,9'ax} = 6.72$, $J_{8',9'eq} = 6.72$, H-9′eq), 4.37−4.29 (m, 2H, H-9ax and H-9′ax), 4.20−4.15 (dd, 1H, $J_{9eq,9ax} = 3.99$, $J_{8,9eq} = 3.75$, H-9eq), 3.46−3.39 (ddd, 1H, $J_{7',8} = 3.72$, $J_{8,9eq} = 4.02$, $J_{8,8'} = 4.02$, H-8), 3.12−3.03 (m, 1H, H-8′); ^{13}C NMR ($CDCl_3$, 200 MHz) δ (ppm) 178.05 (C-7′), 148.21 (C-3), 147.71 (C-4), 132.73 (C-1), 119.6 (C-6), 108.31 (C-5), 106.31 (C-2), 101.23 (OCH_2O), 86.02 (C-7), 70.03 (C-9), 69.77 (C-9′), 48.31 (C-8), 45.98 (C-8′); EIMS *m*/*z* (relative %) 248 [M^+] (100), 218 [$M - CH_2O^+$] (23), 163 (83), 150 [$ArCHO^+$] (74), 149 [$ArCO^+$] (89), 135 [$Ar_2CH_2^+$] (82), 121[Ar_2^+] (36); HREIMS *m*/*z* 248.0684 (calcd for $C_{13}H_{12}O_5$, 248.0685).

(+)-(7*S*,8*R*,8′*R*)-Acuminatolide (3B): colorless needles (EtOAc/hexane); mp 118 °C (lit.[21a,24] mp 118−119 °C); $[\alpha]^{25}_D$ +100.1 (c 0.3, $CHCl_3$) (lit.[21b] $[\alpha]^{25}_D$ +100.2 (*c* 0.11, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1775 (CO), 1610, 1510, 1500 (C═C−Ar), 1245 (C−O) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 6.80 (br s, 1H, Ar−H), 6.70 (br s, 2H, Ar−H), 5.95 (s, 2H, OCH_2O), 4.57 (d, 1H, $J_{7,8} = 6.8$, H-7), 4.46 (dd, 1H, $J_{9eq,9ax} = 6.8$, $J_{8,9eq} = 9.7$, H-9eq), 4.35−4.25 (m, 2H, H-9′ax and H-9ax), 4.14 (dd, 1H, $J_{9'eq,9'ax} = 3.6$, $J_{8',9'eq} = 9.3$, H-9′eq), 3.40 (ddd, 1H, $J_{7,8'} = 3.6$, $J_{8',9'eq} = 9.2$, $J_{8',8} = 9.0$, H-8′), 3.05 (m, 1H, H-8); ^{13}C NMR ($CDCl_3$, 200 MHz) δ (ppm) 178.16 (C-7′), 148.11 (C-3), 147.60 (C-4), 132.73 (C-1), 119.57 (C-6), 108.22 (C-5), 106.27 (C-2), 101.19 (OCH_2O), 85.92 (C-7), 69.93 and 69.77 (C-9/C-9′), 48.86 (C-8), 46.58 (C-8′); EIMS *m*/*z* (relative %) 248 [M^+] (100), 218 [$M - CH_2O^+$] (22), 163 (87), 150 [$ArCHO^+$] (80), 149 [$ArCO^+$] (99), 135 [$Ar_2CH_2^+$] (82), 121 [Ar_2^+] (36); HREIMS *m*/*z* 248.0683 (calcd for $C_{13}H_{12}O_5$, 248.0685).

Compound 4: colorless oil; $[\alpha]^{25}_{D}$ +94 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 6.88−6.70 (3H, m, Ar−H), 5.97 (2H, s, OCH_2O), 4.88 (1H, m, H-7′), 4.05 (2H, m, CH_2, H-9′), 3.72 (2H, m, CH_2, H-9), 2.6 (2H, m, H-8+H-8′); ^{13}C NMR ($CDCl_3$, 200 MHz) δ (ppm) 178.20 (C-7′), 147.75 (C-3), 147.60 (C-4), 133.45 (C-1), 120.81 (C-6), 108.19 (C-5), 101.53 (OCH_2O), 84.56 (C-7), 69.90 (C-9′), 61.09 (C-9), 47.79 (C-8′), 46.78 (C-8); EIMS *m*/*z* (relative %) 266 [M^+] (100); HREIMS *m*/*z* 266.0790 (calcd for $C_{13}H_{14}O_6$, 266.0790).

Compound 5: colorless oil (characterized as acetate derivative); ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.59 (dd, J = 8.1, 1.7 Hz, 1H), 7.20 (d, J = 1.7 Hz, 1H), 7.04 (d, J = 1.6 Hz, 1H), 6.94 (d, J = 8.1 Hz, 1H), 6.80 (dd, J = 8.0, 1.6 Hz, 1H), 6.65 (d, J = 8.0 Hz, 1H), 6.06 (s, 2H), 5.95 (s, 2H), 5.05 (m, 1H), 4.44 (m, 1H), 4.03 (m, 1H), 4.0 (m, 1H), 3.71 (m, 2H), 3.66 (m, 1H), 3.0 (m, 1H), 1.75 (s, 3H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ (ppm) 198.4 (C-7′), 170.7 (CO), 152.2 (C), 148.6 (C), 148.4 (C), 147.7 (C), 133.4 (C), 130.0 (C), 124.9 (CH), 119.9 (CH), 108.8 (CH), 108.0 (CH), 108.0 (CH), 107.0 (CH), 102.0 (OCH_2), 101.0 (OCH_2), 84.9 (C-7), 71.0 (C-9′), 62.0 (C-9), 49.0 (C-8), 48.7 (C-8), 20.8 (CH_3CO); EIMS *m*/*z* (relative %) 412 [M^+] (100); HREIMS *m*/*z* 412.3892 (calcd for $C_{22}H_{20}O_8$, 412.3894).

Cytotoxicity Assay. Cytotoxicity of compounds **3A** and **4** was evaluated using the murine fibroblast cell line (3T3 cells), the plasmocytoma murine cell line (NSO cells), normal human lymphocytes PHA-stimulated, and the human lymphoblastoid cell line (Daudi cells) and compared with that of the parent terahydrofurofuran lignan, asarinin **1**. Data were obtained by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test aimed to analyze cell proliferation in cells cultured in the presence of different compounds.[25,26] No difference was observed for cytotoxicity between data obtained by using the MTT test and DNA synthesis. The data processing included the Student's *t*-test with $p < 0.05$ taken as significance level.

Cell Lines. All cell lines were obtained from ATCC. The cells were cultured in RPMI 1640 supplemented with 5% FCS, 0.1 mM glutamine, 1% penicillin, and streptomycin. Cells were grown in Nunc clone plastic bottles (TedNunc, Roskilde, Denmark) and split twice weekly at different cell densities according to the standard procedure. 3T3 cells were grown as a monolayer and were split by using trypsin. Peripheral blood mononuclear cells (MNC) were separated from heparinized whole blood cells obtained from a healthy donor on a Fycoll-Hypaque gradient as previously described.[41] MNC thus obtained were washed twice with RPMI 1640 supplemented with 10% FCS, glutamine, and antibiotics, suspended at 200.000 viable cells/mL in medium containing, as mitogen, 5 μg/mL PHA (Sigma) and used in toxicity tests.

Chemicals. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Aldrich. It was dissolved at a concentration of 5 mg/mL in sterile PBS at room temperature, and the solution was further sterilized by filtration and stored at 4 °C in a dark bottle. SDS was obtained from Sigma. Lysis buffer was prepared as follows: 20% w/v of SDS was dissolved at 37 °C in a solution of 50% each of DMF and demineralized H_2O; the pH was adjusted to 4.7 by adding 2.5% of an 80% acetic acid and 2.5% 1 N HCl solution.

General Procedure. Cells were plated at different concentrations on flat bottom 96-well microplates (0.1 mL/well). Lymphocytes were plated out at 20 000 cells/well. 3T3 cells (murine fibroblast line) were plated at 10 000 cells/well. NSO cells (plasmocytoma murine cell line) were plated out at 3000 cells/well, and Daudi cells (human lymphoblastoid cell line) were plated at 300 cells/well. Twelve hours after plating, different concentrations of each compound were added to each well. After 48 h, the MTT assay was performed to analyze the cytotoxicity of the different compounds. Some experiments were performed by using confluent cells: compounds were added to the 3T3 monolayer 3 days after plating. Tests were then run as described above.

MTT/Formazan Extraction Procedure. A total of 20 μL of the 5 mg/mL stock solution of MTT was added to each well; after 2 h of incubation at 37 °C, 100 μL of the extraction buffer was added. After an overnight incubation at 37 °C, the optical densities at 570 nm were measured using a Titer-Tech 96-well multiscanner, employing the extraction buffer as the blank.

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