Isolation, structure, and synthesis of combretastatin A-2, A-3, and B-2[1]

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Further investigation of the South African tree *Combretum caffrum* (Combretaceae) for murine P388 lymphocytic leukemia (PS) cell-growth inhibitory substances has led to discovery of three new active constituents designated combretastatins A-2 (**5***a*, PS ED_{50} 0.027 μg/mL), A-3 (**5***b*, PS ED_{50} 0.026 μg/mL), and B-2 (**3***b*, PS ED_{50} 0.32 μg/mL). Both combretastatins A-2 and A-3 were found to markedly inhibit tubulin polymerization. The structure of each combretastatin was firmly established by a combination of high resolution (400 MHz) ^1H and ^{13}C nuclear magnetic resonance and mass spectral analyses followed by total syntheses. The conversion of methyl gallate (**7***b*) to combretastatin A-2 via intermediates **7***c* → **7***d* → **7***e* → **7***a* and **6***a* → **5***a* illustrates the practical synthetic route utilized for obtaining these substances. The Wittig reaction employed as the penultimate step in obtaining combretastatins A-3, afforded predominantly the natural *Z* isomer.

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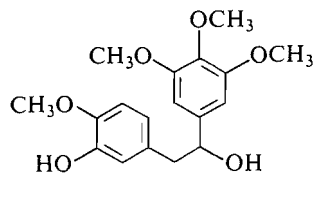
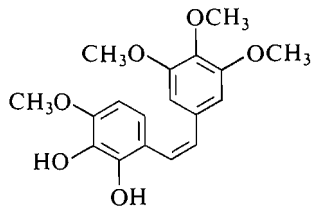
Dans le but d'isoler de nouvelles substances inhibant la croissance des cellules (PS) de la leucémie lymphocytaire de la murine P388, on a procédé à une nouvelle investigation de l'arbre sud-africain *Combretum caffrum* (Combretaceae); cette étude a conduit à la découverte de trois nouveaux constituants actifs que l'on a appelés combrétastatines A-2 (**5***a*, PS ED_{50} 0,027 μg/mL), A-3 (**5***b*, PS ED_{50} 0,026 μg/mL) et B-2 (**3***b*, PS ED_{50} 0,32 μg/mL). On a trouvé que les combrétastatines A-2 ainsi que A-3 inhibent toutes les deux la polymérisation de la tubuline. On a fermement établi la structure de chacune des combrétastatines en faisant appel à une combinaison de résonance magnétique nucléaire ^1H et ^{13}C à haute résolution (400 MHz) et de spectrométrie de masse suivis de synthèses totales. La transformation du gallate de méthyle (**7***b*) en combrétastatine A-2 (via les intermédiaires **7***c* → **7***d* → **7***e* → **7***a* et **6***a* → **5***a*) illustre bien les voies pratiques de synthèse qui peuvent être utilisées pour obtenir ces substances. La réaction de Wittig, utilisée dans l'avant-dernière étape de la synthèse des combrétastatines A-3, fournit principalement l'isomère *Z* naturel.

[Traduit par la revue]

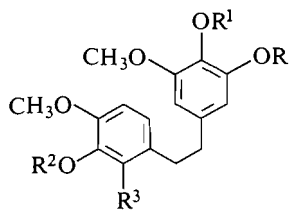
The plant family Combretaceae is comprised of 20 genera (600 species) growing in tropical and subtropical regions as shrubs and trees (2). The genus *Combretum* with 250 climbing or erect species is known for its medicinal properties in Africa and India (3).[3] In 1979 we began an investigation directed at discovering murine P-388 lymphocytic leukemia (PS system) inhibitory components of the South African tree *Combretum caffrum* (Eckl. and Zeyh) Kuntze (or as *Salicifolium* E. Mey), a valuable lead uncovered in the U.S. National Cancer Institute's (NCI) world-wide explorations (5). Eventually we succeeded in isolating combretastatin (**1**), which was found to significantly cause astrocyte reversal in the NCI astrocytoma bioassay (6) and inhibition of tubulin polymerization (7). Isolation of diphenylethanol **1** was followed by discovery of the principal PS in vivo active constituent of *Combretum caffrum* fractions, the powerful tubulin polymerization inhibitor combretastatin A-1 (**2**, ref. 8). Subsequently, we isolated from the same tree the PS cell-growth inhibitory combretastatin B-1 (**3***a*, ref. 8) and a series of phenanthrenes (cf. **4**, ref. 1) with analogous activity. We now report discovery of two new compounds closely related to combretastatin A-1 (**2**), designated combretastatins A-2 (**5***a*) and A-3 (**5***b*), which display significant PS cell-growth and tubulin inhibitory properties. Another constituent active against the PS cell line, named combretastatin B-2 (**3***b*), was also found in this productive plant.



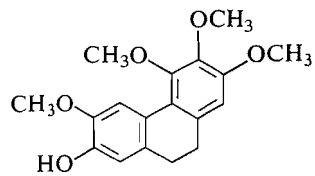
1, Combretastatin
PS ED_{50} 0.011 μg/mL



2, Combretastatin A-1
PS ED_{50} 0.99 μg/mL



3*a*, R = R^1 = CH_3, R^2 = H, R^3 = OH
Combretastatin B-1
b, R = R^2 = R^3 = H, R^1 = CH_3
Combretastatin B-2
c, R, R^1 = —CH_2—, R^2 = R^3 = H



4
PS ED_{50} 2.8 μg/mL

The methylene chloride fraction (8) prepared from the original methylene chloride – methanol extract of *Combretum caffrum* stem wood (77 kg dry wt.) was employed as starting material for the present bioassay (PS) guided research. The active methylene chloride fraction was subjected to steric exclusion chromatography in methanol on Sephadex LH-20 to provide two active fractions (A and B of ref. 8). Several partition chromatography sequences of Sephadex LH-20, employing hexane–toluene–methanol (3:1:1) as solvent, afforded a set of active fractions that were further separated by ambient column and high performance silica gel chromatography, to yield combretastatin A-2 (**5***a*, NSC 383473, ED_{50} 0.027 μg/mL), combretastatin A-3 (**5***b*, NSC 382393, ED_{50} 0.026 μg/mL), and combretastatin B-2 (**3***b*, NSC 601287, ED_{50} 0.32 μg/mL). The structures of these interesting cell-growth inhibitory com-

[1]Antineoplastic agents 130; for part 129 see ref. 1.
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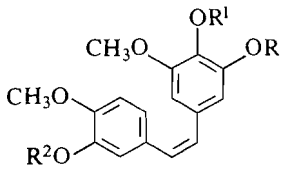
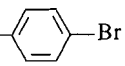
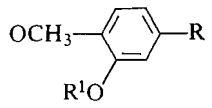
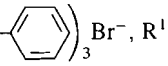


5*a*, R, R^1 = —CH_2—, R^2 = H
Combretastatin A-2
b, R = R^2 = H, R^1 = CH_3
Combretastatin A-3
c, R, R^1 = —CH_2—, R^2 = $COCH_3$
d, R, R^1 = —CH_2—, R^2 = CONH—  —Br
e, R = R^2 = $COCH_3$, R^1 = CH_3
f, R = R^2 = $Si(CH_3)_2C(CH_3)_3$, R^1 = CH_3



6*a*, R = CH_2P^+()$_3$ Br^-, R^1 = H
b, R = CH_2OH, R^1 = H
c, R = CH_2Br, R^1 = H
d, R = CHO, R^1 = $Si(CH_3)_2C(CH_3)_3$

pounds were unequivocally assigned as summarized in the sequel.

Both the ultraviolet and infrared spectra of combretastatins A-2, A-3, and B-2 suggested aromatic systems and this was further supported by high resolution electron impact mass spectra (hreims) that revealed the molecular formulae $C_{17}H_{16}O_5$, $C_{17}H_{18}O_5$, and $C_{17}H_{20}O_5$ respectively. The 400-MHz ^{1}H nmr spectrum of the A-2 (**5***a*) exhibited two methoxyl group protons (δ 3.750 and 3.870), methylenedioxy protons at δ 5.935 (supported by a ^{13}C nmr signal at δ 101.35 and ir band at 930 cm^{-1}), and signals integrating for 7 protons in the aromatic region. Two sets of relatively shielded AB spin systems appeared in the ^{1}H nmr spectrum totalling four protons. One of these sets appeared as a doublet at δ 6.458 (J = 1.32 Hz) with its counterpart at δ 6.483 (J = 1.32 Hz), typical of two *meta*-coupled aromatic protons. The other AB spin system showed doublets at δ 6.383 and 6.420 (J = 12.16 Hz each). Signals for the other three aromatic protons were split into an ABC system with J_{AC} = 0.0 Hz, typical of *ortho–ortho–meta* disposed aromatic protons. A relatively upfield one-proton doublet appeared at δ 6.732 (J_{AB} = 8.4 Hz), a downfield doublet of doublets and a doublet (one proton each) at δ 6.773 (dd, J_{BA} = 8.4 and J_{BC} = 2.0 Hz) and 6.875 (J_{BC} = 2.0 Hz). Another one-proton broad singlet resonated at δ 5.520 and disappeared upon D_2O exchange, suggesting the presence of a phenolic group. Confirmation of this observation was achieved by acetylation (**5***a* → **5***c*) and conversion to *p*-bromophenylcarbamate **5***d*.

When combretastatin A-2 was catalytically hydrogenated, the mass spectrum of the dihydro product (**3***c*) gave a relatively small molecular ion at m/z 302 and two major fragment ions at m/z 165 ($C_9H_9O_3$) and 137 ($C_8H_9O_2$), resulting from cleavage of the benzyl bond. The mass spectral analysis suggested presence of a methoxyl and methylenedioxy group in one aromatic ring and a methoxyl and hydroxyl group in the other. Examination of the ^{1}H nmr spectrum of the dihydro derivative **3***c* revealed the absence of doublets at δ 6.383 and 6.420. Instead a four-proton broad singlet was displayed at δ 2.78, typical of the benzyl protons of a bibenzyl unit. On this basis, combretastatin A-2 (**5***a*) was assumed to be a stilbene.

Interpretation of the ^{13}C nmr spectra (Table 1) of combretastatin A-2 (**5***a*), its acetate (**5***c*), and dihydro derivative (**3***c*) suggested that all contained (on the basis of chemical shift additive rules, ref. 9) a 3,4-methylenedioxy-5-methoxy substitution pattern in one phenyl ring and 3′-hydroxy-4′-methoxy substitution in the other. The 3′-hydroxy-4′-methoxy substitution pattern in the latter ring was further supported by an *ortho–para* shift of +8.3 ppm at C-2′, +5.0 at C-4′, and +6.5 at C-6′ in the ^{13}C nmr, as a result of acetylation. Once the substitution pattern in the aromatic rings was securely established, the geometry at the ethylene bridge was assigned as *Z* based on the olefin ^{1}H nmr coupling constant (J = 12.16 Hz). A coupling constant of 12.2 Hz was observed in the ^{1}H nmr spectrum of combretastatin A-1 and the *cis*-olefin geometry was independently proved by an X-ray crystal structure determination (8). Therefore, combretastatin A-2 was assigned structure **5***a* and this was unequivocally confirmed by total synthesis (*vide infra*).

The 400-MHz ^{1}H nmr spectra of combretastatin A-3 showed signals corresponding to 3-methoxy groups, and in general indicated that combretastatin B-2 was its dihydro derivative. Catalytic hydrogenation of A-3 to B-2 confirmed this relationship. The ^{1}H nmr spectrum of A-3 displayed two *meta*-coupled aromatic protons at δ 6.427 and 6.535 (J = 1.72 Hz each), and a set of ABC spin system protons with J_{AC} = 0 Hz. The most shielded doublet of this set appeared at δ 6.727 (J_{AB} = 8.4 Hz) and the relatively deshielded protons at δ 6.792 (dd, J_{BA} = 8.4 Hz) and 6.897 (d, J_{CB} = 2.0 Hz). The close proximity of their signals to those in combretastatin A-2 suggested a similar substitution pattern. The ^{1}H nmr spectrum also displayed a set of AB spin system protons at δ 6.380 and 6.439 (J = 12.2 Hz each) and two phenolic group protons at δ 5.514 and 5.679, which disappeared upon D_2O exchange. The assumption of two phenol groups in A-3 was confirmed by acetylation (**5***b*–**5***e*).

The mass spectrum of combretastatin B-2 showed a molecular ion at m/z 304 and two major fragments at m/z 167 ($C_9H_{11}O_3$) and 137 ($C_8H_9O_2$) arising from cleavage of the benzyl bond. In turn this suggested that one ring contained two methoxyl and one hydroxyl groups and the other one methoxyl and one hydroxyl group. The lack of an AB spin system with J = 12.2 Hz in the ^{1}H nmr spectrum of the combretastatin A-2 hydrogenation product (*vide supra*) and appearance of a four-proton broad singlet at δ 2.785 ppm was consistent with structure **3***b* for combretastatin B-2.

Finally, the substitution pattern in the aromatic rings of both A-3 (**5***b*) and B-2 (**3***b*) was established as 3-hydroxy-4,5-dimethoxy and 3′-hydroxy-4′-methoxy by application of chemical shift additive rules (9) in interpreting the ^{13}C nmr spectra (Table 1) of these substances and diacetate **5***c*. The assignments were further supported by the acetylation shifts seen in both the ^{13}C and ^{1}H nmr spectra. These shifts were similar to those appearing in the spectra of combretastatin A-2 acetate. Thus the structure of combretastatin A-3 was established as *Z*-stilbene **5***b* and combretastatin B-2 as bibenzyl **3***b*. The structures were confirmed (as summarized below) by unambiguous syntheses. Thus combretastatins A-2 and A-3 represent valuable new additions to the rare (heretofore, seven members) *cis*-stilbene family of plant biosynthetic products (10–12).

A very practical synthesis of combretastatin A-2 was achieved,

TABLE 1. Combretastatin A-2, A-3, B-2, and acetate derivatives, ^{13}C nmr (100.6 MHz) chemical shift (δ) assignments relative to tetramethylsilane in deuteriochloroform

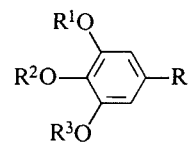
Carbon	3*b*	3*c*	5*a*	5*b*	5*c*	5*e*
1	138.80	136.47	131.83	133.33	131.52	132.42
2	108.05	107.76	108.57	108.92	108.36	155.74
3	145.61[a]	143.40	143.40	145.87[a]	143.51	143.91[a]
4	133.92	134.99	134.40	138.79	134.51	140.39
5	152.20	148.75	148.63	151.98	148.75	153.25
6	104.67	102.52	103.04	105.06	103.08	110.79
1a	37.14	37.48	129.23	129.59	129.38	129.00
1a′	38.07	38.09	128.88	128.96	128.29	128.52
1′	135.23	134.99	130.66	130.66	130.16	129.85
2′	114.81	114.68	115.03	115.16	123.32	123.32
3′	144.98[a]	145.51[a]	145.80[a]	145.25[a]	139.61	139.61[a]
4′	149.16	144.87[a]	145.32[a]	149.05	150.28	150.49
5′	110.82	110.63	110.45	110.45	112.19	112.24
6′	119.82	119.78	121.04	121.14	127.50	127.75

3*b*: 60.97 (OCH_3 at C-4), 56.90, 55.90 (OCH_3).
3*c*: 101.17 (OCH_2O), 56.58, 56.05 (OCH_3).
5*a*: 101.35 (OCH_2O), 56.38, 55.92 (OCH_3).
5*b*: 60.99 (OCH_3 at C-4), 55.92, 55.70 (OCH_3).
5*c*: 168.79, 101.38, 56.36, 55.96, 20.62.
5*e*: 169.05, 168.86, 60.73, 55.99, 55.95, 20.70, 20.61.
Similar superscript in vertical column may be interchanged.

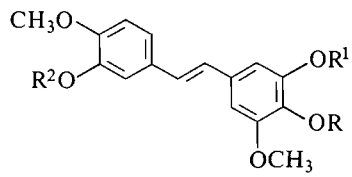
based on condensing the ylide from phosphonium bromide **6***a* with aldehyde **7***a*. Methyl gallate (**7***b*) proved to be a very effective starting point for a more convenient (cf. ref. 13) preparation of aldehyde **7***a*. Reaction of phenol **7***b* with aqueous sodium borate formed the *ortho*-borate ester and allowed specific methylation of the remaining hydroxy group using dimethylsulfate. Acid hydrolysis of the borate ester afforded 3,4-dihydroxy-5-methoxybenzoate (**7***a*, 84.5% yield). Conversion to the methylenedioxy derivative (**7***d*, 98% yield) was accomplished by fluoride-assisted methylenation with dibromomethane and cesium fluoride. Benzoate **7***d* was reduced with lithium aluminum hydride to benzyl alcohol **7***e* in 98% recovery. Oxidation of benzyl alcohol (**7***e*) with pyridinium chlorochromate (PCC) led to the required aldehyde (**7***a*) in 90% yield (73% overall).

Reaction of isovanillyl alcohol **6***b* with phosphorus tribromide in benzene–tetrahydrofuran yielded benzyl bromide **6***c*. The benzyl bromide (**6***c*) was unstable (more so in dichloromethane) and immediately converted to phosphonium bromide **6***a*. The ylide prepared (two equivalents of butyllithium in tetrahydrofuran) from phosphonium bromide **6***a* was treated with aldehyde **7***a* to yield a mixture of olefins **5***a* and **8***a* in 57% yield with a *Z*/*E* ratio of 1:16 (by ^1H nmr analysis). The isomers were separated by chromatography and the yield of *Z* isomer was increased by irradiating the *E* isomer in dioxane solution with long-wave (365 nm) uv light, to provide a *Z*/*E* ratio of 2.5:1.5. The *Z* isomer was identical with natural combretastatin A-2 (**5***a*), by both spectral and chromatographic comparisons. A route analogous to that used for A-2 (**5***a*) was adopted for synthesis of combretastatin A-3 (**5***b*). Therefore, benzoic acid **7***f* was converted to silyl ether **7***g* (14) and reduced to benzyl alcohol **7***h*. Reaction of benzyl alcohol **7***h* with phosphorus tribromide was followed by conversion of benzyl bromide **7***i* to phosphonium bromide **7***j*. The ylide prepared from bromide **7***j* upon reaction with aldehyde **6***d* yielded a mixture of olefins **5***f* and **8***b* in 82% yield with a *Z*/*E* ratio of 5:1 (by ^1H nmr). The geometrical isomers were separated using multiple development preparative layer chromatography. The major product, *Z* isomer **5***f*, was deprotected using tetrabutylammonium fluoride to afford combretastatin A-3 in 84% yield, identical (by tlc, ir, nmr) with the natural product.

The quite different olefin *Z*/*E* isomeric ratios obtained in the



7*a*, R = CHO, R^1, R^2 = —CH_2—, R^3 = CH_3
b, R = CO_2CH_3, R^1 = R^2 = R^3 = H
c, R = CO_2CH_3, R^1 = R^2 = H, R^3 = CH_3
d, R = CO_2CH_3, R^1, R^2 = —CH_2—, R^3 = CH_3
e, R = CH_2OH, R^1, R^2 = —CH_2—, R^3 = CH_3
f, R = CO_2H, R^1 = R^2 = CH_3, R^3 = H
g, R = $CO_2Si(CH_3)_2\,C(CH_3)_3$, R^1 = R^2 = CH_3, R^3 = $Si(CH_3)_2\,C(CH_3)_3$
h, R = CH_2OH, R^1 = R^2 = CH_3, R^3 = $Si(CH_3)_2\,C(CH_3)_3$
i, R = CH_2Br, R^1 = R^2 = CH_3, R^3 = $Si(CH_3)_2\,C(CH_3)_3$
j, R = $CH_2P^+(C_6H_5)_3\,Br^-$, R^1 = R^2 = CH_3, R^3 = $Si(CH_3)_2\,C(CH_3)_3$



8*a*, R, R^1 = —CH_2—, R^2 = H
b, R^1 = R^2 = $Si(CH_3)_2\,C(CH_3)_3$, R = CH_3

Wittig reactions leading to A-2 and A-3 are noteworthy. In both the A-2 and A-3 syntheses, the *E* isomer(s) would normally be predicted as major product with a reaction performed in the presence of a lithium salt (15). However, in the synthesis of combretastatin A-3, the predominant product was the *Z* isomer. Apparently, the reaction is kinetically controlled due to the steric bulk (16) of the *tert*-butyldimethylsilyl group, thereby stabilizing the intermediate oxaphosphetane in the *erythro* (17) form to yield the *Z* isomer. Analogous results were realized in our parallel synthesis of combretastatin A-1 (8). Alternatively, the rate of dissociation to *cis*-stilbenes may be faster than reversal to the *threo*-oxaphosphetanes or starting ylides (18).

In experiments conducted by Dr. E. Hamel, both combretastatins A-2 and A-3 strongly inhibited tubulin polymerization (7, 8). Other biological studies with these new *cis*-stilbenes are in progress.

Experimental

Each of the synthetic intermediates was used as received from Sigma–Aldrich or from Lancaster Synthesis. All chromatographic solvents were redistilled. Sephadex LH-20 (particle size 25–100 μm) was obtained from Pharmacia Fine Chemicals AB (Uppsala, Sweden) and silica gel 60 (70–230 mesh) was supplied by E. Merck (Darmstadt, Germany). Analtech, Inc. (Newark, Delaware) silica gel GHLF U (0.25 mm layer thickness) was employed for thin-layer chromatograms and developed with an anisaldehyde – acetic acid or ceric sulfate – sulfuric acid spray reagent (heated at approximately 150°C for 5–10 min) and (or) by use of ultraviolet light.

In each of the synthetic procedures, solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate. Ether refers to diethyl ether. Each pure specimen was colorless. The mutual identity of natural and synthetic specimens was established by comparison of infrared (NaCl plates) and ^{1}H nmr spectra combined with results from thin-layer chromatographic (tlc) comparisons in several solvents.

All melting points are uncorrected and were observed with a Kofler-type hot-stage apparatus. Ultraviolet spectra were obtained using a Hewlett–Packard model 8540A uv/vis spectrophotometer. Infrared spectra were measured with a Nicolet FT-IR model MX-1 unit and nuclear magnetic resonance spectra were obtained with a Bruker AM-400 instrument (deuteriochloroform as solvent and tetramethylsilane as the internal standard with chemical shifts recorded using the δ scale). The SFORD technique was used for determining multiplicities in ^{13}C nmr spectra. The mass spectral measurements were performed with a MS-50 instrument at the NSF Regional Facility, University of Nebraska, Lincoln, Nebraska. Elemental microanalyses were determined at MicAnal, Tucson, Arizona.

Plant taxonomy

Combretum caffrum (Eckl. and Zeyh.) Kuntze stem wood was collected (1979) and identified as part of the National Cancer Institute–U.S. Department of Agriculture research program directed by Drs. John Douros, Matthew I. Suffness, and James A. Duke.

Extract and solvent partition procedures

The stem wood (77 kg dry wt.) of *Combretum caffrum* was extracted with 1:1 methylene chloride – methanol and converted to a methylene chloride fraction that was partitioned between hexane and methanol–water (9:1) followed by adjustment to 3:2 methanol–water and extraction with methylene chloride as previously described (8). The methylene chloride fraction (827.9 g) from the solvent partitioning sequence was separated by steric exclusion chromatography on Sephadex LH-20 to obtain fractions A and B reported earlier (8).

*Isolation of combretastatin A-2 (**5**a), A-3 (**5**b), and B-2 (**3**b)*

Fraction A (28.6 g) was further separated on a column of Sephadex LH-20 (2.5 kg) by partition chromatography employing hexane–toluene–methanol (3:1:1) to furnish an active fraction (1.97 g, PS ED_{50} 1.8×10^{-2} μg/mL) that was redissolved in 3:1:1 hexane–toluene–methanol (20 mL), and the solution was filtered. The filtrate was chromatographed on a Sephadex LH-20 (200 g) column with the same solvent system. The resulting active fraction (1.35 g, PS ED_{50} 2.4×10^{-2} μg/mL) was dissolved in hexane – ethyl acetate (1:1, 5 mL) and chromatographed on a column (60 × 2.5 cm) of silica gel (60 g). Gradient elution, from 4:1 to 1:1 hexane – ethyl acetate, afforded in a 3:1 fraction the next PS (0.7 g, ED_{50} 1.0×10^{-2} μg/mL) active material. Rechromatography in acetone (2 mL) over a long column (100 × 1.2 cm) of silica gel (45 g) using the gradient hexane – ethyl acetate 9:1–4:1 yielded in a 4:1 fraction a pure specimen of combretastatin A-2 (**5***a*, 0.442 g, 5.74×10^{-4}% yield based on the dried plant, PS ED_{50} 2.7×10^{-2} μg/mL) as a viscous oil; R_f 0.46 (1:1 hexane – ethyl acetate); uv (CH_3OH) λ_{max} (ε): 223 (17 175), 303 (7190); ir ν_{max} (NaCl): 3490, 1508, 1452, 1440, 1427, 1272, 1196, 1129, 1114, 1085, 1042, 930 cm^{-1}; ^{1}H nmr (400 MHz): 3.750 (3H, s, OCH_3), 3.870 (3H, s, OCH_3), 5.520 (1H, s, OH, disappeared upon adding D_2O), 5.935 (2H, s, -OCH_2O-), 6.383 (1H, d, J = 12.16 Hz, -CH=CH-), 6.420 (1H, d, J = 12.16 Hz, -CH=CH-), 6.458 (1H, d, J = 1.32 Hz, H-2 or H-6), 6.483 (1H, d, J = 1.32 Hz, H-6 or H-2), 6.731 (1H, d, J = 8.4 Hz, H-5′), 6.773 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 6.875 (1H, d, J = 2.0 Hz, H-2′); ^{13}C nmr (see Table 1); hreims (*m*/*z*): 300.1001 (100, M^+, calcd. for $C_{17}H_{16}O_5$: 300.0998), 285.0767 (4, $C_{16}H_{13}O_5$), 267.0666 (10, $C_{16}H_{11}O_4$), 239.0714 (17, $C_{15}H_{11}O_3$).

Active fraction B (30.6 g) was also separated in hexane–toluene–methanol (3:1:1) by partition chromatography on Sephadex LH-20 (2.5 kg). The PS active components were concentrated in two fractions: 8.11 g (PS, ED_{50} 2.7 μg/mL) and 1.57 g (ED_{50} 0.36 μg/mL). The latter fraction was chromatographed on a column (70 × 2.5 cm) of silica gel (70 g) and eluted with hexane – ethyl acetate (4:1–1:1). A fraction eluted with 4:1 hexane – ethyl acetate gave a pure specimen of combretastatin A-3 (**5***b*, 480.7 mg, 6.24×10^{-4}% yield, PS ED_{50} 2.6×10^{-2} μg/mL) as a viscous oil; R_f 0.40 (1:1 hexane – ethyl acetate); uv (CH_3OH) λ_{max} (ε): 251 (8090), 295 (8895); uv (CH_3OH + $NaOCH_3$) λ_{max}: 259 (9078), 279 (7383), 296 (7402); ir ν_{max} (NaCl): 3430, 1583, 1509, 1458, 1441, 1430, 1274, 1234, 1201, 1114, 1104 cm^{-1}; ^{1}H nmr (400 MHz): 3.668 (3H, s, OCH_3), 3.867 (3H, s, OCH_3), 3.886 (3H, s, OCH_3), 5.514, 5.680 (1H, each, brs, OH, D_2O exchanged), 6.381 (1H, d, J = 12.2 Hz, -CH=CH-), 6.427 (1H, d, J = 1.72 Hz, H-6), 6.439 (1H, d, J = 12.2 Hz, -CH=CH-), 6.535 (1H, d, J = 1.72 Hz, H-2), 6.72 (1H, d, J = 8.4 Hz, H-5′), 6.792 (1H, dd, J = 8.4 Hz, 2.0 Hz, H-6′), 6.897 (1H, d, J = 2.0 Hz, H-2′); ^{13}C nmr (refer to Table 1); hreims (*m*/*z*): 302.1156 (100, M^+, calcd. for $C_{17}H_{18}O_5$: 302.1154), 287.0919 (14, $C_{16}H_{15}O_5$), 269.0813 (14, $C_{16}H_{13}O_4$).

The active fraction weighing 8.11 g was chromatographed in ethyl acetate (20 mL) on a column of silica gel (200 g). Elution with hexane – ethyl acetate (3:1) and combination of earlier fractions furnished a 0.181-g fraction (PS, ED_{50} 0.19 μg/mL) that was further purified on a Whatman hplc column (500 × 10 mm) packed with Partisil (M-9). Elution with hexane-2-propanol (9:1) at a flow rate of 0.56 mL/min afforded pure combretastatin B-2 (**3***b*, 51.7 mg, 6.71×10^{-5}% yield, PS, ED_{50} 0.32 μg/mL) as another viscous oil; R_f 0.42 (1:1 hexane – ethyl acetate); uv (CH_3OH) λ_{max} (ε): 220 (22 902), 280 (6120); ir ν_{max} (NaCl): 3437, 1595, 1512, 1461, 1442, 1430, 1351, 1278, 1237, 1150 cm^{-1}; ^{1}H nmr (400 MHz): 2.785 (1H, brs, -CH_2—CH_2-), 3.819 (3H, s, OCH_3), 3.856 (3H, s, OCH_3), 3.866 (3H, s, OCH_3), 5.605, 5.748 (1H each, brs, OH, D_2O exchanged), 6.264 (1H, d, J = 1.88 Hz, H-6), 6.465 (1H, d, J = 1.88 Hz, H-2), 6.648 (1H, dd, J = 8.12, 2.0 Hz, H-6′), 6.760 (1H, d, J = 8.12 Hz, H-5′), 6.794 (1H, d, J = 2.0 Hz, H-2′); ^{13}C nmr (see Table 1); hreims (*m*/*z*): 304.1326 (30, M^+, calcd. for $C_{17}H_{20}O_5$: 304.1311), 167.0708 (100, $C_9H_{11}O_3$), 137.0604 (65, $C_8H_9O_2$).

*Acetylation of combretastatin A-2 (**5**a) and A-3 (**5**b)*

Both combretastatin A-2 (**5***a*, 12.0 mg) and combretastatin A-3 (**5***b*, 18.0 mg) were acetylated (separately) with acetic anhydride (1.0 mL) – pyridine (0.5 mL) at room temperature (72 h). Solvent was evaporated under a stream of nitrogen to afford acetate **5***c* and diacetate **5***e* as viscous oils; *combretastatin A-2 acetate* **5***c* displayed R_f 0.60 (1:1

hexane – ethyl acetate); ir ν_{max} (NaCl): 1767, 1510, 1430, 1264, 1199, 1127, 1112, 1086, 1042, 930, 773 cm^{-1}; ^1H nmr (400 MHz): 2.273 (3H, s, $COCH_3$), 3.731 (3H, s, OCH_3), 3.809 (3H, s, OCH_3), 5.940 (2H, s, $-OCH_2O-$), 6.410 (2H, s, -CH=CH-), 6.453 (1H, d, J = 1.1 Hz, H-2, or H-6), 6.473 (1H, d, J = 1.1 Hz, H-6 or H-2), 6.840 (1H, d, J = 8.56 Hz, H-5′), 6.977 (1H, d, J = 2.0 Hz, H-2′), 7.107 (1H, dd, J = 8.56, 2.0 Hz, H-6′); ^{13}C nmr (see Table 1); hreims (m/z): 342.1101 (59, M^+, calcd. for $C_{19}H_{18}O_6$: 342.1103), 300.0987 (100, $C_{17}H_{16}O_5$); *combretastatin A-3 diacetate* (**5***e*) showed R_f 0.54 (1:1 hexane – ethyl acetate); ir ν_{max} (NaCl): 1769, 1510, 1370, 1284, 1265, 1242, 1203, 1132, 1113, 1092 cm^{-1}; ^1H nmr (400 MHz): 2.270 (3H, s, $COCH_3$), 2.287 (3H, s, $COCH_3$), 3.675 (3H, s, OCH_3), 3.807 (3H, s, OCH_3), 3.813 (3H, s, OCH_3), 6.390 (1H, d, J = 12.2 Hz, -CH=CH-), 6.445 (1H, d, J = 12.2 Hz, -CH=CH-), 6.634 (1H, d, J = 1.76 Hz, H-6), 6.708 (1H, d, J = 1.76 Hz, H-2), 6.849 (1H, d, J = 8.46 Hz, H-5′), 7.037 (1H, d, J = 2.0 Hz, H-2′), 7.115 (1H, dd, J = 8.46, 2.0 Hz, H-6′); ^{13}C nmr (in Table 1); hreims (m/z): 386.1365 (71, M^+, calcd. for $C_{21}H_{22}O_7$: 386.1366), 344.1252 (56, $C_{19}H_{20}O_6$), 302.1146 (100, $C_{17}H_{18}O_5$).

*Hydrogenation of combretastatin A-2 (***5**a*) and combretastatin A-3 (***5**b*)

In separate experiments combretastatin A-2 (**5***a*, 12.0 mg) and combretastatin A-3 (**5***b*, 10 mg) in methanol (10 mL) and 5% Pd/C (10 mg) were each treated with a positive pressure of hydrogen at ambient temperature overnight. Catalyst was removed by filtering the solution and the product purified by preparative layer chromatography on regular (250 μm) Analtech plates with hexane – ethyl acetate (1:1) as mobile phase. The oily product from combretastatin A-3 was identical with natural *combretastatin B-2* while the dihydro product (*bibenzyl* **3***c*) from combretastatin A-2 (**5***a*) was a viscous oil exhibiting R_f 0.58 (1:1, hexane – ethyl acetate); ir ν_{max} (NaCl): 3478, 1633, 1590, 1510, 1451, 1441, 1429, 1274, 1194, 1129, 1089, 925 cm^{-1}; ^1H nmr (90 MHz): 2.78 (4H, s, $-CH_2CH_2-$), 3.86 (3H, s, OCH_3), 5.93 (2H, s, $-OCH_2O-$), 6.30 (H, d, J = 1.5 Hz, H-2 or H-6), 6.38 (1H, d, J = 1.5 Hz, H-6 or H-2), 6.63 (1H, dd, J = 8.2, 1.9 Hz, H-6′), 6.76 (1H, d, J = 8.2 Hz, H-5′), 6.77 (1H, d, J = 1.9 Hz, H-2′); ^{13}C nmr (entered in Table 1); hreims (m/z): 302.1149 (29, M^+, calcd. for $C_{17}H_{18}O_5$: 302.1154), 165.0547 (100, calcd. for $C_9H_9O_3$: 165.0552), 137.0597 (67, calcd. for $C_8H_9O_2$: 137.0603).

*Combretastatin A-2/3′-*O*-p-bromophenylcarbamate (***5**b*)

To a solution of combretastatin A-2 (**5***a*, 10 mg) in dry methylene chloride (1 mL) was added a solution of *p*-bromophenyl isocyanate (80 mg) in methylene chloride (1 mL). The mixture was stirred for 5 days at room temperature and heated at reflux for 24 h. The reaction mixture was cooled to room temperature and the solution filtered. The filtrate was concentrated and chromatographed (preparative layer) with hexane – ethyl acetate (1:1) as mobile phase. The band was eluted with acetone and crystallized from methylene chloride to afford an amorphous powder, mp 138–140°C; ir ν_{max} (NaCl): 3310, 1734, 1722, 1533, 1509, 1491, 1431, 1398, 1202, 1128, 1114, 924, 750 cm^{-1}; ^1H nmr (90 MHz): 3.75 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 5.93 (2H, s, OCH_2O), 6.43 (2H, brs, -CH=CH-), 6.48 (2H, AB_q, J = 1.1 Hz, H-2, H-6), 6.65–7.0 (3H, M, H-2′, -5′, -6′), 7.09 (1H, s, NH), 7.35 (2H, d, J = 8.5 Hz, ArH), 7.41 (2H, d, J = 8.5 Hz, ArH).

*Methyl 3,4-dihydroxy-5-methoxybenzoate (***7**c*)

Methyl gallate (**7***b*, 10 g, 54.3 mmol) was added to a solution of borax (80 g) in 800 mL of water with stirring (30 min). Dimethyl sulfate (30 mL) and a solution of sodium hydroxide (13 g in 50 mL of water) were added from two separate dropping funnels over 2.5 h and stirring was continued overnight. Concentrated sulfuric acid (50 mL) was added and stirring continued an additional hour. The product was extracted with chloroform (5 × 1 L and each time stirring the solution for 20 min). The combined chloroform extracts were washed with brine (500 mL), dried, and concentrated, and the residue crystallized from methanol–benzene to yield methyl ether **7***c* (9.1 g, 84.5%); mp 110–111°C (lit. (19) mp 112°C); ir ν_{max} (NaCl): 3380, 1700, 1696, 1611, 1436, 1341, 1314, 1229, 1204, 1089 cm^{-1}; ^1H nmr (90 MHz): 3.88 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 5.50–6.0 (2H, OH), 7.22 (1H, d, J = 1.8 Hz, ArH), 7.34 (1H, d, J = 1.8 Hz, ArH).

Under analogous experimental conditions but using continuous extraction with ethyl acetate in place of chloroform yielded a mixture of methyl gallate **7***c* (7.0 g) and 3-*O*-methyl gallic acid (1.4 g).

*Methyl 3,4-methylenedioxy-5-methoxybenzoate (***7**d*)

Cesium fluoride (24.5 g, 161.5 mmol) was added to a stirring solution of phenol **7***c* (7.3 g, 36.8 mmol) in dimethylformamide (90 mL) under argon. After stirring 20 min, dibromomethane (2.8 mL, 40.5 mmol) was added and the mixture heated for 2 h. The reaction was allowed to cool to room temperature. Ether (300 mL) was added and the ethereal solution was washed with cold water (3 × 50 mL), dried, and concentrated to afford methylenedioxy derivative **7***d* as a powder (7.62 g, 98% yield), which was recrystallized from acetone–hexane, mp 89–91°C; ir ν_{max} (NaCl): 1714, 1636, 1507, 1436, 1369, 1327, 1245, 1177, 1107, 1041 cm^{-1}; ^1H nmr (90 MHz): 3.89 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.06 (2H, s, $-CH_2-$), 7.21 (1H, d, J = 1.4 Hz, ArH), 7.33 (1H, d, J = 1.4 Hz, ArH); eims, m/z (rel. int.%): 210 (100, M^+), 179 (75, $M^+ - OCH_3$). *Anal.* calcd. for $C_{10}H_{10}O_5$: C 57.15, H 4.80; found: C 57.10, H 4.74.

*3,4-Methylenedioxy-5-methoxybenzyl alcohol (***7**e*)

Lithium aluminum hydride (0.50 g) was added to a stirred solution of methyl ester **7***d* (1.7 g) in ether–tetrahydrofuran (2:1, 50 mL). After stirring for 30 min, the reaction mixture was cooled to 5°C and saturated aqueous sodium sulfate was carefully added until a white solid appeared. The precipitate was collected by filtration, the solution was dried, and the solvent evaporated to give crystalline alcohol **7***e* (1.45 g, 98% yield). Recrystallization from ethyl acetate – hexane afforded an analytical sample melting at 66–67°C (lit. (4) mp 66°C); ir ν_{max} (NaCl): 3220, 1632, 1467, 1453, 1435, 1323, 1203, 1133, 1093, 1008, 918 cm^{-1}; ^1H nmr (90 MHz): 1.75 (1H, brs, OH), 3.90 (3H, s, OCH_3), 4.58 (2H, brs, $-CH_2OH$), 5.96 (2H, s, $-CH_2-$), 6.55 (2H, s, ArH).

*3,4-Methylenedioxy-5-methoxybenzaldehyde (***7**a*)

To a stirred yellow mixture of pyridinium chlorochromate (20) (1.72 g, 7.99 mmol) and anhydrous sodium acetate (0.655 g, 7.99 mmol) in methylene chloride CH_2Cl_2 (30 mL) was added at once a solution of benzyl alcohol **7***e* (1.32 g, 7.26 mmol) in methylene chloride (10 mol). The greyish solution, which formed immediately, was stirred for 2 h, and the reaction was monitored by tlc (1:1 hexane – ethyl acetate). After filtering the solution through a small silica column, colorless aldehyde **7***a* (1.18 g, 91% yield) was eluted with hexane – ethyl acetate (7:3). Recrystallization from acetone afforded needles, mp 132–133°C (lit. (13) mp 130–131°C, 129–130°C); ir ν_{max} (NaCl): 1694, 1676, 1622, 1508, 1474, 1451, 1362, 1325, 1134, 1190 cm^{-1}; ^1H nmr (90 MHz): 3.95 (3H, s, OCH_3), 6.09 (2H, s, $-CH_2-$), 7.04 (1H, d, J = 1.4 Hz, ArH), 7.12 (1H, d, J = 1.4 Hz, ArH), 9.78 (1H, s, -CHO).

*3-Hydroxy-4-methoxybenzyltriphenylphosphonium bromide (***6**a*)

A solution of phosphorus tribromide (8.51 mL) in a mixture of tetrahydrofuran–benzene (1:2, 330 mL) was added to a cool (0°C) solution of 3-hydroxy-4-methoxybenzyl alcohol (**6***b*, 6.13 g, 40 mmol) in the same solvent (75 mL) under argon. The colorless solution was stirred at room temperature for 2 h, poured onto ice water (100 mL), and extracted with ether (2 × 100 mL). The ethereal layer was washed once with water (50 mL) followed by brine (2 × 50 mL), dried, and evaporated to dryness to afford bromide **6***a* as an amorphous powder. A solution prepared from the crude bromide, anhydrous benzene (150 mL), and triphenylphosphine (15.72 g, 60 mmol) was stirred for 10 min at room temperature and heated at reflux for 2 h. On cooling to room temperature, a viscous oil separated. The upper solvent phase was decanted and the oil was crystallized from ethanol–ether to give bromide **6***a* as a powder (10.0 g, 52.4% from alcohol **6***b*); mp 262–264°C; ir ν_{max} (NaCl): 3158, 1604, 1589, 1527, 1512, 1437, 1279, 1255, 1128, 1111, 743 cm^{-1}; ^1H nmr (90 MHz, $CDCl_3$ + D_2O): 3.77 (3H, s, OCH_3), 4.95 (2H, d, J_{PCCH} = 13.7 Hz, $-CH_2-$), 6.57 (2H, brs, ArH), 6.83 (1H, brs, ArH), 7.56–7.77 (15H, ArH). *Anal.* calcd. for $C_{26}H_{24}O_2PBr$: C 65.6, H 5.05, Br 16.67; found: C 64.50, H 5.07, Br 16.78.

*3'-Hydroxy-3,4-methylenedioxy-4',5-dimethoxy-(E)- and -(Z)-stilbene (**5**a and **8**a, combretastatin A-2 (**5**a), and E isomer (**8**a)*

Phosphonium bromide **6***a* (3.35 g, 7.0 mmol) was suspended in tetrahydrofuran (100 mL), stirred, and cooled to −50°C (under argon), and *n*-butyllithium (10 mL, 15 mmol) was added using a syringe and septum technique. The solution became deep red immediately and upon reaching room temperature was stirred 20 min prior to adding a solution of aldehyde **7***a* (1.15 g, 6.39 mmol) in tetrahydrofuran (30 mL). All the aldehyde was consumed in 30 min. Cold hydrochloric acid (1 *M*, 50 mL) was added followed by water (100 mL), and the product was extracted with ethyl acetate (3 × 100 mL) from the colorless solution. The ethyl acetate extract was washed with water (50 mL), brine (50 mL), dried, and the solvent evaporated. The crude product was chromatographed on a silica gel (50 g) column. Elution with hexane – ethyl acetate (17:3) afforded a mixture of *Z*- and *E*-stilbenes (1.09 g, 57% yield, ratio *Z*/*E* 1:16). Half of the product was rechromatographed on a longer silica gel column, and elution with hexane – ethyl acetate (9:1) provided first combretastatin A-2 (40 mg) as a viscous oil identical with natural combretastatin A-2 (**5***a*) and later the *E* isomer **8***a* (0.20 g) as needles from ethyl acetate – hexane, mp 145–150°C; ir ν_{max} (NaCl): 3490, 1622, 1509, 1463, 1440, 1429, 1319, 1279, 1263, 1254, 1133 cm^{-1}; ^1H nmr (400 MHz): 3.908 (3H, s, OCH_3), 3.941 (3H, s, OCH_3), 5.598 (1H, brs, OH), 5.978 (2H, s, $-OCH_2O-$), 6.629 (1H, d, J = 1.30 Hz, H-2 or H-6), 6.729 (1H, d, J = 1.30 Hz, H-6 or H-2), 6.825 (1H, d, J = 8.32 Hz, H-5'), 6.848 (2H, s, -CH=CH-), 6.945 (1H, d, J = 8.32, 2.0 Hz, H-6'), 7.113 (1H, d, J = 2.0 Hz, H-2'). *Anal.* calcd. for $C_{17}H_{16}O_5$: C 68.00, H 5.37; found: C 67.63, H 5.37.

Photochemical isomerization of E*-stilbene **8**a to combretastatin A-2 (**5**a)*

A solution of *E* isomer **8***a* (40 mg) in dioxane (30 mL) – water (1 mL) was stirred and irradiated (directly into the solution from above) with long-wavelength (365 nm) uv for 5 h. The ultraviolet source was an uv lamp used for visualizing tlc plates equipped with both short-wave (254 nm) and long-wave (365 nm) lamps. The solvent was removed and ^1H nmr examination of the residue revealed an isomeric mixture in the ratio *Z*/*E* of 2.5:1.5. Separation by chromatography on a silica gel column and elution with hexane – ethyl acetate (9:1) yielded combretastatin A-2 (15 mg).

*3-*tert*-Butyldimethylsilyloxy-4,5-dimethoxybenzyl alcohol (**7**h)*

Diisopropylethylamine (11.3 mL, 77 mmol) was added to a stirred solution of 3-hydroxy-4,5-dimethoxybenzoic acid (**7***f*, 5.0 g, 25 mmol) in dimethylformamide (50 mL, under argon) followed by addition of *tert*-butyldimethylsilyl chloride (8.32 g, 55 mmol), and the reaction mixture was stirred for 1 h. Ice (50 g) was added and the reaction mixture was extracted with ethyl ether (200 mL). The ethereal solution was washed with cold water (3 × 50 mL), sodium bicarbonate solution (10%, 50 mL), water (50 mL), dried, and solvent evaporated to yield *tert*-butyldimethylsilyl 3-*tert*-butyldimethylsilyloxy-4,5-dimethoxybenzoate (**7***g*) as a chromatographically homogeneous oil (10.4 g, 97% yield). Attempts at high vacuum distillation (100°C at 1.0 Torr; 1 Torr = 133.3 Pa) failed and resulted in desilylation. However, the oil showed the correct ir and ^1H nmr spectral data for silane **7***g*; ir ν_{max} (NaCl): 2932, 1700, 1590, 1420, 1350, 1254, 1230, 1221, 1118, 839, 775 cm^{-1}; ^1H nmr (90 MHz): 0.107 (6H, s, 2 × $SiCH_3$), 0.276 (6H, s, 2 × $SiCH_3$), 0.922 (18H, s, 6 × CH_3), 3.751 (3H, s, OCH_3), 3.790 (3H, s, OCH_3), 7.170 (2H, AB_q, J_{AB} = 1.5 Hz, ArH).

Silyl ester **7***g* (10.0 g, 23 mmol) was dissolved in ether (300 mL) and stirred (under argon) with lithium aluminum hydride (2.0 g) at room temperature for 1 h. Saturated ammonium chloride solution (ice-cold) was added and the ether layer separated. The aqueous phase was extracted with ether (3 × 200 mL) and the combined ether extract was washed with sodium bicarbonate solution, cold water, and dried. After solvent removal, the residual oil was found to be chromatographically homogeneous alcohol **7***h* (6.0 g, 86% yield); the oil distilled at 210°C (0.04 Torr) and displayed ir ν_{max} (NaCl): 3450, 2931, 1587, 1501, 1427, 1233, 1118, 1004, 837, 782 cm^{-1}; ^1H nmr (90 MHz): 0.201 (6H, s, 2 × CH_3), 1.026 (9H, s, 3 × CH_3), 1.716 (1H, t, J = 5.9 Hz, OH, D_2O exchanged), 3.794 (3H, s, OCH_3), 4.596 (2H, d, J = 5.9 Hz, $-CH_2OH$, collapsed to a broad singlet upon D_2O exchange), 6.510 (1H, d, J = 1.9 Hz, ArH), 6.610 (1H, d, J = 1.9 Hz, ArH). *Anal.* calcd. for $C_{16}H_{26}O_4Si$: C 60.39, H 8.78; found: C 60.06, H 8.78.

*3-*tert*-Butyldimethylsilyloxy-4,5-dimethoxybenzyltriphenylphosphonium bromide (**7**j)*

Before adding phosphorus tribromide (0.95 mL) in methylene chloride (5 mL) a solution of silyloxybenzyl alcohol **7***h* (6.0, 20 mmol) in methylene chloride (anhydrous, 100 mL) was stirred and cooled (−10°C, ice–salt bath) for 15 min. The mixture was stirred 10 min and 10% aqueous sodium bicarbonate solution (50 mL) was added (slowly). The methylene chloride layer was washed with cold water (2 × 50 mL), dried, and solvent evaporated to give 3-*tert*-butyldimethysilyloxy-4,5-dimethoxybenzyl bromide (**7***i*) as a colorless oil (6.4 g, 88% yield), homogeneous by tlc. While the product (**7***i*) was heat sensitive, and distillation was unsuccessful, it did give the correct spectral data for silane **7***i*; ir ν_{max} (NaCl): 2932, 1586, 1500, 1427, 1348, 1250, 1234, 1127, 1111, 838 cm^{-1}; ^1H nmr (90 MHz): 0.183 (6H, s, 2 × CH_3), 1.006 (9H, s, 3 × CH_3), 3.777 (3H, s, OCH_3), 3.855 (3H, s, OCH_3), 4.409 (2H, s, $-CH_2-$), 6.561 (2H, AB_q, J = 2.0 Hz, ArH); ms (*m*/*z*, rel. amt.) 362, 360 (40%, M^+), 305, 303 (80, $M^+ - C_4H_9$), 281 (60, $M^+ - Br$), 253 (75, $M^+ - Br - 28$), 209 (100, $M^+ - Br - C_5H_{12}$), 166 (45, $M^+ - Br - Si(CH_3)_2C(CH_3)_3$).

To a solution of bromide **7***i* (6.0 g, 16.6 mmol) in toluene (50 mL) was added (stirring) a solution of triphenylphosphine (4.36 g, 16.6 mmol) in toluene (10 mL). The mixture was heated to reflux for 15 min. When the clear solution started to become turbid, heating was discontinued and the mixture was stirred overnight at room temperature. The solid phosphonium bromide **7***j* (7.17 g, 69% yield) was recovered by filtration as a powder melting at 248°C; ir ν_{max}(NaCl): 2957, 1586, 1502, 1453, 1436, 1344, 1253, 1113, 836, 742, 721 cm^{-1}; ^1H nmr (90 MHz): 0.58 (6H, s, 2 × CH_3), 1.48 (9H, s, 3 × CH_3), 4.15 (3H, s, OCH_3), 4.32 (3H, s, OCH_3), 5.89 (2H, d, J_{PCH} = 14 Hz, CH_2), 6.71 (1H, t, J = 2.2 Hz, ArH), 7.36 (1H, t, J = 2.2 Hz, ArH), 8.23–8.45 (15H, ArH). *Anal.* calcd. for $C_{33}H_{40}BrO_3PSi$: C 63.56, H 6.46, Br 12.81; found: C 64.04, H 6.57, Br 12.47.

*Combretastatin A-3 (**5**b)*

Butyllithium (2.47 mL, 2.2 mmol) was added to a stirred and cooled (−10°C) suspension of phosphonium bromide **7***j* (1.31 g, 2.1 mmol) in tetrahydrofuran (100 mL). The orange-red solution was stirred at room temperature 10 min. Aldehyde **6***d* (0.532 g, 2.0 mmol) was added and stirring continued another 10 min while the red solution changed to yellow. A tlc examination (4:1, hexane – ethyl acetate) showed completion of reaction. Ice water (100 mL) was added and the product extracted with ether (3 × 100 mL). The ethereal solution was washed with water (100 mL), and concentrated to a gum, which upon silica gel column (40 g) chromatography and elution with hexane – ethyl acetate (97:3) afforded a mixture of 3,3'-bis-(*tert*-butyldimethylsilyloxy)-4',4,5-trimethoxy-(*Z*)- and (*E*)-stilbene (**5***f* and **8***b*) in a ratio of 5:1 (0.870 g, 82% yield). The isomers were separated by preparative layer chromatography on silica gel (20 × 20 cm, 500 μm, E-Merck plates) employing 19:1 hexane – ethyl acetate. The short uv positive upper band was the major product and elution with hexane – ethyl acetate yielded *Z* isomer **5***f* as an oil (423 mg); ir ν_{max} (NaCl): 2930, 1575, 1509, 1250, 1231, 1118, 838, 782 cm^{-1}; ^1H nmr (400 MHz): 0.070 (6H, s, 2 × CH_3), 0.105 (6H, s, 2 × CH_3), 0.932 (9H, s, 3 × CH_3), 0.958 (9H, s, 3 × CH_3), 3.666 (3H, s, OCH_3), 3.761 (3H, s, OCH_3), 3.774 (3H, s, OCH_3), 6.366 (1H, d, J = 12 Hz, -CH=CH-), 6.413 (1H, d, J = 1.84 Hz, H-6), 6.429 (1H, d, J = 12 Hz, -CH=CH-), 6.471 (1H, d, J = 1.84 Hz, H-2), 6.718 (1H, d, J = 8.3 Hz, H-5'), 6.778 (1H, d, J = 2.0 Hz, H-2'), 6.840 (1H, dd, J = 8.3, 2.0 Hz, H-6'). The lower long uv positive band was also eluted with hexane – ethyl acetate to give *E* isomer **8***b* as an oil (80 mg); ir ν_{max} (NaCl): 2930, 1578, 1509, 1427, 1272, 1251, 1117, 838, 782 cm^{-1}; ^1H nmr (400 MHz): 0.183 (6H, s, 2 × CH_3), 0.207 (6H, s, 2 × CH_3), 1.024 (9H, s, 3 × CH_3), 1.028 (9H, s, 3 × CH_3), 3.794 (3H, s, OCH_3), 3.823 (3H, s, OCH_3), 3.901 (3H, s, OCH_3), 6.626

(1H, d, J = 1.88 Hz, H-6), 6.693 (1H, d, J = 1.88 Hz, H-2), 6.800 (1H, d, J = 16.2 Hz, -CH=CH-), 6.825 (1H, d, J = 8.3 Hz, H-5′), 6.845 (1H, d, J = 16.2 Hz, -CH=CH-), 7.020 (1H, d, J = 2.1 Hz, H-2′), 7.047 (1H, dd, J = 8.3 Hz, 2.1 Hz, H-6′). *Anal.* calcd. for $C_{29}H_{46}O_5Si_2$: C 65.62, H 8.73; found: C 66.09, H 8.97.

To a stirred solution of silyl-*Z*-stilbene **5***f* (0.25 g, 0.47 mmol) in tetrahydrofuran (10 mL under argon) was added a 1 *M* tetrahydrofuran solution of tetrabutylammonium fluoride (1 mL, 1.0 mmol). Instantaneously the solution became yellow and reaction was complete as evidenced by tlc (hexane – ethyl acetate 3:2). Ice (5 g) – water (5 mL) was added to the mixture and the product extracted with ether (2 × 25 mL). The ethanol extract was washed with cold water (20 mL) and dried. After evaporation of solvent, the residue in 1:1 hexane – ethyl acetate was filtered through a pipette filled with silica gel (2 g) to afford combretastatin A-3 as an oil (0.13 g, 91% yield), homogeneous by tlc and identical with the natural product.

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