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ISOLATION, STRUCTURE, SYNTHESIS, AND ANTIMITOTIC PROPERTIES OF COMBRETASTATINS B-3 AND B-4 FROM *COMBRETUM CAFFRUM*[1]

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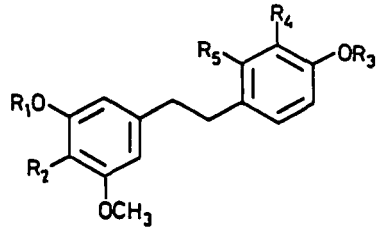
ABSTRACT.—Further investigation of a CH_2Cl_2 fraction prepared from the South African tree *Combretum caffrum* for substances inhibitory to the murine P-388 lymphocytic leukemia (PS system) cell line has led to the isolation of two new bibenzyls, designated combretastatins B-3 [**3**] and B-4 [**4**], accompanied by the previously known bibenzyls **7**, **8**, and **9**. The structure of each substance was ascertained by results of mass and nmr spectral analyses and confirmed by crystal structure determination (for **7**) or synthesis. Combretastatins B-3 and B-4 gave PS ED_{50} values of 0.4 and 1.7 ug/ml, respectively, and bibenzyls **7**, **8**, and **9** were comparably cell growth inhibitory against the PS cell line with ED_{50} results of 1.7, 2.5, and 0.25 ug/ml, respectively. All the bibenzyls caused leukemia cells to accumulate in mitosis at cytotoxic drug concentrations; however, a wide range of in vitro activity against the protein tubulin (the major component of the mitotic spindle) was observed.

Although shrubs and trees of the *Combretum* genus (250 species) represent more than one third of the Combretaceae and a number have found application in primitive medical treatment (2), the structures of chemical constituents have rarely been reported. Illustrative is the west African drug "kinkeliba" (3) prepared from *Combretum micranthum* (4,5). Other chemical studies of the *Combretum* genus have included examination of the cycloartane glycosides (6), tannins (7), and phenanthrenes (8) of *Combretum molle*, the triterpenoids of *Combretum elaeagnoides* (9), the phenanthrenes and 9,10-dihydrophenanthrenes of *Combretum hereroense* (10), *Combretum psidioides* (11), and *Combretum apiculatum* (12), and our detailed examination of *Combretum caffrum* (Eckl. and Zeyh) Kuntze (1,2,13–15) for murine P-388 lymphocytic leukemia (PS system) antineoplastic and/or cell growth inhibitory constituents.

In our earlier studies of *C. caffrum* we characterized a series of unique stilbenes, named combretastatins A-1, A-2, and A-3, active against the PS leukemia, and these proved to be potent inhibitors of tubulin polymerization (1,2,13,14). Two bibenzyl components, designated combretastatins B-1 and B-2, were also found to inhibit growth of the PS cell line. Further careful separation guided by PS cell line bioassay of fractions leading to these active stilbenes and bibenzyls revealed the presence of five more cell growth inhibitory (PS) bibenzyls. As summarized in the sequel, two of these, namely combretastatins B-3 [**3**] and B-4 [**4**], proved to be new biosynthetic products. The other three bibenzyls, **7**, **8**, and **9**, were found upon assignment of structures to have been previously described.

The structures of bibenzyls **7**, **8**, and **9** were deduced by hrms and ^{1}H-nmr spectroscopy. The structure of bibenzyl **7** was confirmed by crystal structure analysis (Figure 1), and each of the bibenzyls **7–9** was synthesized. As bibenzyls **7–9** were found to have been reported previously, bibenzyl **7** represents another of these rare natural prod-

[1]Number 131 in the series Antineoplastic Agents. For Number 130, see Pettit and Singh (1).



1 R_1=R_3=Me, R_2=OMe, R_4=R_5=OH
2 R_1=R_5=H, R_2=OMe, R_3=Me, R_4=OH
3 R_1=Me, R_2=OMe, R_3=R_5=H, R_4=OH
4 R_1=Me, R_2=R_3=R_5=H, R_4=OH
5 R_1=R_3=Me, R_2=R_4=OMe, R_5=H
6 R_1=R_3=Me, R_2=R_5=H, R_4=OMe
7 R_1=R_3=Me, R_2=R_5=H, R_4=OH
8 R_1=Me, R_2=R_3=R_4=R_5=H
9 R_1=Me, R_2=OMe, R_3=R_4=R_5=H

ucts (17) synthesized (18) prior to its discovery in nature. Bibenzyl **8** was isolated in 1940 as a hydrogenation product (19), and bibenzyl **9** was previously found in the heartwood of *C. psidioides* (11). The crystal structure determination of bibenzyl **7** proceeded well, and a molecular representation with atomic numbering appears as Figure 1. Bond lengths and angles are the expected order of magnitude, similar to those observed for the related styrene, combretastatin A-1 (2). Neither of the aryl rings deviates significantly from planarity, and the dihedral angle between their least-squares planes is only 2.3(2)°. Although the hydroxyl hydrogen on O-3′ was not located, the intramolecular close contact O-3′. . . .O-4′, 2.673(9) Å implies the existence of an internal O-3′–H. . . .O-4′ hydrogen bond.

Combretastatins B-3 [**3**] and B-4 [**4**] proved to be new substances and required structural elucidation employing principally mass, 400 MHz ^{1}H-nmr, and ^{13}C-nmr spectral analyses. The combretastatin B-3 mass spectral data showed two major fragment ions at *m/z* 181 ($C_{10}H_{13}O_3$) and 123 ($C_7H_7O_2$), obtained by cleavage of its bibenzyl bridge, thereby pointing to one ring with three methoxyl and the other with two hydroxyl groups. The substitution pattern was finally established on the basis of ^{13}C-

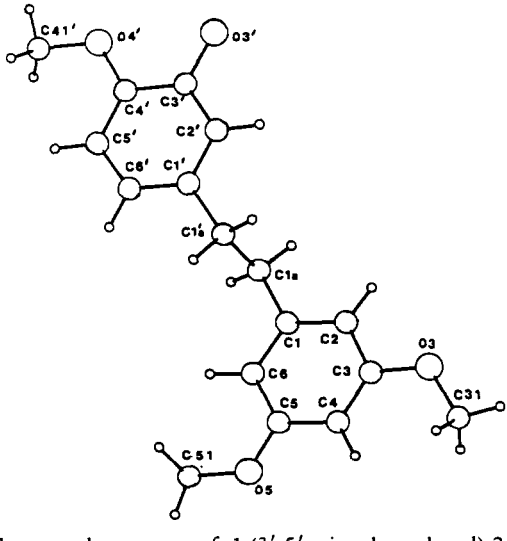
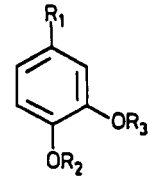


FIGURE 1. The crystal structure of 1-(3′,5′-trimethoxyphenyl)-2-(3″-hydroxy-4″-methoxyphenyl)-ethane [**7**].



10 R_1=CHO, R_2=R_3=Bz
11 R_1=CH_2OH, R_2=Me, R_3=Bz
12 R_1=CH_2Br, R_2=Me, R_3=Bz
13 R_1=$CH_2\bar{P}Ph_3\bar{B}r$, R_2=Me, R_3=Bz

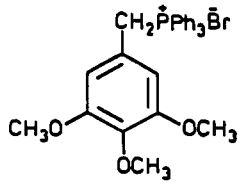
nmr (Table 1) chemical shift calculations (20). The B-3 ^{13}C-nmr spectrum showed 12 carbon signals for 14 skeletal carbons and two for the three methoxy groups, producing clear evidence for a symmetrical 3,4,5-trimethoxy substitution pattern in one aromatic ring. Chemical shift calculations based on additive rules suggested 3,4-dihydroxy substitution in the other ring, and this was further confirmed by methylation of B-3 to permethyl ether derivative **5**. Therefore, combretastatin B-3 was assigned structure **3** and verified by total synthesis. Parallel methods were used for the structural determination of combretastatin B-4 [**4**]. Permethylation of B-4 produced permethyl ether **6**, which was found identical with the methyl ether derivative of bibenzyl **7** (18). Therefore, combretastatin B-4 was assigned structure **4** and confirmed by total synthesis of its methyl ether derivative **7**. The initial assignment for both combretastatins B-3 and B-4 based on spectral evidence was assisted by the crystal structure of bibenzyl **7** (Figure 1) serving as an unequivocal reference.

Synthesis of combretastatin B-3 [**3**] was achieved using a Wittig reaction sequence as a key step. Protection of 3,4-dihydroxybenzaldehyde as the dibenzyl ether **10** constituted the first step. Next, 3,4,5-trimethoxybenzylphosphonium bromide [**14**] was prepared from trimethoxybenzyl bromide, and the phosphonium bromide [**14**] was

TABLE 1. ^{13}C-nmr Chemical Shift Assignments[a] for Combretastatins B-3 [**3**] and B-4 [**4**] and Bibenzyls **7** and **8**.

Carbon	Compound			
	3	**4**	**7**	**8**
C-1	137.62	144.24[b]	144.32[b]	146.1
C-2	105.63	106.73	106.59	105.8
C-3	153.08	160.77	160.81	160.9
C-4	134.86	98.10	98.05	98.0
C-5	153.08	160.77	160.81	160.9
C-6	105.63	106.73	106.59	105.8
C-1a	38.42	38.26	38.35	38.4
C-1b	37.23	36.89	37.08	36.7
C-1′	134.86	135.02	135.23	133.8
C-2′	115.32[b]	115.41[b]	114.70	129.5
C-3′	141.70[c]	141.65[c]	145.57[b]	115.2
C-4′	143.53[c]	143.48[c]	144.91[b]	150.8
C-5′	115.70[b]	115.71[b]	110.69	115.2
C-6′	120.92	120.91	119.79	129.5
3,5-diOMe	56.15		55.27	55.27
4-OMe	60.94	55.31		
4′-OMe	54.1			

[a]Chemical shifts are in ppm from TMS; solvent was $CDCl_3$.
[b,c]Assignments are tentative and may be interchanged.



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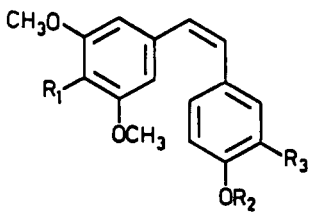
treated with NaH in *N,N*-dimethylimidazolidinone. The resulting ylide was allowed to react with aldehyde **10** to produce a 1:1 mixture of *Z/E* stilbenes **15** and **17** in 61% yield. The mixture of geometrical isomers was hydrogenated over 5% Pd/C in EtOAc-MeOH (1:1) to afford (91% yield) combretastatin B-3 identical with the natural product.

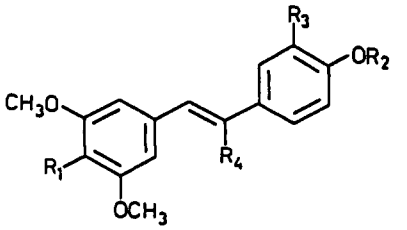
An analogous synthetic route was used to obtain combretastatin B-4 methyl ether [7]. Here, isovanillyl alcohol was converted to 3-benzyloxy-4-methoxy-benzyl alcohol [**11**] in 95% yield. Bromination of alcohol **11** with phosphorous tribromide provided benzyl bromide **12**, and treatment with triphenylphosphine furnished phosphonium bromide **13** (94% yield). The Wittig ylide was generated with NaH in *N,N*-dimethylimidazolidinone and was allowed to react with 3,5-dimethoxybenzaldehyde. The product (78% yield) was a mixture of *Z/E* stilbenes **16** and **18**. The isomers were individually characterized following chromatographic separation (Si gel). However, hydrogenation of the *Z/E* mixture was most practical for obtaining bibenzyl **7**, which proved to be identical with the natural product. As noted above, the methyl ether derivative **6** of bibenzyl **7** was found to be identical with the permethylation product from combretastatin B-4; as noted above the structure of bibenzyl **7** was unequivocally established by an X-ray crystal structure determination.



15 R_1=OMe, R_2=Bz, R_3=OBz
16 R_1=H, R_2=Me, R_3=OBz

Bibenzyl **8** was synthesized by a Perkin condensation between *p*-hydroxyphenylacetic acid and 3,5-dimethoxybenzaldehyde. The resulting cinnamic acid **19** (66% yield) was decarboxylated using a copper/quinoline procedure to give *trans* stilbene **20**. Catalytic (Pd/C) hydrogenation of stilbene **20** yielded bibenzyl **8** identical with the natural product. Similarly, a Perkin condensation between *p*-hydroxyphenylacetic acid and 3,4,5-trimethoxy-benzaldehyde followed by decarboxylation (**21–22**) and catalytic reduction afforded bibenzyl **9**.

The isolation of combretastatins B-3 and B-4 and the related bibenzyls **7**, **8**, and **9** summarized herein brings to 15 the total number of related bibenzyls, stilbenes, dihydrophenanthrenes, and phenanthrenes we have isolated from *C. caffrum* that inhibit growth of the PS cell line. As evidenced by the astrocyte reversal shown by combretastatin, the PS in vivo activity of combretastatin A-1, and the mitotic arrest and inhibition of tubulin polymerization caused by previously examined combretastatins (13,14), it is clear this plant genus can produce a variety of small molecular weight substances with



17 R_1=OMe, R_2=Bz, R_3=OBz, R_4=H
18 R_1=H, R_2=Me, R_3=OBz, R_4=H
19 R_1=R_2=R_3=H, R_4=CO_2H
20 R_1=R_2=R_3=R_4=H
21 R_1=OMe, R_2=R_3=H, R_4=CO_2H
22 R_1=OMe, R_2=R_3=R_4=H

significant biological activities. Unless the *N*-methyl-L-tyrosine (21) and the 3-aminomethyl-L-phenylalanine (22) isolated from *Combretum zeyheri* seeds are derived from fungal intrusions, the peptide components of *Combretum* species may also prove to be interesting.

In view of the strong inhibition of microtubule assembly previously observed with combretastatin (14), combretastatin A-1 (2), and combretastatin B-1 (2), we examined all the compounds described herein for effects on this reaction (Table 2). These substances were all compared to podophyllotoxin (2). Significant inhibition was observed only with bibenzyl **7**, and the much more potent inhibition previously observed with combretastatins A-1 and B-1 [**1**] was confirmed.

Previous studies have demonstrated that combretastatin, combretastatin A-1, and combretastatin B-1 [**1**] mediate their inhibition of microtubule assembly by binding at

TABLE 2. Effects of Compounds Isolated from *Combretum caffrum* on Microtubule Assembly, the Binding of Colchicine to Tubulin, and Growth of L1210 Leukemia Cells in Culture.

Compound	IC_{50} microtubule assembly[a] (μM)	Inhibition of colchicine binding[b] (% of control) inhibitor: colchicine			IC_{50} cell growth[c] (μM)
		1:1	10:1	100:1	
Podophyllotoxin	4	11	0	—	—
Combretastatin A-1[d]	4	3	0	—	0.6
1	4	13	0	—	2
2	> 100	71	19	7	3
3	> 100	92	81	48	3
4	> 100	75	31	10	1
5	> 100	88	63	37	20
6	> 100	90	80	34	30
7	30	38	8	3	1
8	> 100	87	45	13	4
9	> 100	89	77	43	50

[a]The IC_{50} is defined as the drug concentration which inhibited the extent of microtubule assembly by 50% after 15 min at 37° (2).

[b]Experimental conditions were described in detail previously (2). The control reaction mixtures contained 1 μM tubulin and 5 μM [^{3}H] colchicine, with inhibiting drugs added at the concentrations indicated in the table. Incubation was for 10 min at 37°.

[c]The IC_{50} is defined as the drug concentration which inhibited cell growth by 50% at 24 h. For experimental details see Hamel and Lin (14).

[d]The structure of combretastatin A-1 is given in Pettit *et al.* (2).

the colchicine site of tubulin, with the latter two compounds being particularly potent inhibitors of the binding of radiolabeled colchicine to the protein (2,14). When the Table 2 series were evaluated for inhibitory effects on the colchicine binding reaction, all were effective, provided the ratio of potential inhibitor to radiolabeled colchicine was high enough (Table 2). There was good correlation, moreover, between the two in vitro biochemical assays. The most potent inhibitors of colchicine binding were also the most potent inhibitors of microtubule assembly.

The bibenzyls described here, as well as combretastatin A-1, were also examined for their effects on the growth of L-1210 leukemia cells in culture (Table 2). A different pattern emerged from that observed with the tubulin assays. The most cytotoxic compounds were **4** and **7** with an IC_{50} of 1 μM; the least was **9** with an IC_{50} of 50 μM. The most active compounds with tubulin in vitro, combretastatins A-1 and B-1 [**1**], had IC_{50} values of 2 μM. Three additional bibenzyls **2**, **3**, and **8** had comparable cytotoxic effects.

The bibenzyls, as well as combretastatin A-1, were evaluated for their effects on the mitotic index of L-1210 cells after 6 h of drug exposure at comparable cytotoxic doses (approximately 2 to 7 times the IC_{50} value obtained with each drug). All nine bibenzyls and combretastatin A-1 caused a marked rise in the mitotic index, ranging from 25% mitoses with **1** to 56% mitoses with **6**. The control culture without drug had 4% mitotic figures.

Thus, despite the incomplete agreement between the results of the in vitro tubulin assays and the cell culture studies, it remains probable that all members of the combretastatin series of drugs act as antimitotic agents. The discrepancies between the two types of assay cannot be readily explained at the present time, but they could be related to drug transport or stability in the culture medium or to intracellular metabolism.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—A previous report (2) provides a summary of chromatographic, synthetic, and instrumental techniques utilized in the present investigation. The mutual identity of natural and synthetic specimens was established by comparison of ir (KBr) and nmr spectra and chromatographic behavior.

PLANT MATERIAL.—Dry *C. caffrum* stemwood material (77 kg) was collected in 1979, and identified through the U.S. National Cancer Institute and U.S. Department of Agriculture joint research program. The herbarium specimen corresponds to NCI NSC B-817373 and is maintained by the USDA. The original CH_2Cl_2/MeOH extract of the stemwood was processed through the 9:1→3:2 MeOH-H_2O partition sequence with hexane followed by CH_2Cl_2 as previously described (2). The latter CH_2Cl_2 fraction was further separated by steric exclusion chromatography, in MeOH followed by hexane-toluene-MeOH (3:1:1) partition chromatography, both on Sephadex LH-20 as noted earlier (2,16). Further careful separation guided by PS cell line bioassay of the fractions previously employed from this partition chromatogram to isolate combretastatins A-1 to A-3 and the dihydrophenanthrenes (15) were used for the experiments reported here.

Evaluation of drug effects on microtubule assembly (2), on the binding of radiolabeled colchicine to tubulin (2), on growth of L1210 leukemia cells (14), and on the mitotic index of L1210 cells (14) was performed as described previously.

ISOLATION OF COMBRETASTATINS B-3 [**3**], B-4 [**4**], AND BIBENZYLS **7**, **8**, AND **9**.—The *C. caffrum* fraction previously found (2) to contain combretastatin A-1 was submitted to further separation on a Partisil M-9 column by hplc with hexane-iPrOH (9:1) as solvent at a flow rate of 0.1 ml/min to furnish 12.0 mg of combretastatin B-3 [**3**] as a powder from EtOH/Et_2O, mp 113–115°; PS ED_{50} 0.4 μg/ml; uv (MeOH) λ max 241 (ε 8450), 281 (6907); uv (MeOH + NaOMe) λ max 246 (10190), 293 (5699); ir λ max 3400, 1590, 1507, 1457, 1420, 1240, 1126 cm^{-1}; ^1H nmr (400 MHz) 2.796 (4H, s, $-CH_2-CH_2-$), 3.817 (6H, s, 2 × OMe), 3.829 (3H, s, OMe), 5.240, 5.350 (1H, each, br s, OH, D_2O exchangeable), 6.355 (2H, s, H-2,6), 6.610 (1H, dd, J = 7.88, 1.88 Hz, H-6′), 6.684 (1H, d, J = 1.88 Hz, H-2′), 6.777 (1H, d, J = 7.88 Hz, H-5′); ^{13}C nmr see Table 1; hreims *m/z* $[M]^+$ 304.1308 (15%), (calcd for $C_{17}H_{20}O_5$, 304.1311), 181.0863 (100) (calcd for $C_{10}H_{13}O_3$, 181.0865), 123.0449 (9) (calcd for $C_7H_7O_2$, 123.0446).

The mixture remaining from the original separation of 2,7-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene (15) was further separated by a series of chromatographic procedures starting with a Lobar-A Si gel column and hexane-CH_2Cl_2-MeOH (3:7:0.1) as solvent followed by separation on three Lobar-A columns in series using hexane-$CHCl_3$-Me_2CO (6:4:0.5) as eluent. Where necessary, preparative tlc on Si gel with CH_2Cl_2-MeOH (99:1) effected final separation. By this means 35.8 mg of combretastatin B-4 [**4**] was obtained as a viscous oil with PS ED_{50} 1.7 μg/ml; uv (MeOH) λ max 221 (ε 19,188), 280 (4942); uv (MeOH + NaOMe) ν max 221 (24803), 280 (4111), 290 (3810); ir ν max 3400, 1595, 1512, 1460, 1444, 1430, 1350, 1277, 1203, 1148 cm^{-1}; ^1H nmr (400 MHz) 2.786 (4H, s, $-CH_2-CH_2-$), 3.757 (6H, s, 2 × OMe), 5.196 (2H, broad, 2 × OH, D_2O exchangeable), 6.307 (1H, dd, $J = 2.0$ Hz each, H-4), 6.322 (2H, br d, $J = 2.0$ Hz, H-2,6), 6.608 (1H, br d, $J = 7.8$ Hz, H-6′), 6.687 (1H, br s, H-2′), 6.755 (1H, d, $J = 7.8$ Hz, H-5′); ^{13}C nmr see Table 1; hreims *m/z* $[M]^+$ 274.1208 (34.5%) (calcd for $C_{16}H_{18}O_4$, 274.1205), 152.0822 (29) (calcd for $C_9H_{12}O_2$, 152.0837), 151.0746 (15) (calcd for $C_9H_{11}O_2$, 151.0759), 123.0450 (100) (calcd for $C_7H_7O_2$, 123.0446).

In final separation of the combretastatins, bibenzyl **7** was isolated and recrystallized from Me_2CO/hexane to afford small needles melting at 108°: PS ED_{50} 1.7 μg/ml; uv (MeOH) λ max 222 (25412), 280 (6854); ir ν max 3485, 1609, 1595, 1511, 1469, 1452, 1425, 1207, 1146 cm^{-1}; ^1H nmr (90 MHz) 2.82 (4H, s, $-CH_2-CH_2-$), 3.77 (6H, s, 2 × OMe), 3.86 (3H, s, OMe), 5.57 (1H, br s, OH, D_2O exchangeable), 6.33 (3H, br s, H-2,4,6), 6.64 (1H, dd, $J = 8.14$, 1.8 Hz, H-6′), 6.77 (1H, d, $J = 8.14$ Hz, H-5′), 6.80 (1H, d, $J = 1.8$ Hz, H-2′); ^{13}C nmr see Table 1; and hreims *m/z* $[M]^+$ 288.1364 (22%) (calcd for $C_{17}H_{20}O_4$, 288.1362), 151.0756 (5%) (calcd for $C_9H_{11}O_2$, 151.0759), 137.0603 (100) (calcd for $C_8H_9O_2$, 137.0603).

The crystal structure of 1-(3,5-dimethoxy-phenyl)-2-(3′-hydroxy-4′-methoxy-phenyl)-ethane [**7**] was determined as follows: A suitable single crystal (Pc space group) was irradiated with MoK (λ = 0.7107 Å) radiation, using an Enraf-Nonius CAD4 diffractometer. Cell parameters were obtained by least-squares analysis of the setting angles of 24 reflections in the range $16 \leq \theta \leq 17°$. During the data collection (1217 reflections collected with 1037 observed), intensities of three standard reference reflections were monitored every hour, and centering was checked every 100 measured reflections. Intensities were corrected for Lorentz and polarization effects but not for absorption.

The structure was solved by direct methods using a preliminary version of SHELX-84[2] and refined using SHELX-76 (23). In the final refinements (R = 0.038, Rw = 0.036), all non-hydrogen atoms were treated anisotropically. Ring and methylene hydrogens were placed in calculated positions with a single temperature factor, and the methyls were treated as rigid groups, again with a single temperature factor. Attempts to locate and refine the single hydroxyl hydrogen on O-3′ were unsuccessful, and it has been omitted from the final model. In the final cycles of refinement, a weighting scheme $(\sigma^2 F)^{-1}$ was employed: shift/esd for parameters <0.02, maximum residual electron density = 0.15 $eÅ^{-3}$.

Complex neutral atom scattering factors were taken from Cromer and Mann (24) for C and O and from Stewart *et al.* (25) for H, with dispersion corrections from Cromer and Liberman (26). PARST (27) was used for the calculation of molecular parameters and PLUTO[3] for a drawing of the molecule.[4]

The original fraction (15) bearing 2-hydroxy-3,4,6,7-tetramethoxy-9,10-dihydrophenanthrene was chromatographed on a column of Si gel in hexane-EtOAc (3:1) to isolate bibenzyl **8** (1.15 g) as a viscous oil; uv (MeOH) λ max 236 (ε 3555), 280 (3212), uv (MeOH + NaOMe) λ max 247 (6433), 280 (2506), 294 (1795); ir ν max 3417, 1607, 1596, 1514, 1461, 1429, 1204, 1150, 1066, 850, 690 cm^{-1}; ^1H nmr (90 MHz) 2.85 (4H, s, $-CH_2-CH_2-$), 3.76 (6H, s, 2 × OMe), 5.15 (1H, br s, OH, D_2O exchangeable), 6.32 (3H, s, H-2,4,6), no aromatic solvent induced shift was observed when the spectrum was obtained in a mixture of $C_6D_6/CDCl_3$; ^{13}C nmr see Table 1; hreims *m/z* $[M]^+$ 258.1255 (25%) (calcd for $C_{16}H_{18}O_3$, 258.1256), 152.0831 (33) (calcd for $C_9H_{12}O_2$, 152.0837), 107.0495 (100) (calcd for C_7H_7O, 107.0497).

A companion fraction from isolation of bibenzyl **2** was rechromatographed in hexane-EtOAc (4:1) on a column of Si gel. One of the fractions thereby prepared was used for final separation in hexane-iPrOH (9:1) by hplc on a column of Partisil M-9 with a flow rate of 1 ml/min. The result was 10 mg of bibenzyl **9** that recrystallized as needles from Me_2CO/hexane: mp 110–112°; PS ED_{50} 0.25 ug/ml; ir ν max 3411, 1612, 1591, 1514, 1457, 1420, 1328, 1236, 1125, 1098, 840, 750 cm^{-1}; ^1H nmr (90 MHz) 2.82 (4H, s, $-CH_2-CH_2-$), 3.82 (9H, s, 3 × OMe), 4.99 (1H, br s, OH, D_2O exchangeable), 6.35 (2H, s, H-2,6), 6.75 (2H, d, $J = 8.6$ Hz, H-3′,5′), 7.04 (2H, d, $J = 8.6$ Hz, H-2′,6′); ^{13}C nmr see Table 1; hreims *m/z*

[2]G.M. Sheldrick, SHELXS-84 direct methods, 1983, personal communication.

[3]W.D.S. Motherwell, PLUTO, Cambridge University, England, 1974, unpublished.

[4]Atomic coordinates have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

$[M]^+$ 288.136 (17%) (calcd for $C_{17}H_{20}O_4$, 288.1362), 181.0863 (100) (calcd for $C_{10}H_{13}O_3$, 181.0865), 107.0499 (100) (calcd for C_7H_7O, 107.0497).

METHYLATION OF COMBRETASTATINS B-2 AND B-3.—Combretastatin B-2 (10 mg) and combretastatin B-3 (2.0 mg) were separately methylated in a refluxing (5 h) mixture composed of excess MeI, K_2CO_3, and Me_2CO. The K_2CO_3 was collected by filtration, and the permethyl ether derivative **5** was isolated by passing the filtrate through a pipette filled with Si gel. The products from both reactions were found to be identical viscous oils. Compound **5**: ir ν max 1589, 1514, 1509, 1464, 1457, 1419, 1261, 1236, 1127 cm^{-1}; ^1H nmr (400 MHz) 2.849 (4H, m, $ArCH_2$), 3.825 (9H, s, OMe), 3.844 (3H, s, OMe), 3.863 (3H, s, OMe), 6.368 (2H, s, ArH), 6.662 (1H, d, J = 1.88 Hz, ArH), 6.724 (1H, dd, J = 8.10, 1.88 Hz, ArH), 6.802 (1H, d, J = 8.10 Hz, ArH); hreims m/z $[M]^+$ 332.1621 (18%) (calcd for $C_{19}H_{24}O_5$, 332.1624), 181.0864 (100%) (calcd for $C_{10}H_{13}O_3$, 181.0865), 151.0762 (59%) (calcd for $C_9H_{11}O_2$, 151.0759).

COMBRETASTATIN B-4 PERMETHYL ETHER [**6**].—Combretastatin B-4 [**4**] (20 mg) and bibenzyl **7** (6.2 mg) were each permethylated as described above to give identical products: permethyl ether **6** as a viscous oil: ir ν max 1606, 1594, 1514, 1463, 1428, 1419, 1204, 1151 cm^{-1}; ^1H nmr (400 MHz) 2.843 (4H, s, $ArCH_2$), 3.763 (6H, s, 2 × OMe), 3.843 (3H, s, OMe), 3.857 (3H, s, OMe), 6.311 (1H, t, J = 2.4 Hz, ArH), 6.338 (2H, d, J = 2.4 Hz, ArH), 6.672 (1H, d, J = 1.9 Hz, ArH), 6.728 (1H, dd, J = 8.2, 1.9 Hz, ArH), 6.792 (1H, d, J = 8.2 Hz, ArH); hreims m/z $[M]^+$ 302.1511 (14%) (calcd for $C_{18}H_2O_4$, 302.1518), 151.0761 (100%) (calcd for $C_9H_{11}O_2$, 151.0759).

3,4-DIBENZYLOXY-BENZALDEHYDE [**10**].—A mixture of 3,4-dihydroxy-benzaldehyde (2.76 g, 20 mmol) in dry Me_2CO (50 ml), K_2CO_3 (5.52 g, 40 mmol), and benzylbromide (5 ml, 42 mmol) was heated at reflux for 12 h. The mixture was cooled to room temperature, K_2CO_3 was removed by filtering the solution, and the filtrate was concentrated to a powder that was recrystallized from Me_2CO to yield ether **10** (5.4 g, 81%) as prisms, mp 89–90° [lit. (28) mp 90°]; ir ν max 1685, 1595, 1483, 1508, 1454, 1434, 1269, 1132, 695, 650 cm^{-1}; ^1H nmr (90 MHz), 5.21 (2H, s, $ArCH_2O$), 5.26 (2H, s, $ArCH_2O$), 7.02 (1H, d, J = 7.9 Hz), 7.30–7.50 (12H, ArH), 9.81 (1H, s, CHO). *Anal.* calcd for $C_{21}H_{18}O_3$: C 79.23, H 5.70; found C 79.26, H 5.68.

3′,4′-DIBENZYLOXY-3,4,5-TRIMETHOXY-(*Z*)- [**15**] AND -(*E*)-STILBENE [**17**].—To a stirred suspension of NaH (0.75 g, 31.4 mmol) in *N,N*-dimethylimidazolidinone (10 ml) was added phosphonium bromide **14** (29), (8.26 g, 15.7 mmol) under Ar. Aldehyde **10** (4.0 g, 12.6 mmol) was added to the deep red solution followed by 2 ml of *N,N*-dimethylimidazolidinone. The mixture was stirred overnight. Then ice H_2O (10 g ice in 75 ml H_2O) was added. Upon extraction with EtOAc (3 × 100 ml), the organic phase was washed with H_2O (3 × 75 ml), dried, and evaporated to a dark mass (9.0 g). The crude product in hexane-EtOAc (9:1) was chromatographed on a column of Si gel (200 g) to afford a mixture of *E*- and *Z*-isomers (3.70 g, 61% yield). A 1.1 g sample of the mixture was rechromatographed on a column of Si gel and eluted with hexane-EtOAc (19:1) to furnish *Z*-isomer **15** (0.25 g) and *E*-isomer **17** (0.32 g). The *Z*-isomer was further purified by preparative tlc (hexane-EtOAc, 7:3) to yield **15** as a chromatographically homogeneous and viscous oil: ir ν max 1581, 1509, 1462, 1454, 1412, 1265, 1237, 1128, 1007 cm^{-1}; ^1H nmr (400 MHz) 3.731 (6H, s, 2 × OMe), 3.856 (3H, s, OMe), 4.856 (2H, s, $ArCH_2$), 5.121 (2H, s, $ArCH_2$), 6.589 (2H, s, ArH), 6.600 (3H, m, ArH), 6.747 (1H, d, J = 8.6 Hz, ArH), 6.758 (1H, br s, ArH), 7.273–7.407 (10H, ArH).

The *E*-isomer crystallized from Me_2CO/MeOH as granules melting at 105–106°: ir ν max 1581, 1509, 1454, 1413, 1241, 1128, 1008 cm^{-1}; ^1H nmr (400 MHz) 3.874 (3H, s, OMe), 3.909 (6H, s, 2 × OMe), 5.219 (4H, s, 2 × $ArCH_2$), 6.855 (2H, s, ArH), 6.942 (1H, d, J = 8.4 Hz, ArH), 7.209 (1H, dd, J = 8.4, 2.2 Hz, ArH), 7.261 (2H, s, -CH=CH-), 7.318 (1H, d, J = 2.2 Hz, ArH), 7.325–7.500 (10H, m, ArH). *Anal.* calcd for $C_{31}H_{30}O_5$, C 77.16, H 6.27; found C 77.50, H 5.83.

COMBRETASTATIN B-3 [**3**].—A mixture composed of *Z*- and *E*-isomers **15** and **17** (0.35 g), 5% Pd/C (0.10 g), and MeOH-EtOAc (1:1, 20 ml) was saturated (ambient temperature) with H_2 at a slightly positive pressure. The reaction mixture was stirred overnight, catalyst was removed by filtration, and the crude product was chromatographed (Si gel column). Elution with hexane-EtOAc (4:1) yielded combretastatin B-3 (0.20 g, 91%) as a sticky oil which crystallized from EtOH/Et_2O as rods (mp 114–116°) and was identical with the natural product.

3-BENZYLOXY-4-METHOXYBENZYL-TRIPHENYLPHOSPHONIUM BROMIDE [**13**].—To a solution of bromide **12** (25 g, 0.081 mol) prepared (30) by benzylation (95% yield, mp 89–90°) of isovanillyl alcohol followed by bromination (99% yield, mp 93–96°) (29) in CCl_4 (150 ml) at 60° was added a solution of Ph_3P (23 g, 0.087 mol) in the same solvent. Heating was discontinued and stirring (24 h) begun when the clear solution started to become turbid. The powdery product (44.0 g, 95%) was collected and found to

melt at 252–253° following recrystallization from EtOH/Et_2O: ir ν max 1516, 1438, 1264, 1138, 1111, 727, 690, 620, 612 cm^{-1}; ^1H nmr (90 MHz), 3.78 (3H, s, OMe), 4.75 (2H, s, $ArCH_2$), 5.25 (2H, d, J = 13.8 Hz, -CH_2P-), 6.64 (2H, br s, ArH), 6.83 (1H, br s, ArH), 7.29 (5H, br s, ArH), 7.57–7.81 (15H, ArH). *Anal.* calcd for $C_{33}H_{30}BrO_2P \cdot \frac{1}{2}H_2O$: C 68.52, H 5.40, Br 13.81; found C 68.22, H 5.33, Br 14.05.

3′-BENXYLOXY-3′,4′,5′-TRIMETHOXY-(*Z*)- [**16**] AND -(*E*)-STILBENE [**18**].—Phosphonium salt **13** (3.98 g, 7.0 mmol) was added to a stirred suspension of NaH (250 mg, 10.4 mmol) in *N,N*-dimethylimidazolidinone (5 ml) under Ar. The deep red solution was stirred for 10 min and heated at 40–50° for 5 min, and 3,5-dimethoxybenzylaldehyde (1.0 g, 5.0 mmol) was added. After allowing the reaction mixture to reach room temperature, stirring was continued overnight. Ice (5 g) and H_2O (50 ml) were added; the product was extracted with CH_2Cl_2 (3 × 50 ml), washed with cold H_2O (50 ml), dried, and solvent evaporated. The crude product in hexane-EtOAc (4:1) was chromatographed using a Si gel (80 g) column to provide a mixture of *Z*- and *E*-isomers (1.76 g, 78% yield). A 0.50-g sample of the mixture was rechromatographed (Si gel column). Elution with hexane-EtOAc (19:1) afforded chromatographically pure *Z*-isomer **16** (0.15 g) as a viscous oil: ir ν max 1599, 1590, 1513, 1455, 1427, 1257, 1236, 1205, 1155, 1141 cm^{-1}; ^1H nmr (400 MHz) 3.682 (6H, s, 2 × OMe), 3.858 (3H, s, OMe), 4.894 (2H, s, $ArCH_2$), 6.337 (1H, t, J = 2.2 Hz, H-4), 6.433 (2H, d, J = 2.2 Hz, H-2,6), 6.447 (1H, d, J = 12.5 Hz, -CH=CH-), 6.453 (1H, d, J = 12.5 Hz, -CH=CH-), 6.768 (1H, d, J = 8.24 Hz, H-5′), 6.844 (1H, dd, J = 8.24, 2.0 Hz, H-6′), 6.874 (1H, d, J = 2.0 Hz, H-2′), 7.282–7.320 (5H, m, ArH).

Continued elution of the preceding chromatogram led to the *E*-isomer **18** (0.15 g). Recrystallization from Me_2CO/hexane gave a pure specimen as flakes, mp 96–98°: ir ν max 1597, 1513, 1454, 1441, 1427, 1262, 1155, 1137 cm^{-1}; ^1H nmr (400 MHz) 3.829 (6H, s, 2 × OMe), 3.909 (3H, s, OMe), 5.204 (2H, s, $ArCH_2$), 6.375 (1H, t, J = 2.2 Hz, H-4), 6.635 (2H, d, J = 2.2 Hz, H-2,6), 6.822 (1H, d, J = 16.24 Hz, -CH=CH-), 6.886 (1H, d, J = 8.3 Hz, H-5′), 6.978 (1H, d, J = 16.24 Hz, -CH=CH-), 7.068 (1H, dd, J = 8.3, 1.92 Hz, H-6′), 7.096 (1H, d, J = 1.92 Hz, H-2′), 7.301–7.492 (5H, m, ArH). *Anal.* calcd for $C_{24}H_{24}O_4$, C 76.58, H 6.43; found C 76.69, H 6.41.

1-(3,5-DIMETHOXYPHENYL)-2-(3′-HYDROXY-4′-METHOXYPHENYL)-ETHANE [**7**].—A mixture prepared from *Z*-isomer **16** and *E*-isomer **18** (0.10 g), 5% Pd/C (50 mg), and MeOH (10 ml) was reduced at ambient temperature with a slightly positive pressure of H_2 over 24 h with stirring. The catalyst was collected, the product (70.6 mg, 92% yield) was purified by preparative tlc (3:2, hexane-EtOAc, mobile phase), and crystallized from Me_2CO/hexane to yield needles melting at 108° [lit. (18) mp 107–107.5°] and identical with natural bibenzyl **7**.

3,5-DIMETHOXY-2′-(4″-HYDROXYPHENYL)-(*E*)-CINNAMIC ACID [**19**].—A mixture of 4-hydroxyphenylacetic acid (0.92 g, 6.02 mmol), 3,5-dimethoxybenzyaldehyde (1.0 g, 6.02 mmol), Ac_2O (1 ml), and triethylamine (0.5 ml) was heated at 130–140° for 20 h. Course of the reaction was monitored by tlc using hexane-EtOAc (3:2). Upon completion, the reaction mixture was cooled to room temperature, and 5 ml of concentrated HCl was added. The precipitate (1.8 g) was dissolved in $CHCl_3$ (50 ml) and extracted with 10% aqueous NaOH (3 × 25 ml), and the basic solution was acidified (pH 1) with concentrated HCl. After stirring 2 h, the precipitate (1.2 g, 66% yield) was collected and recrystallized from EtOH/H_2O to afford cinnamic acid **19** as fine needles; mp 226–228°: ir ν max (KBr) 3428, 1669, 1627, 1589, 1514, 1426, 1286, 1208, 1155, 1075, 1055, 825 cm^{-1}; ^1H nmr ($CDCl_3$ -MeOD), 3.57 (6H, s, 2 × OMe), 6.29 (3H, br s, H-5,7,9), 6.84 (2H, d, J = 8.8 Hz, H-3″,5′), 7.09 (2H, d, J_{AB} = 8.8 Hz, H-2′,6′), 7.73 (1H, s, H-3). *Anal.* calcd for $C_{17}H_{16}O_5 \cdot \frac{1}{4}H_2O$, C 66.99, H 5.45; found C 67.32, H 5.25.

4′-HYDROXY-3,5,-DIMETHOXY-(*E*)-STILBENE [**20**].—The aforementioned cinnamic acid **19**, (0.38 g) was heated under Ar for 5 h in refluxing quinoline (10 ml) with Cu powder (1.0 g). The mixture was cooled to room temperature; the Cu was collected and concentrated HCl (25 ml) was added to the stirred filtrate. Stirring was continued 2 h, H_2O (20 ml) was added, and the product was extracted with $CHCl_3$ (3 × 50 ml). The $CHCl_3$ extract was washed with 5 N HCl (2 × 25 ml), H_2O (50 ml), dried, and concentrated (reduced pressure). The crude product (0.30 g) in hexane-EtOAc (4:1) was chromatographed on a small column of Si gel. Elution with the same solvent led to slightly impure stilbene **20** which was purified by preparative tlc (Si gel, hexane-EtOAc, 3:2) to yield stilbene **20** as a homogeneous oil (0.10 g, 31% yield): ir ν max 3386, 1590, 1513, 1457, 1204, 1171, 1151, 1066, 834 cm^{-1}; ^1H nmr (90 MHz) 3.82 (6H, s, 2 × OMe), 5.0–6.0 (1H, br s, OH, D_2O), 6.38 (1H, t, J = 2.2 Hz, H-4); 6.65 (2H, d, J = 2.2 Hz, H-2,6), 6.81 (2H, d, J_{AB} = 8.7 Hz, H-3′,5′), 6.82 (1H, d, J_{AB} = 16 Hz, CH=CH), 7.06 (1H, d, J_{AB} = 16 Hz, -CH=CH-), 7.39 (2H, d, J_{AB} = 8.7 Hz, H-2′,6′); and hreims *m/z* $[M]^+$ 256.1105 (15%) (calcd for $C_{16}H_{16}O_3$, 256.1099).

1-(3′,5′-DIMETHYOXYPHENYL)-2-(4′-HYDROXYPHENYL)-ETHANE [**2**].—A solution of 4′-hydroxy-3,5-dimethoxy-stilbene (20 mg) in MeOH (12 ml) was hydrogenated using 5% Pd/C (20 mg) as

described above for compound **7**. The viscous oily product (18 mg, 90% yield) was chromatographically homogeneous and found to be identical with the natural product **8**.

3,4,5-TRIMETHOXY-2′-(4″-HYDROXYPHENYL)-(*E*)-CINNAMIC ACID [**21**].—The Perkin condensation between 4-hydroxy-phenylacetic acid (3.08 g, 20.2 mmol), 3,4,5-trimethoxy-benzaldehyde (3.96 g, 20.2 mmol), Ac_2O (4 ml), and triethylamine (2 ml) was allowed to proceed (monitored by tlc using hexane-EtOAc, 1:2) for 72 h at 130–140°. Addition of concentrated HCl (25 ml) gave a solution that was diluted with H_2O (100 ml) and extracted with EtOAc (4 × 50 ml), followed by $CHCl_3$ (4 × 75 ml). The EtOAc extract was concentrated and the residue dissolved in $CHCl_3$ (100 ml). The combined $CHCl_3$ solution was extracted with 1 N NaOH (3 × 100 ml), and the basic solution was acidified with concentrated HCl to pH 1. After stirring at room temperature for 30 min, the precipitate (1.9 g) was collected, and the filtrate was saturated with NaCl and extracted with $CHCl_3$ (5 × 100 ml). The $CHCl_3$ extract was washed with brine (100 ml), dried, and concentrated to a 2.77 g (41% yield) residue that was recrystallized from EtOH to give very fine needles, mp 216–218°: ir ν max (KBr) 3400, 1684, 1617, 1580, 1515, 1505, 1420, 1331, 1266, 1248, 1125, 1000, 850 cm^{-1}; ^1H nmr ($CDCl_3/CD_3OD$) 3.59 (6H, s, 2 × OMe), 3.80 (3H, s, OMe), 6.38 (2H, s, H-5,9), 6.87 (2H, s, J_{AB} = 8.8 Hz, H-3′,5′), 7.11 (2H, d, J_{AB} = 8.8 Hz, H-2′,6′), 7.73 (1H, s, H-3). *Anal.* calcd for $C_{18}H_{18}O_{16}\cdot\frac{1}{4}H_2O$, C 64.58, H 5.56; found C 64.30, H 5.23.

4′-HYDROXY-3,4,5-TRIMETHOXY-(*E*)-STILBENE [**22**].—Cinnamic acid **21** (0.10 g) was decarboxylated in quinoline (5 ml) with Cu powder (500 mg) and the product purified as summarized above for stilbene **20**. Stilbene **22** (39.5 mg, 46% yield) was obtained as flaky needles from Me_2CO/hexane, mp 193–194° [lit. (31–33) mp 190°]: ir ν max (KBr) 3400, 1608, 1582, 1513, 1463, 1456, 1453, 1419, 1231, 1126, 850, 760 cm^{-1}; ^1H nmr (90 MHz) 3.87 (3H, s, OMe), 3.91 (6H, s, 2 × OMe), 6.71 (2H, s, H-2,6), 6.83 (2H, d, J_{AB} = 8.7 Hz, H-3′,5′), 6.91 (2H, s, -CH=CH-), 7.39 (2H, d, J_{AB} = 8.7 Hz, H-2′,6′). *Anal.* calcd for $C_{17}H_{18}O_4\cdot\frac{1}{4}H_2O$, C 70.22, H 6.41; found C 70.71, H 6.19.

1-(3′,4′,5′-TRIMETHOXYPHENYL)-2-(4″-HYDROXYPHENYL)-ETHANE [**9**].—By the same procedure used to obtain ethane **7** (see above) stilbene **22** (10 mg) was hydrogenated over 5% Pd/C (10 mg) in MeOH (5 ml). Pure bibenzyl **9** (8.8 mg, 88% yield), recrystallized as needles from Me_2CO/hexane, mp 110–112° [lit. (11) mp 113–115°] and was found identical with the natural product **9** from *C. caffrum*. *Anal.* calcd for $C_{17}H_{20}O_4$, C 70.81, H 6.99; found C 70.74, H 6.94.

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