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# Convergent synthesis and preliminary biological evaluations of the stilbenolignan (±)-aiphanol and various congeners

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**Treatment of an equimolar mixture of stilbene 7 and cinnamyl alcohol 8 with silver carbonate in acetone–benzene afforded a *ca.* 2 : 1 : 2 : 1 mixture of the stilbenolignan (±)-aiphanol (1) and congeners 2–4 each of which show significant anti-angiogenic and COX-2 inhibitory properties.**

Kinghorn and co-workers have recently reported[1] the bioassay-guided isolation of the stilbenolignan (−)-aiphanol (**1**) from the seeds of *Aiphanes aculeata* Willd. (Arecaceae) collected in Peru. The compound not only possesses the unprecedented stilbenolignan skeleton in which a stilbene unit is connected to a phenylpropane unit *via* a dioxane bridge but it also shows potent inhibition of cyclooxygenase-1 and -2 (COX-1 and -2) with $IC_{50}$ values of 1.9 and 9.9 μM, respectively. Since compounds exhibiting COX-2 activity can also act as anti-angiogenic agents (*i.e.* they inhibit the growth of blood vessels)[2,3] and because certain dihydrobenzofuran lignans have recently been shown[4] to function in the latter mode we became interested in developing simple syntheses of compound **1** and various congeners with a view to establishing preliminary structure–activity profiles for this novel type of natural product. In this connection we now describe a simple and convergent (but non-selective) route to (±)-aiphanol [(±)-**1**] and the regio- and/or stereo-isomeric systems **2**–**4**. Preliminary biological evaluations of compounds **1**–**4**, and certain substructures as COX-2 and angiogenesis inhibitors are also reported herein.

The synthetic route employed in obtaining the racemic modifications of compounds **1**–**4** is shown in Scheme 1. Thus, the previously reported[5] and readily available ylide **5** was reacted with the known[5a] benzaldehyde **6** to give, after an extractive work up and a fluoride ion treatment which resulted in two-fold desilylation of the primary product, the stilbene piceatannol (itself a natural product[6]) in 67% yield (mp 232–234 °C; lit.[5b] mp 229 °C). Following Stermitz's modification[7] of the classic procedures developed by Merlini *et al.*,[8] silver carbonate-promoted oxidative coupling of compound **7** with commercially available sinapyl alcohol (**8**) gave a *ca.* 2 : 1 : 2 : 1 mixture of compounds **1**–**4** (67% combined yield)[9] which could be separated into its component parts *via* HPLC (Fig. 1). The most mobile and second most abundant component, F1, proved to be *rac*-aiphanol [(±)-**1**] as judged by comparison of the derived $^1H$ and $^{13}C$ NMR spectral data with those reported[1] for the natural product. The major product, F3, is most likely the regio-isomeric system **3** which is assigned as such by virtue of the observation of a vicinal coupling of 8.1 Hz between H2 and H3 (the equivalent coupling in *rac*-aiphanol is the same) suggesting a *trans*-relationship between the aryl and hydroxymethyl substituents on the newly formed dioxane ring. In compounds **2** and **4**, arising from fractions F2 and F4, respectively, $J_{2,3}$ = 2.6–2.7 Hz suggesting a *cis*-relationship between these same groupings. The similarities in the $^{13}C$ NMR spectral data (Fig. 2) derived from compounds **1** and **2** suggest they possess the same regiochemical relationships between the styrene, hydroxymethyl and aryl moieties in terms of their positions of attachment to the 1,4-benzodioxin core. In principle the use of horseradish peroxidase to promote oxidative coupling of compounds **7** and **8** could deliver (−)-aiphanol selectively but related experiments by Fukuyama *et al.*[9] suggest that the enantiomeric excesses likely to be observed in such processes would be very modest indeed.

For the sake of establishing more comprehensive structure–activity relationships within the aiphanol "class", compound

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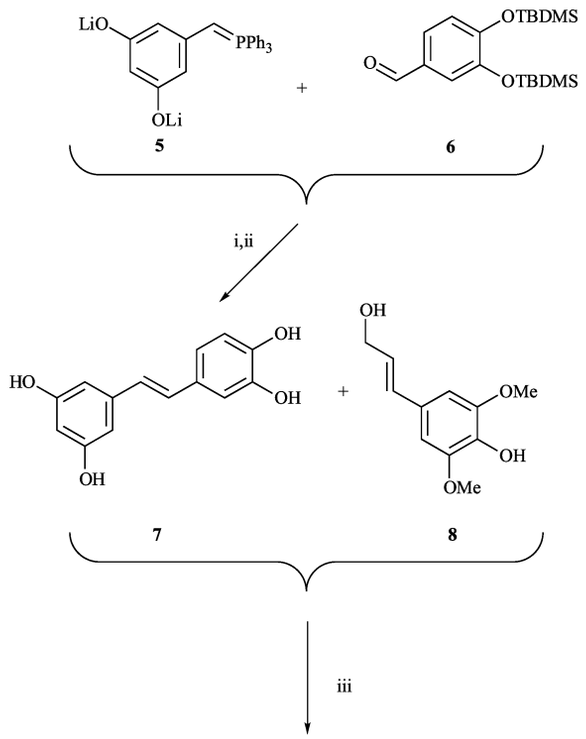



**Scheme 1** *Reagents and conditions*: (i) THF, 18 °C, 18 h then HCl (aq.); (ii) TBAF (4 mole equiv.), THF, 0 °C, 5 min; (iii) $Ag_2CO_3$ (1 mole equiv.), 1 : 2 v/v acetone–benzene, 60 °C, 18 h.

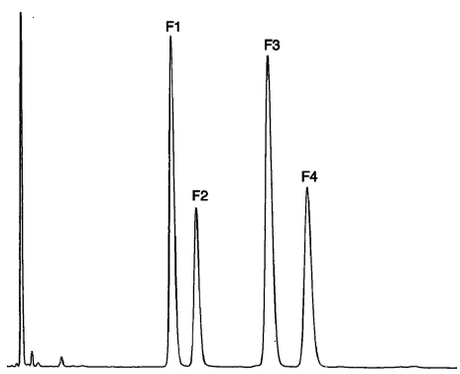
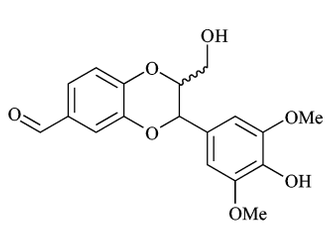



**Fig. 1** HPLC trace derived from analysis of the reaction mixture obtained on oxidative coupling of compounds **7** and **8** with silver carbonate (see Experimental section for details).

**9** (71%), embodying the 1,4-benzodioxin core plus the appended aryl and hydroxymethyl substituents of aiphanol, was prepared (as a *ca*. 1 : 1 and chromatographically inseparable mixture of *cis*- and *trans*-isomers) by oxidative coupling of 3,4-dihydroxybenzaldehye with sinapyl alcohol. The absence of any of the regio-isomeric coupling products in this reaction is consistent with earlier work[7a] where it has been noted that the presence of an aldehyde moiety in the 4-position on the catechol residue results in a completely regioselective conversion.




**9**

With serviceable quantities of lignans **1**–**4** as well as congeners **7** and **9** available *via* the pathways described above, biological evaluations could be carried out. Assessment of compounds **1**–**4** as inhibitors of COX-1 and -2 was conducted *in vitro* under conditions defined previously[10] and the results presented in Table 1. Interestingly, (±)-aiphanol is a significant inhibitor of COX-2 but only a modest inhibitor of COX-1. This appears to be the reverse of the situation observed for the naturally occurring (−)-form and suggests that (+)-aiphanol could be a more potent COX-2 inhibitor. Whilst definitive comment on this matter must await the preparation and testing of (+)-**1**, it is worth noting that the enantiomeric forms of other chiral ligands have been shown[11] to vary in their COX-1 and -2 inhibitory properties. The anti-angiogenic properties of compounds **1**–**4**, **7** and **9** were determined in an *in vitro* angiogenesis assay using rat aorta rather than human placental blood vessel fragments[12] and the results shown in Table 2 indicate that *rac*-aiphanol [(±)-**1**] completely inhibited blood vessel growth at 100 μg $mL^{-1}$ while isomers **2** and **4** behaved similarly. Compound **3** proved a lot less active as did "substructure" **9**. Interestingly, piceatannol (**7**) was almost as active as *rac*-aiphanol thus further emphasizing the pharmaceutical potential of hydroxystilbenes.[13] It is also worth noting that compounds **1**–**3** and **7** are all more active, at the 100 μg $mL^{-1}$ level, than PI-88, a polysulfated oligosaccharide which exhibits anti-angiogenic properties and is now in clinical development as an agent for the treatment of certain cancers.[14]

**Table 1** COX-1/2 inhibitory properties of compounds (±)-**1**–**4**[a]

| Compound | $IC_{50}$ for COX-1/μM | $IC_{50}$ for COX-2/μM |
|---|---|---|
| **1** | 7.3 | 0.17 |
| **2** | 6.3 | 9.5 |
| **3** | 9.0 | 41[b] |
| **4** | 7.7 | 7.7 |

[a] Assays conducted according to the method of Dannhardt *et al.*[10] [b] % Inhibition at 10 μM concentration.

## Experimental

### Compounds 1–4

A magnetically stirred solution of stilbene **7** (94 mg, 0.38 mmol) and alcohol **8** (81 mg, 0.38 mmol) in benzene–acetone (30 mL of a 2 : 1 v/v mixture) was heated at 60 °C for 0.25 h then treated, in one portion, with silver carbonate (107 mg, 0.38 mmol). The resulting mixture was heated at 60 °C for 18 h then cooled and the precipitate removed by filtration. The filtrate was then concentrated under reduced pressure to give a brown gum. Subjection of this material to flash chromatography (silica, 8 : 2 v/v ethyl acetate–hexane elution) yielded, after concentration of the appropriate fractions ($R_f$ 0.35), 108 mg of a brown solid comprising a *ca*. 2 : 1 : 2 : 1 mixture of compounds **1**–**4**. Subjection of this material to HPLC (using a 300 × 10 mm 5 μm C18 Alltech Alltima column, 50 : 49.9 : 0.1 v/v/v water–methanol–acetic acid elution, solvent flow rate of 5 mL $min^{-1}$, UV peak detection at 325 nm) afforded four fractions (F1–F4 – see Fig. 1).

Concentration of F1 ($R_t$ 15.06 min) under reduced pressure gave (±)-aiphanol (**1**) (30 mg, 17%) as a brown solid, mp 162–164 °C. $\nu_{max}$ (KBr)/$cm^{-1}$ 3370, 1595, 1505, 1463, 1270, 1115; $\delta_H$ (300 MHz, $CD_3COCD_3$) 7.14 (1H, d, *J* 2.1), 7.09 (1H, dd, *J* 8.5 and 2.1), 7.02 (1H, d, *J* 16.2), 6.94 (1H, d, *J* 16.2), 6.90 (1H, d, *J* 8.4), 6.84 (2H, s), 6.55 (2H, d, *J* 2.1), 6.28 (1H, br t, *J* 2.1), 4.97 (1H, d, *J* 8.1), 4.14 (1H, m), 3.86 (6H, s), 3.78 (1H, m), 3.53 (1H, m); $\delta_C$ (75 MHz, $CD_3COCD_3$) 159.2, 148.4, 144.7, 144.1, 140.2, 136.9, 131.5, 128.3, 127.8, 127.7, 120.5, 117.4, 115.0, 105.7, 105.4, 102.6, 79.4, 77.2, 61.6, 56.5; *m*/*z* 452.1471 ($M^{+\bullet}$, $C_{25}H_{24}O_8$ requires 452.1471, 58%), 255 (13), 210 (40), 167 (100), 73 (35).

Concentration of F2 ($R_t$ 16.65 min) under reduced pressure gave compound **2** (20 mg, 12%) as a brown solid, mp 162–164 °C.

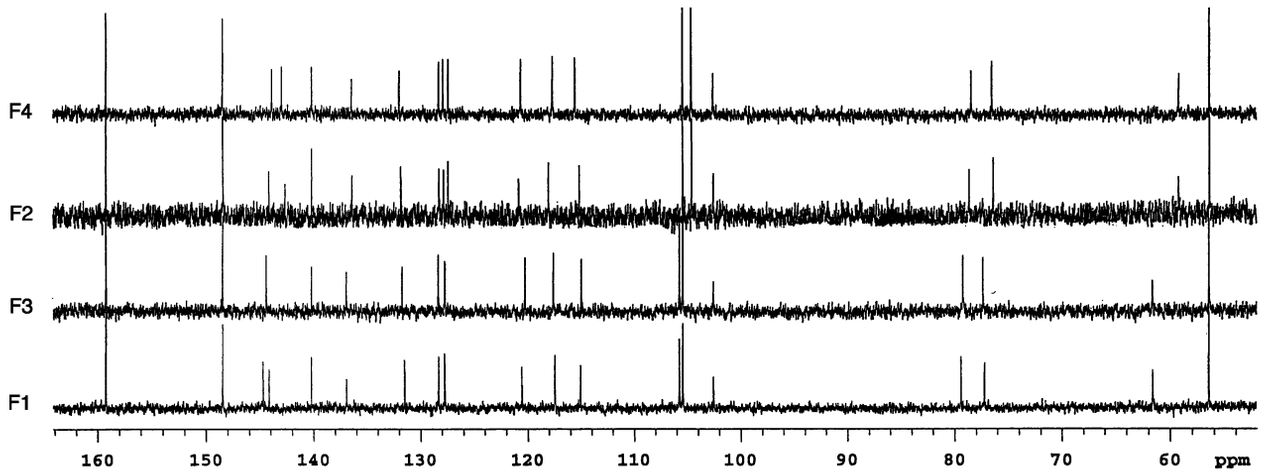



**Fig. 2** Stacked-plot presentation of the 75 MHz $^{13}C$ NMR spectra of compounds (±)-**1**–**4** ($CD_3COCD_3$ as solvent).

**Table 2** Anti-angiogenic properties of compounds (±)-**1**–**4**, **7** and **9** as determined in a rat aorta assay.[a]

| Compound | Inhibition of vessel growth at 100 μg $mL^{-1}$ (%) | Inhibition of vessel growth at 10 μg $mL^{-1}$ (%) |
|---|---|---|
| **1** | 100 | 42 |
| **2** | 99 | 25 |
| **3** | 50 | 38 |
| **4** | 96 | 32 |
| **7** | 80 | 34 |
| **9** | 57 | 35 |
| **PI-88** | 62 | 30 |

[a] Assays conducted according to the method of Parish *et al.*[12b]

$\nu_{max}$ (KBr)/$cm^{-1}$ 3339, 1596, 1505, 1463, 1272, 1117; $\delta_H$ (300 MHz, $CD_3COCD_3$) 7.17 (1H, d, *J* 2.1), 7.11 (1H, dd, *J* 8.5 and 2.2), 7.03 (1H, d, *J* 16.2), 6.95 (1H, d, *J* 15.2), 6.91 (1H, d, *J* 8.4), 6.79 (2H, s), 6.56 (2H, d, *J* 2.1), 6.28 (1H, br t, *J* 2.2), 5.29 (1H, d, *J* 2.7), 4.54 (1H, m), 3.81 (6H, s), 3.65 (1H, m), 3.54 (1H, m); $\delta_C$ (75 MHz, $CD_3COCD_3$) 159.2, 148.4, 144.2, 142.5, 140.2, 136.4, 131.8, 128.3, 127.9, 127.4, 120.8, 118.1, 115.2, 105.5, 104.6, 102.6, 78.7, 76.4, 59.3, 56.5; *m*/*z* 452.1473 ($M^{+\bullet}$, $C_{25}H_{24}O_8$ requires 452.1471, 50%), 346 (19), 255 (15), 199 (45), 167 (100), 151 (84), 73 (71).

Concentration of F3 ($R_t$ 21.36 min) under reduced pressure gave compound **3** (32 mg, 19%) as a brown solid, mp 161–163 °C. $\nu_{max}$ (KBr)/$cm^{-1}$ 3386, 1594, 1505, 1463, 1270, 1216, 1114; $\delta_H$ (300 MHz, $CD_3COCD_3$) 7.13 (1H, d, *J* 1.9), 7.08 (1H, dd, *J* 8.4 and 1.9), 7.03 (1H, d, *J* 16.2), 6.94 (1H, d, *J* 16.2), 6.89 (1H, d, *J* 8.4), 6.83 (2H, s), 6.56 (2H, d, *J* 2.1), 6.29 (1H, br t, *J* 2.1), 4.97 (1H, d, *J* 8.1), 4.15 (1H, m), 3.86 (6H, s), 3.75 (1H, dd, *J* 12.0 and 4.1), 3.53 (1H, dd, *J* 12.0 and 4.1); $\delta_C$ (75 MHz, $CD_3COCD_3$) 159.2, 148.4, 144.4, 144.3, 140.1, 136.9, 131.7, 128.3, 127.7, 127.7, 120.2, 117.6, 114.9, 105.7, 105.4, 102.6, 79.2, 77.3, 61.6, 56.4; *m*/*z* 452.1475 ($M^{+\bullet}$, $C_{25}H_{24}O_8$ requires 452.1471, 35%), 346 (15), 241 (32), 210 (42), 167 (100), 151 (47), 73 (44).

Concentration of F4 ($R_t$ 23.89 min) under reduced pressure gave compound **4** (22 mg, 14%) as a brown solid, mp 161–163 °C. $\nu_{max}$ (KBr)/$cm^{-1}$ 3351, 1594, 1505, 1462, 1269, 1115; $\delta_H$ (300 MHz, $CD_3COCD_3$) 7.15 (1H, d, *J* 2.1), 7.10 (1H, dd, *J* 8.4 and 2.1), 7.03 (1H, d, *J* 16.4), 6.95 (1H, d, *J* 16.4), 6.92 (1H, d, *J* 8.2), 6.78 (2H, s), 6.56 (2H, d, *J* 2.1), 6.29 (1H, br t, *J* 2.1), 5.29 (1H, d, *J* 2.6), 4.54 (1H, m), 3.81 (6H, s), 3.65 (1H, dd, *J* 12.0 and 3.9), 3.49 (1H, dd, *J* 12.0 and 3.9); $\delta_C$ (75 MHz, $CD_3COCD_3$) 159.2, 148.4, 143.8, 142.9, 140.2, 136.4, 131.9, 128.3, 127.9, 127.4, 120.6, 117.7, 115.5, 105.4, 104.6, 102.6, 78.5, 76.5, 59.2, 56.4; *m*/*z* 452.1469 ($M^{+\bullet}$, $C_{25}H_{24}O_8$ requires 452.1471, 30%), 434 (13), 346 (5), 210 (26), 167 (65), 100 (100), 73 (65).

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