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AMORFRUTIN A AND B, BIBENZYL ANTIMICROBIAL AGENTS FROM *AMORPHA FRUTICOSA*

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Key Word Index—*Amorpha fruticosa*; Leguminosae; false indigo fruit; amorfrutin A and amorfrutin B; bibenzyl metabolites; antimicrobial agents; structure elucidation.

Abstract—Bioassay directed fractionation of the waxy fractions derived from the fruit of *Amorpha fruticosa* resulted in the isolation of amorfrutin A and B, active new antimicrobial agents. Spectroscopic studies, chemical degradation and synthesis showed them to be new bibenzyl metabolites.

INTRODUCTION

Amorpha fruticosa is an indigenous American shrub finding local Amerindian use for bedding and as a mat for keeping freshly butchered meat clean. Ethanolic extracts of the powdered fruits, stems and leaves showed reproducable activity *in vitro* against *Mycobacterium smegmatis* (ATCC 607) and *Staphylococcus aureus* (ATCC 13709) [1]. Similar extracts from related species, *A. canescens* and *A. nana*, were inactive. Initial study, reported briefly elsewhere, resulted in the discovery of a new, active, rotenoid, 11-hydroxytephrosin [2]. The waxy constituents from the fruits contained additional bioactive substances so further fractionation was carried out and we report herein the properties and structures of the active constituents, the new bibenzyl metabolites, amorfrutin A (**1**) and B (**2**).

RESULTS AND DISCUSSION

Amorpha fruticosa was collected in Douglas County, Kansas, by Ralph Brooks of the Kansas Biological Survey, Lawrence, where there is a voucher specimen on deposit. The air-dried, ground fruits were exhaustively percolated with ethanol and then fractionated essentially as previously described [1]. The biological activity was found in the *n*-hexane soluble portions which primarily contain non-polar lipids. Extensive chromatography over silica gel produced active components **1** and **2**.

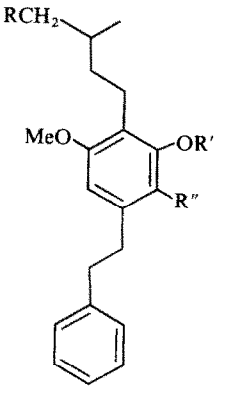
Amorfrutin A (**1**) has an empirical formula of $C_{21}H_{24}O_4$ based upon microanalysis and mass spectrometry (M^+, 340). The ^1H NMR spectrum contained typical absorption peaks for a OMe group, a prenyl moiety, a pentasubstituted aromatic ring, a monosubstituted aromatic ring, two exchangeable acidic hydrogens and a symmetrical non-first order AA′BB′ multiplet (δ 2.94, 2 H; δ 3.28, 2 H). These data suggested a bibenzyl structure for amorfrutin A. While as yet uncommon in nature, bibenzyls are being isolated with increasing frequency [3–15]. The base peak in the mass spectrum occurred at *m/e* 91 (tropylium ion) in agreement with this provisional assignment. A permanent colour with alcoholic ferric chloride, solubility in sodium bicarbonate, a hydrogen-bonded carboxylic acid peak at 1610 cm^{-1} in the infra-red and ready mass spectrometric loss of water ($M^+ - 18$, *m/e* 322) and carbon dioxide ($M^+ - 44$, *m/e* 294) indicated that the two exchangeable protons were part of a salicylic acid moiety. In agreement, brief treatment with diazomethane produced ester **3** (δ 3.91, 3 H, *s*, CO_2CH_3; *v* 1650 cm^{-1}; $M^+ - 32$, *m/e* 322; etc.). In further confirmation and in order to locate the various substituents, amorfrutin A was decarboxylated to phenol **4** by heating under nitrogen for 1 hr at 155–60°. The UV spectrum of phenol **4** (λ_{max} 274, 228 nm) is typical of previously known bibenzyls as is the collapse of the AA′BB′ multiplet of **1** to a 4 proton singlet at δ 2.85 in **4** and, most revealingly, the presence of a pair of *meta* coupled (1.5 Hz) aromatic proton doublets at δ 6.27 and 6.35 identifies the spacial relationship between the original aromatic proton and carboxyl group of **1**. Compound **4** also gave a positive Gibbs' test (blue) indicating that the phenolic hydroxyl group was *para* to an Ar–H group.

The position of the prenyl group relative to the phenolic OH was established by BF_3 mediated cyclization of **4** to *gem*-diMe substituted dihydropyranyl degradation product **5**. The differences in the ^1H NMR spectra of **4** and **5** consisted in the loss of the exchangeable OH of **4** and replacement of the prenyl signals by a 6-proton *gem*-diMe group and a pair of A_2M_2-type triplets representing the two methylenes of the dihydropyran moiety. There are two possible structures consistent with these findings (**5** and **6**). Biogenetic considerations strongly favour structure **5**. Because materials were now rather limited, unambiguous proof of structure **5** (and hence **1** for amorfrutin A) was sought by synthesis.

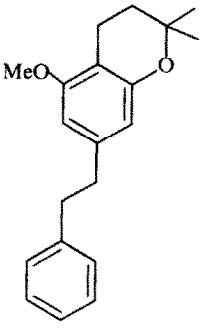
Reaction of 3,5-dimethoxybenzaldehyde (**7**) with the Wittig reagent prepared from benzyltriphenylphosphorane led to styrene **8** in 85% yield as a mixture of *Z*- and *E*-isomers. Catalytic reduction produced a quantitative yield of 3,5-dimethoxybibenzyl (**9**). Ether cleavage with BBr_3 gave a 90% yield of 3,5-dihydroxybibenzyl (**10**) which was subsequently prenylated in the usual way by BF_3-catalysed reaction of **10** with 3-methyl-2-buten-1-ol to give phenolic

dihydropyran **11** [13] in 12% yield, after chromatographic separation from its isomers, particularly **13**. The desired compound (**11**) was readily distinguished from **13** by giving a positive (purple) Gibbs' test. Further, it is well known that chemical shifts for aromatic hydrogens in ^1H NMR studies of phenols are sensitive to the addition of base [16–18]. In such studies $\Delta\delta$ values of greater numerical value are observed for hydrogens *para* to phenolic OH groups as compared with *ortho* hydrogens. In isomer **11**, a $\Delta\delta$ value of 0.34 and another of 0.53 (δ DMSO-d_6 − δ DMSO-d_6/NaOD) were observed consistent with that expected for one *ortho* and one *para* H respectively. With isomer **13**, the corresponding $\Delta\delta$ values observed were 0.34 and 0.24, consistent with that expected for two *ortho* hydrogens.

Methylation of isomer **11** in the usual way produced **5**, identical to the product obtained by degradation of amorfrutin A (**1**). Compound **13** produced an isomeric substance on methylation, which was easily distinguished from **5**. With more material, particularly with both isomers available, added distinction of **12** from **14** and structural information of **5** (**12**) was possible from nuclear Overhauser experiments [19]. Irradiation of the $OC\underline{H}_3$ group of **5** gave an enhancement of the integrated area for ArH_6 of 46% while similar irradiation of the $ArC\underline{H}_2C\underline{H}_2Ar'$ bridge hydrogens gave a 45% enhancement. The corresponding changes for ArH_4 were 17 and 47%, respectively. Similar irradiation of the $OC\underline{H}_3$ moiety of **14** gave a 42 and 37% integral enhancement of the two aromatic hydrogens, and of the $ArC\underline{H}_2C\underline{H}_2Ar'$



1 R = R′ = H, R″ = CO_2H
2 R = $CH_2CH{=}C(Me)_2$, R′ = H, R″ = CO_2H
3 R = R′ = H, R″ = CO_2Me
4 R = R′ = R″ = H
15 R = $CH_2CH{=}C(Me)_2$, R′ = CO_2Me
16 R = $CH_2CH{=}C(Me)_2$, R′ = R″ = H



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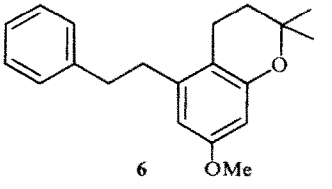

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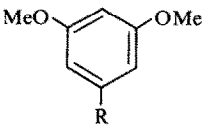
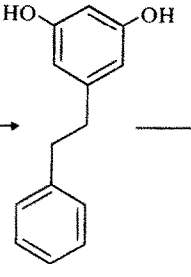


7 R = CHO
8 R = $CH{=}CHC_6H_5$
9 R = $CH_2CH_2C_6H_5$



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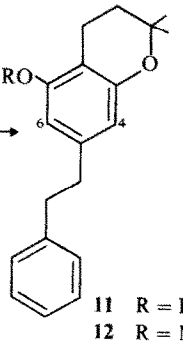
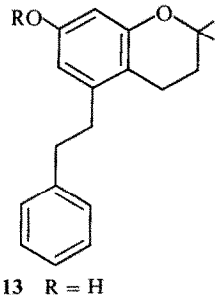
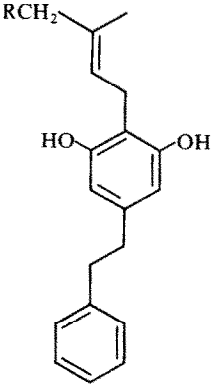


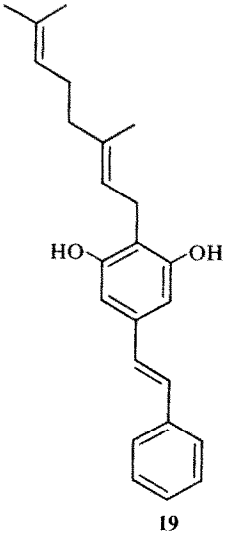
11 R = H
12 R = Me



13 R = H
14 R = Me



17 R = H
18 R = $CH_2CH{=}C(Me)_2$



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bridge hydrogens gave a 56 and a 29% enhancement. These results are consistent with the structures assigned, and the structure proposed for amorfrutin A is secure.

Amorfrutin B (**2**) differs from A in having a geranyl side-chain in place of the prenyl group. This distinction was made on spectroscopic grounds because of the limited quantity of B present in the extracts. In particular, the UV spectra of amorfrutin A and B are closely similar, suggesting possession of the same chromophore, and the mass spectrum, in addition to many similarities in fragmentation, indicated possession of a second C_5H_9 (prenyl) unit. Because the ^1H NMR spectra of the two antimicrobial agents differed only in the δ 1.0 to 5.5 region, this unit was obviously joined to the first prenyl unit and the detailed pattern showed that the attachment was clearly head-to-tail. The spectrum showed 3 Me singlets (δ 1.53, 1.57 and 1.73), a broad 4 proton singlet at δ 1.93 due to the geranyl $C\underline{H}_2C\underline{H}_2$ unit and an overlapping signal for a two hydrogen pair of vinyl triplets at δ 5.07. Formation of a mono Me ester (**15**), useful for purification as well as for characterization, and thermal decarboxylation to give phenol **16** were both attended by the expected spectroscopic changes in parallel with experience with amorfrutin A. These considerations lead to structure **2** for amorfrutin B.

The most closely precedent structures in the literature are those of the phenols **17**, recently discovered in *Helichrysum umbraculigerum* [15] and the European liverwort *Radula complanata*, [13] and **18**, also from *H. umbraculigerum* [15]. In light of the biosynthetic origin of the bibenzyls, the recent discovery of amorphastilbol (**19**) from various *Amorpha* species including *A. fruticosa*, is especially noteworthy [20].

The *in vitro* antimicrobial activity of the amorfrutins and related substances is given in Table 1. From this it can be seen that activity is limited to Gram-positive and acid-fast microorganisms and that the amorfrutins are essentially equipotent. Curiously, Me ester formation abolishes activity whereas decarboxylation does not. The specific potency, rather higher than usual for plant products, suggest *in vivo* studies would be worthwhile. However, the relative narrowness of spectrum is discouraging.

Table 1. Antimicrobial data for the amorfrutins and related materials. Potency was determined by an agar-dilution streak method [1] and data are expressed in μg/ml

	Microorganism*					
	1	2	3	4	5	6
1, amorfrutin A	6.25	i	i	i	6	i
3, amorfrutin A Me ester	i	i	i	i	i	i
4	3	i	i	i	12	i
2, amorfrutin B	6.25	i	i	i	6.25	i
14, amorfrutin B Me ester	i	i	i	i	i	i
8	i	i	i	i	i	i
9	i	i	i	i	i	i
10	100	i	i	100	50	i
11	25	i	i	i	12	i
12	25	i	i	i	12	i

* Microorganism: 1, *Staphylococcus aureus* (ATCC 13709); 2, *Escherichia coli* (ATCC 9637); 3, *Salmonella gallinarum* (ATCC 9184); 4, *Klebsiella pneumoniae* AD (ATCC 10031); 5, *Mycobacterium smegmatis* (ATCC 607); 6, *Candida albicans* (ATCC 10231).

i: Denotes no activity.

EXPERIMENTAL

Isolation of amorfrutin A (**1**) *and B* (**2**). *Amorpha fruticosa* seeds (810 g) were percolated to exhaustion with 95% EtOH. Evapn of the biologically active percolate left 46 g of a dark, thick liquid. This was partitioned between 5% HCl soln and $CHCO_3$. The $CHCl_3$ layer was dried (Na_2SO_4), filtered and evapd under red. pres. to give 43.8 g of a bioactive dark oil. This was partitioned between 90% MeOH and *n*-hexane to produce 13.7 g of 90% MeOH solubles and 21.1 g of *n*-hexane solubles. The bioactive *n*-hexane soluble fraction was chromatographed on a Si gel column (300 g, 5 × 60 cm) using EtOAc–hexane mixtures as eluent, with 20-ml fractions being collected. Fractions 351–470, eluted with EtOAc–*n*-hexane (1:1), were combined and evapd to give 0.63 g of a thick semi-solid. This material was bioactive and was further purified by chromatography on a Si gel G column, using C_6H_6–Et_2O (17:3) as eluent, to yield 150 mg of amorfrutin A (**1**), mp 145–6° (cyclohexane); λ_{max}^{MeOH} 223 nm: (log ε 4.63), 258 (3.99) and 301 (3.62); ν_{max}^{KBr} 3300–2500 cm^{-1}: (CO_2H), 2920, 2850, 1630 (CO_2H .. HO), 1600, 1565, 1490, 1450, etc.; ^1H NMR ($CDCl_3$): δ 1.70 (3 H, *s*, $C\underline{H}_3$), 1.81 (3 H, *s*, $C\underline{H}_3$), 2.94 (2 H, *m*, AA′ of AA′BB′ system), 3.28 (2 H, *m*, BB′ of AA′BB′ system), 3.35 (2 H, *br. d*, J = 7 Hz, =CHC$\underline{H}_2$Ar), 3.80 (3 H, *s*, $OC\underline{H}_3$), 5.23 (1 H, *br. t*, J = 7 Hz, =C$\underline{H}$CH$_2$Ar), 6.22 (1 H, *s*, Ar$\underline{H}$), 7.26 (5 H, *s*, $C_6\underline{H}_5$), 12.06 (2 H, *s* (exch.), O$\underline{H}$); EI–MS *m/e* (rel. int.) 340 (M^+, 14), 322 ($M^+ - H_2O$, 8), 307 ($M^+ - H_2O - CH_3$, 20), 296 ($M^+ - CO_2$, 34), 267 ($M^+ - H_2O - C_4H_7$, 14), 241 ($M^+ - CO_2 - C_4H_7$, 69), 205 ($M^+ - CO_2 - CH_2C_6H_5$, 38), 105 ($^+CH_2CH_2C_6H_5$, 38), 91 ($^+CH_2C_6H_5$, 100). (Calc. for $C_{21}H_{24}O_4$: C, 74.09; H, 7.10 Found: C, 74.05; H, 7.36.)

Fractions 266–300, eluted with EtOAc–*n*-hexane (3:17), were combined and evapd to give 1.9 g of a thick residue. This material was bioactive and was further chromatographed on a Si gel column with EtOAc–*n*-hexane (1:4). The bioactive fractions eluted late and gave 324 mg on evapn. These were chromatographed on a silica gel G column using benzene–ether (17:3) as eluant to give 70 mg of amorphous, nearly pure, amorfrutin B (**2**); λ_{max}^{MeOH} 222 nm: (log ε 4.37), 261 (3.78), 300 (3.41); ν_{max}^{KBr} 3200–2400 cm^{-1}: (CO_2H), 2920, 2850, 1635 (CO_2H .. HO), 1610, 1570, etc.; ^1H NMR ($CDCl_3$): δ 1.53 (3 H, *s*, $C\underline{H}_3$), 1.57 (3 H, *s*, $C\underline{H}_3$), 1.73 (3 H, *s*, $C\underline{H}_3$), 1.93 (4 H, *br.s*, $-C\underline{H}_2C\underline{H}_2$), 2.87 (2 H, *m*, AA′ of AA′BB′ system), 3.23 (2 H, *m*, BB′ of AA′BB′ system), 3.28 (2 H, *br.d*., J = 6 Hz, =CHC$\underline{H}_2$Ar), 3.71 (3 H, *s*, $OC\underline{H}_3$), 5.07 (2 H, *m*, 2X=C$\underline{H}$), 6.10 (1 H, *s*, Ar$\underline{H}$), 7.13 (5 H, *s*, $C_6\underline{H}_5$), 12.00 (2 H, *s* (exch.), O$\underline{H}$); EI–MS *m/e* (rel. int.) 408 (M^+, 1), 390 ($M^+ - H_2O$, 4), 364 ($M^+ - CO_2$, 7); 339 ($M^+ - C_5H_9$, 13), 321 ($M^+ - H_2O - C_5H_9$, 8), 307 ($M^+ - H_2O - C_6H_{11}$, 16), 295 ($M^+ - CO_2 - C_5H_9$, 51), 241 (*a*, 100), 204 (*b*, 43), 105 ($^+CH_2CH_2C_6H_5$, 50), 91 ($^+CH_2C_6H_5$, 75). (Calcd. for $C_{26}H_{32}O_4$: C, 76.43; H, 7.89. Found: C, 75.23; H, 8.29.)

Methyl ester of amorfrutin A (**3**). Amorfrutin A (20 mg) was dissolved in $CHCl_3$ (5 ml) and treated with CH_2N_2–Et_2O at room temp. for 5 min. After evapn, the residue was crystallized from MeOH to give 17 mg of ester **3**, mp 72–3°; λ_{max}^{MeOH} 225 nm: (log ε 4.42), 268 (4.24) and 307 (3.78); $\nu_{max}^{CHCl_3}$ 2940 cm^{-1}, 2860, 1650 (CO_2CH_3 .. HO), 1610, 1570, etc.; ^1H NMR ($CDCl_3$) δ 1.64 (3 H, *s*, $C\underline{H}_3$), 1.75 (3 H, *s*, $C\underline{H}_3$), 2.80 (2 H, *m*, AA′ of AA′BB′ system), 3.10 (2 H, *m*, BB′ of AA′BB′ system), 3.31 (2 H, *br.d*, J = 7 Hz, =CHC$\underline{H}_2$Ar), 3.75 (3 H, *s*, $OC\underline{H}_3$), 3.91 (3 H, *s*, $CO_2C\underline{H}_3$), 5.13 (1 H, *br.t*, J = 7 Hz, =C$\underline{H}$CH$_2$), 6.13 (1 H, *s*,

ArH), 7.18 (5H, *s*, C_6H_5), 11.67 (1H, *s*, D_2O exch., OH); EI–MS *m/e* (rel. int.) 354 (M^+, 65), 322 ($M^+ - CH_3OH$, 59), 307 ($M^+ - CH_3OH - CH_3$, 100), 279 (307 − CO, 53), 267 ($M^+ - CH_3OH - C_4H_7$, 56), 321 ($M^+ - CH_3OH - CH_2C_6H_5$, 9), 91 ($^+CH_2C_6H_5$). (Calcd. for $C_{22}H_{26}O_4$: C, 74.55; H, 7.39. Found: C, 74.42; H, 7.38.)

Methyl ester of amorfrutin B (**15**). Amorfrutin B (30 mg) was treated with CH_2N_2–Et_2O in the same way as amorfrutin A to give 28 mg of ester **15** as a semi-solid, after purification using $CHCl_3$ by prep. TLC on Si gel G using $CHCl_3$. λ_{max}^{MeOH} 226 nm (log ε 4.34), 268 (3.97), 308 (3.55); ν_{max}^{film} cm^{-1}: 2960, 2920, 2850, 1648 (CO_2CH_3 .. HO), 1605, 1567, etc.; ^1H NMR ($CDCl_3$) δ 1.53 (3H, *s*, CH), 1.60 (3H, *s*, CH), 1.73 (3H, *s*, CH), 1.95 (4H, *br.s*, $-CH_2CH_2-$), 2.77 (2H, *m*, AA′ of AA′BB′ system), 3.10 (2H, *m*, BB′ of AA′BB′ system), 3.29 (2H, *d*, $J = 7$ Hz, $-CHCH_2Ar$), 3.71 (3H, *s*, OCH_3), 3.88 (3H, *s*, CO_2CH_3), 5.07 (2H, *m*, 2X=CHCH$_2$–), 6.07 (1H, *s*, ArH), 7.13 (5H, *s*, C_6H_5), 11.58 (1H, *s*, exch., OH); EI–MS *m/e* (rel. int.) 422 (M^+, 100), 390 ($M^+ - CH_3OH$, 13), 321 ($M^+ - CH_3OH - C_5H_9$, 21), 299 (M^+–MeOH–$CH_2C_6H_5$, 42), 267 (M^+ − MeOH–C_9H_{15}, 100), 105 (34), 91 (60). (Calcd. for $C_{27}H_{34}O_4$: C, 76.74; H, 8.10. Found: C, 77.04; H, 8.20.)

Decarboxylation of amorfrutin A and B. Because of scarcity of material a mixed fraction containing both amorfrutin A and B (229 mg) was heated for 1 hr at 155–60° under a N_2 stream whereupon A and B were then detectable only in trace amounts by TLC. The resulting brown gum (220 mg) was dissolved in a small vol. of $CHCl_3$ and chromatographed on two thick layer (0.5 mm) Si gel G plates using $CHCl_3$. The band at R_f 0.57 was removed and eluted with $CHCl_3$ to give 12 mg of oily phenol **4**; λ_{max}^{MeOH} 228 nm: (ε 6023), 274 (890); ν_{max}^{film} 3420 cm^{-1}: (OH), 2920, 2850, 1615, 1590, 1510, 1490, etc.; ^1H NMR δ 1.70 (3H, *s*, CH), 1.78 (3H, *s*, CH), 2.85 (4H, *s*, $ArCH_2CH_2C_6H_5$), 3.35 (2H, *br.d*, $J = 7$ Hz, $=CHCH_2Ar$), 3.74 (3H, *s*, OCH_3), 5.19 (1H, *s*, exch, OH), 6.27 (1H, *m*, ArH), 6.35 (1H, *m*, ArH), 7.22 (5H, *s*, C_6H_5); EI–MS (rel. int.) *m/e* 296 (M^+, 67), 281 ($M^+ - CH_3$, 19), 241 ($M^+ - C_4H_7$, 100), 205 ($M^+ - C_6H_5CH_2$, 57), 105 (24), 91 (42). Blue Gibbs test.

The band at R_f 0.64 was removed and eluted with $CHCl_3$ to give 2 mg of oily phenol **16**; ν_{max}^{film} 3420 cm^{-1}: (OH), 2960, 2920, 2850, 1610, 1590, etc.; ^1H NMR ($CDCl_3$) δ 1.58 (3H, *s*, CH), 1.68 (3H, *s*, CH), 1.80 (3H, *s*, CH), 2.07 (4H, *br.s*, $-CH_2CH_2$), 2.90 (4H, *s* $ArCH_2CH_2C_6H_5$), 3.43 (2H, *br. d*, $J = 7$ Hz, $=CHCH_2C_6H_5$), 3.81 (3H, *s*, OCH_3), 5.28 (2H, *m*, 2X=CHCH$_2$Ar), 5.35 (1H, *s*, exch, OH), 6.42 (1H, *m*, ArH), 6.53 (1H, *m*, ArH), 7.40 (5H, *s*, C_6H_5); EI–MS *m/e* (rel. int.) 364 (M^+, 3), 295 ($M^+ - C_5H_9$, 18), 241 ($M^+ - C_9H_{15}$, 100), 204 ($M^+ - C_5H_9$–$CH_2C_6H_5$, 20), 105 (37), 91 (39).

Acid-catalyzed cyclization of **4**. To a soln of the bibenzyl **4** (25 mg) in 2 ml of dioxane was added 1 ml of $BF_3 \cdot Et_2O$ in dioxane and the reaction mixture stirred for 3 hr at 55°. After dilution with Et_2O and several H_2O washings, the Et_2O layer was dried and evapd. The crude residue was purified by thick layer chromatography on Si gel G using Et_2O–*n*-hexane (19:1). The band at R_f 0.31 was collected to give 8 mg of cyclization product **5** as a viscous oil; ν_{max}^{film} 2980 cm^{-1}, 2940, 2850, 1620, 1583, 1500, etc.; ^1H NMR ($CDCl_3$) δ 1.31 (6H, *s*, 2XCH$_3$), 1.76 (2H, *t*, $J = 7$ Hz, A_2 of A_2X_2 system), 2.61 (2H, *t*, $J = 7$ Hz, X_2 of A_2X_2 system), 2.86 (4H, *s*, $ArCH_2CH_2C_6H_5$), 3.77 (3H, *s*, OCH_3), 6.20 (1H, *d*, $J = 1.5$ Hz, ArH), 6.36 (1H, *d*, $J = 1.5$ Hz, ArH), 7.25 (5H, *s*, C_6H_5); EI–MS (rel. int.) 296 (M^+, 50), 241 ($M^+ - C_4H_8 + H$, 100), 205 ($M^+ - CH_2C_6H_5$, 34), 191 ($M^+ - CH_2CH_2C_6H_5$, 1) 137 (7), 105 (8), 91 (42). (Calcd. for $C_{20}H_{24}O_2$: C, 81.04; H, 8.16. Found: C, 81.10; H, 8.20.)

Synthesis of 3,5-*dimethoxystilbene* (**8**). To a suspension of benzyl triphenyl phosphonium chloride (3.889 g, 10 mM) in 60 ml of freshly dist. THF, 8 ml of 1.6 M *n*-butyl Li in hexane was added dropwise and the resulting mixture stirred for 30 min under a N_2 stream. To the orange-red soln was added at 0° a soln of 3,5-dimethoxybenzaldehyde (1.66 g, 10 mM) in 20 ml of THF. After 30 min, the solvent was removed *in vacuo* and the residue stirred with Et_2O, the resulting white ppt filtered and washed several times with Et_2O. The combined filtrate and washings were washed successively with H_2O, 0.1 N HCl and H_2O and then dried and evapd to give 3.3 g of a yellow oil. Chromatography over Si gel using $CHCl_3$ gave 2.04 g of pure 3,5-dimethoxystilbene (**8**) as a colourless oily mixture of *Z* and *E* isomers; ^1H NMR δ 3.60 (*s*, OCH_3), 3.80 (*s*, OCH_3) (area ratio 1:2), 6.4–7.6 (*m*); EI–MS *m/e* (rel. int.) 240 (M^+, 100), 239 ($M^+ - H$, 56), 225 ($M^+ - CH_3$, 22), 224 ($M^+ - H - CH_3$, 18), 209 ($M^+ - 31$,30), 208 ($M^+ - 32$,20). (Calcd. for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: 80.00; H, 6.61.)

Synthesis of 3,5-*dimethoxybibenzyl* (**9**). A suspension of 2.04 g of the stilbene **8** and 300 mg of 5% Pd–C in 45 ml EtOH was hydrogenated at room temp. and pres. After 1 hr, the reaction was filtered through a pad of diatomaceous earth and evapd to give 2.03 g (100%) of **9** as a colourless viscous oil; ^1H NMR δ 2.90 (4H, *s*, Ar $CH_2CH_2C_6H_5$), 3.79 (6H, *s*, 2X OCH_3), 6.45 (3H, *s*, 3X ArH), 7.35 (5H, *s*, C_6H_5); EI–MS *m/e* (rel. int.) 242 (M^+, 28), 151 ($M^+ - CH_2C_6H_5$, 100), 91 ($^+CH_2C_6H_5$, 43). (Calcd. for $C_{16}H_{18}O_2$: C, 79.30; H, 7.49. Found: C, 79.50, H, 7.29.)

Synthesis of 3,5-*dihydroxybibenzyl* (**10**). To a soln of bibenzyl **9** (2.03 g) in 40 ml of CH_2Cl_2 at −70° was added a soln of 10 g of BBr_3 in 20 ml of CH_2Cl_2 and this was stirred for 1.5 hr at room temp. After evapn and chromatography on a Si gel column (eluting with 1–4% MeOH in $CHCl_3$), 1.49 g (90%) of oily 3,5-dihydroxybibenzyl was obtained: ν_{max}^{nujol} 3380, 1610, 1590 cm^{-1}; ^1H NMR δ 2.67 (4H, *s*, $ArCH_2CH_2C_6H_5$), 6.13 (3H, *s*, 3X ArH), 6.2–6.8 (2H, *br. s*, exch., 2X OH), 7.07 (5H, *s*, C_6H_5), EI–MS *m/e* (rel. int.) 214 (M^+, 84), 123 ($M^+ - CH_2C_6H_5$, 84), 91 ($^+CH_2C_6H_5$, 100).

Isoprenylation of 3,5-*dihydroxybibenzyl* (**10**). To a soln of 800 mg of **10** in 30 ml of dioxane was added dropwise at 52° a mixture of 290 mg of 3-methyl-2-buten-1-ol and 2 ml of $BF_3 \cdot Et_2O$. After stirring for 20 min, an additional 1 ml of $BF_3 \cdot Et_2O$ in 3 ml of dioxane was added and stirring continued for a further 20 min. The reaction mixture was then cooled to room temp. diluted with Et_2O, and extracted several times with H_2O. The Et_2O layer was dried (Na_2SO_4) and evapd under red. pres. to give 1.15 g of a brown, viscous oil which was further purified over a Si gel column using $CHCl_3$ to give 108 mg (12% yield) of cyclization product **11** [12]; ν_{max}^{film} 3400 cm^{-1}, 2960, 2920, 2840, 1620, 1580, 1510, 1490, etc.; ^1H NMR δ 1.35 (6H, *s*, 2XCH$_3$), 1.80 (2H, *t*, $J = 7$ Hz, A_2 of A_2X_2 system), 2.64 (2H, *t*, $J = 7$ Hz, X_2 of A_2X_2 system), 2.83 (4H, *br. s*, $ArCH_2CH_2C_6H_5$), 4.73 (1H, *s*, exch, OH), 6.18 (1H, *d*, $J = 1.5$ Hz, ArH), 6.33 (1H, *d*, $J = 1.5$ Hz, ArH), 7.25 (5H, *s*, C_6H_5); EI–MS *m/e* (rel. int.) 282 (M^+, 40), 227 ($M^+ - C_4H_8 + H$, 58), 191 ($M^+ - CH_2C_6H_5$, 33), 135 ($M^+ - C_4H_8$–$CH_2C_6H_5$, 50), 105 ($^+CH_2CH_2C_6H_5$, 35), 91 ($^+CH_2C_6H_5$, 100). (Calcd. for $C_{19}H_{22}O_2$: C, 80.81; H, 7.85. Found: C, 80.44; H, 8.08.) The results of a Δδ study (δ DMSO-d_6 − δ DMSO-d_6/NaOD) are given in the text. Gibbs test = purple. Also eluted was 197 mg (19%) of isomer **13** as an oil; ν_{max}^{film} 3380 cm^{-1}, 2990, 2940, 2860, 1610, 1590, 1480; ^1H NMR ($CDCl_3$) δ 1.23 (6H, *s*, *s*, 2X CH$_3$), 1.67 (2H, *t*, $J = 7$ Hz, X_2 of A_2X_2 system), 2.73 (4H, *s*, $ArCH_2CH_2C_6H_5$), 4.90 (1H, *s*, exch., OH), 6.00 (1H, *d*, $J = 3$ Hz, ArH), 6.12 (1H, *d*, $J = 3$ Hz, ArH), 7.07 (5H, *s*, C_6H_5); EI–MS *m/e* (rel. int.) 282 (M^+, 65), 227 ($M^+ - C_4H_8 + H$, 100), 191 ($M^+ - CH_2C_6H_5$, 18), 135 ($M^+ - C_4H_8$–$CH_2C_6H_5$, 21), 105 ($^+CH_2CH_2C_6H_5$, 21), 91 ($^+CH_2C_6H_5$, 64). (Calcd. for $C_{19}H_{22}O_2$: C, 80.81; H, 7.85. Found: C, 77.95; H, 7.71.) The results of a Δδ study (δ DMSO-d_6

– δ DMSO-d_6/NaOD) are given in the text. Gibbs test = red (negative).

Methylation of bibenzyl **11** *to give synthetic degradation product* **12** (**5**). To a soln of 30 mg of **11** and 0.5 ml of Me_2SO_4 in 10 ml of EtOH, was added dropwise over 1 hr at room temp. sufficient N NaOH to keep the reaction mixture alkaline. The reaction mixture was treated with excess N NaOH and extracted with $CHCl_3$. The $CHCl_3$ layers were washed several times with H_2O, dried and evapd to give 23 mg of **12** as a viscous oil. This proved to be identical by co-TLC, IR, ^{1}H NMR and MS to **5** obtained by degradation of amorfrutin A (**1**). The results of a nuclear Overhauser experiment are reported in the text.

Methylation of bibenzyl **13** *to give synthetic ether* **14**. In the same manner as described above for **11**, **13** (50 mg) gave 45 mg of synthetic bibenzyl **14**; ν_{max}^{film} 2970, 2930, 2850, 1610, 1580, 1480 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (6 H, *s*, $2XC\underline{H}_3$), 1.75 (2 H, *t*, $J = 7$ Hz, A_2 of A_2X_2 system), 2.55 (2 H, *t*, $J = 7$ Hz, X_2 of A_2X_2 system), 2.83 (4 H, *s*, $ArC\underline{H}_2C\underline{H}_2C_6H_5$), 3.73 (3 H, *s*, $OC\underline{H}_3$), 6.25 (1 H, *d*, $J = 3$ Hz, Ar$\underline{H}$), 6.37 (1 H, *d*, $J = 3$ Hz, Ar$\underline{H}$), 7.23 (5 H, *s*, $C_6\underline{H}_5$); EI–MS *m/e* (rel. int.) 296 (M^+, 60), 241 ($M^+ - C_4H_8 + H$, 100), 205 ($M^+ - CH_2C_6H_5$, 19), 191 ($M^+ - CH_2CH_2C_6H_5$, 13), 137 (28), 105 (15), 91 (37). (Calcd. for $C_{20}H_{24}O_2$: C, 81.04; H, 8.16. Found: C, 80.88; H, 8.30.) The results of a nuclear Overhauser experiment are reported in the text.

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