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A RESVERATROL DIMER FROM *ANIGOZANTHOS PREISSII* AND *MUSA CAVENDISH*

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Key Word Index—*Anigozanthos preissii*; *Musa cavendish*; Haemodoraceae; Musaceae; anigopreissin A; benzofurane; root cultures; stilbene.

Abstract—A novel resveratrol dimer, named anigopreissin A, was isolated from root cultures of *Anigozanthos preissii* and from rhizomes of *Musa cavendish* plants. The structure was established by spectrometric methods including assignments of ^{1}H and ^{13}C NMR data as a completely unsaturated benzofuran derivative. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The Haemodoraceae plant family, including *Anigozanthos* spp, is characterized by the phenylphenalenones as the major chemotaxonomic markers [1]. Root cultures of *A. preissii* contain anigorufone and hydroxyanigorufone which were used to study the biosynthesis of the phenylphenalenones [2, 3].

Stilbene dimers of various constitutions occur in several plant species [4, 5]. ε-Viniferin, which was first found in *Vitis vinifera* (Vitaceae) [6] and *Vatica affinis* (Dipterocarpaceae) [7], and scirpusin A and B from *Scirpus fluviatilis* and *S. maritimus* (Cyperaceae) [8, 9] are characterized by coupling of two stilbene moieties to form a *trans*-2-aryl-2,3-dihydrobenzofuran ring system.

In this paper, the isolation and structure elucidation of the first resveratrol dimer of the unsaturated benzofuran type, anigopreissin A, are described.

RESULTS AND DISCUSSION

Cultured roots of *A. preissii* were extracted with MeOH and partitioned between $CHCl_3$–H_2O and EtOAc–H_2O. From the EtOAc extract, compound **1** was separated by MPLC, TLC, and finally purified by reversed phase HPLC. The EI-mass spectrometry revealed a molecular mass of *m/z* 452 (rel. int. 100) $[M]^+$, and the HR-EI-mass spectrometry *m/z* 452.1267 (100) $[M]^+$ indicated the molecular formula of $C_{28}H_{20}O_6$. Acetylation gave the pentaacetyl derivative as indicated by the parent peak and a characteristic fragmentation pattern in the EI-MS. The UV spectrum suggested a highly conjugated system. The IR spectrum exhibited absorption bands due to hydroxyl, double bond and aromatic ring moieties. The constitution of the molecule was established by NMR spectrometric methods. The ^{1}H NMR and $^{1}H,^{1}H$ COSY (acetone-d_6) spectra revealed five spin systems: two *p*-substituted aromatic rings A_1 and B_1 (Fig. 1) (two pairs of doublets, δ 7.46/6.80 and δ 7.45/6.85, each doublet integrating for two protons); a 3,5-disubstituted aro-

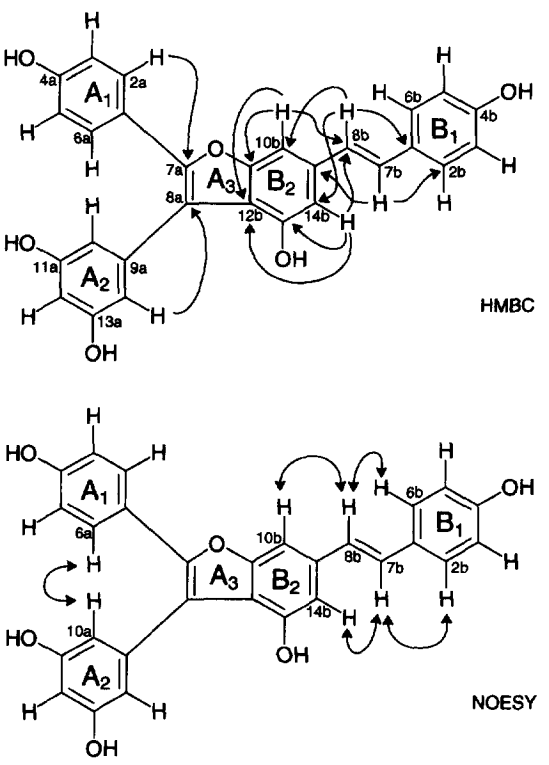

Fig. 1. Structure and selected HMBC and NOESY correlations of anigopreissin A (**1**) (500.13 MHz, acetone-d_6–benzene-d_6 5:3, TMS).

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matic ring A_2 which is characterized by a doublet of two protons (δ 6.49) and a triplet of one proton (δ 6.41); two *meta*-coupling aromatic protons (δ 6.87 and 7.25); two protons at a C–C double bond (δ 7.06 and 7.13) with *trans*-configuration as shown by their large coupling constants of 16.3 Hz. Broad-band decoupled ^{13}C NMR and DEPT spectra exhibited 10 signals of methine carbons representing five pairs of equivalent and five single carbons (15 carbon atoms in total) as shown by integration of HMQC correlated proton signals, six resonances of single quarternary carbons in the range of aromatic and double bond carbon atoms, as well as another six signals of quarternary carbon atoms at lower field between δ 150 and 160, indicating substitution by hydroxyl or ether functions. One of these signals (δ 159.5) is due to two equivalent carbons since it exhibits double intensity and, furthermore, in the HMBC spectrum (acetone-d_6) correlates with the proton signal δ 8.38 which integrates for two hydroxyl protons. Further cross signals of δ 159.5 with δ 6.41 and 6.49 confirmed the occurrence of the 3,5-dihydroxyphenyl ring A_2.

The linkages between the spin systems were established by HMBC and NOESY experiments as shown in Fig. 1. A series of HMBC correlations and NOESY cross signals proved the position of the *p*-hydroxyphenylethenyl moiety at C-9b of ring B_2 which is part of the benzofuran ring system, and also confirmed the *trans*-configuration of the double bond. The carbon atoms C-7a and C-11b carry the ether bridge of the benzofuran ring system and, therefore, were at significantly lower field compared with C-8a and C-12b. Thus, HMBC correlations of H-2a/H-6a with C-7a and H-10a/H-14a with C-8a proved the positions of rings A_1 and A_2 at the corresponding carbon atoms C-7a and C-8a of the furan ring A_3. Finally, the positions of the hydroxyl groups were also established by HMBC correlations with adjacent lower field carbons. Because of overlapping signals in acetone-d_6 of doublets H-2a/H-6a (δ 7.46) with H-2b/H-6b (δ 7.45) and of H-3a/H-5a (δ 6.80) with H-3b/H-5b (δ 6.85) a second series of spectra were recorded in acetone-d_6–benzene-d_6 (5:3). These exhibited improved resolution of the corresponding ^1H resonances and cross signals in the 2D spectra. The spectral data unambiguously proved the suggested structure of anigopreissin A.

Anigopreissin A was found also in rhizomes of *Musa cavendish* using the same extraction procedure. HPLC analysis, UV data and ^1H NMR spectra (acetone-d_6; acetone-d_6–benzene-d_6 5:3) were identical with those of anigopreissin A from *A. preissii*. Interestingly, as in the case of *A. preissii*, Musaceae species contain phenylphenalenones also [10–12]. The co-occurrence of a stilbene derivative with phenylphenalenones both in root cultures of *A. preissii* and in rhizomes of *M. cavendish* plants may give rise to speculations of a biogenetic relationship between both classes of natural products.

This is the first report on a naturally occurring dimeric stilbene derivative of a novel type containing a completely unsaturated benzofuran ring system. Anigopreissin A is also the first resveratrol dimer from the Haemodoraceae and the Musaceae.

EXPERIMENTAL

Plant material. Root cultures of *Anigozanthos preissii* (L.) were grown in liquid LS medium [13] (140 ml in 300-ml Erlenmeyer flasks) at 22° on a gyratory shaker (100 rpm) under permanent light (600 lux). Plants of *Musa cavendish*, subground (AAA) 'Giant Cavendish' were obtained from the Institute of Crop Science, University of Kassel, and grown under greenhouse conditions at a minimum temp of 22°.

Isolation and purification of **1**. Aseptically grown cultured roots of *A. preissii* (250 g fr. wt) were frozen with liquid N_2, ground, and exhaustively extracted with MeOH at room temp. The MeOH extract was evaporated and partitioned between $CHCl_3$ and H_2O followed by partition between EtOAc and H_2O. MPLC (RP-18; MeOH–H_2O 1:1), TLC (silica gel 60 F_{254}, 0.25 mm; EtOAc–MeOH–HCOOH 10:1:1; R_f 0.75), and prep. HPLC (Nucleosil 7 C18, 250 × 20 mm; MeCN–H_2O 17:3; UV 284 nm) of the EtOAc fraction yielded compound **1** (2.4 mg; mp 159–161). HPLC (analytical mode) on LiChrospher 100 RP-18, 250 × 4 mm, 5 μm; MeOH–H_2O 13:7; 0.6 ml min^{-1}; diode array detection; R_t 13.5 min.

Rhizomes of *M. cavendish* plants (50 g fr. wt) yielded 100 μg of pure compound **1**.

Compound **1**. UV (MeOH) λ_{max} nm: 217, 251, 292, 359; IR ν_{max}^{KBr} cm^{-1}: 3408, 1695, 1608, 1512, 1440, 1233, 1171, 1068, 1001, 958, 836; EI-MS (70 eV): *m/z* 452 (rel. int. 100) $[M]^+$; HR-EI-MS (70 eV): *m/z*

Table 1. ^1H (500.13 MHz) and ^{13}C NMR (125.75 MHz) data for compound **1**

Position	^1H	^{13}C
1a	—	123.0
2/6a	7.46 (7.59*) *d*, 8.8	128.6
3/5a	6.80 (6.85) *d*, 8.8	116.1
4a	8.73 *br s*, OH	158.4
7a	—	150.6
8a	—	116.3
9a†	—	136.5
10/14a	6.49 (6.70) *d*, 2.2	109.7
11/13a	8.38 *br s*, OH	159.5
12a	6.41 (6.62) *t*, 2.2	102.9
1b	—	130.0
2/6b	7.45 (7.42) *d*, 8.5	128.9
3/5b	6.85 (6.94) *d*, 8.5	116.4
4b	8.54 *br s*, OH	158.1
7b	7.13 (7.15) *d*, 16.3	128.7
8b	7.06 (7.07) *d*, 16.3	126.8
9b†	—	136.4
10b	7.25 (7.27) *d*, 0.8	101.4
11b	—	156.5
12b	—	118.6
13b	7.96 *br s*, OH	152.4
14b	6.87 (6.99) *d*, 0.8	107.4

Solvent: acetone-d_6 (* parentheses denote δ values obtained in acetone-d_6–benzene-d_6 5:3).

†May be reversed.

452.1267 (100) $[M]^+$; NMR (Bruker DRX 500): 500.13 MHz (^1H), 125.75 MHz (^{13}C), acetone-d_6 or acetone-d_6–benzene-d_6 (5:3), TMS was used as int. standard. ^1H, ^1H–^1H COSY, HMBC, HMQC and NOESY experiments were recorded in a 2.5 mm inverse detection microprobehead; broadband decoupled ^{13}C and DEPT spectra were run using a 2.5 mm broadband microprobehead. For NMR data see Table 1.

Acetylation of **1**. Acetylation was performed with Ac_2O–pyridine (1:1) at room temp. EI-MS: *m/z* 662 (rel. int. 26) $[M]^+$, 620 (72) $[M - acetyl]^+$, 578 (71) $[M - 2\ acetyl]^+$, 536 (100) $[M - 3\ acetyl]^+$, 494 (56) $[M - 4\ acetyl]^+$, 452 (48) $[M - 5\ acetyl]^+$.

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REFERENCES

1. Cooke, R. G. and Edwards, J. M. (1980) *Fortschr. Chem. Org. Naturst.* **40**, 153.
2. Hölscher, D. and Schneider, B. (1995) *J. Chem. Soc., Chem. Comm.* 525.
3. Hölscher, D. and Schneider, B. (1995) *Nat. Prod. Letters* **7**, 177.
4. Gorham, J. (1989) in *Methods in Plant Biochemistry* (Harborne, J. B., ed.), Vol. 1, p. 159. Academic Press, London.
5. Sotheswaran, S. and Pasupathy, V. (1993) *Phytochemistry* **32**, 1083.
6. Langcake, P. and Pryde R. J. (1977) *Experientia* **33**, 151.
7. Sotheswaran, S., Sultanbawa, M. U. S., Surendrakumar, S. and Bladon, P. (1985) *J. Chem. Soc., Perkin I*, 159.
8. Nakajima, N., Taguchi, H., Endo, T. and Yosioka, I. (1978) *Chem. Pharm. Bull.* **26**, 3050.
9. Powell, R. G., Bajaj, R. and McLaughlin, J. L. (1987) *J. Nat. Prod.* **50**, 293.
10. Luis, J. G., Echeverri, F., Quinones, W., Brito, I., Lopez, M., Torres, F., Cardona, G., Aguiar, Z., Pelaez, C. and Rojas, M. (1993) *J. Org. Chem.* **58**, 4306.
11. Hira, N., Ishida, H. and Koshimizu, K. (1994) *Phytochemistry* **37**, 383.
12. Luis, J. G., Quinones, W., Echeverri, F., Grillo, T. A., Kishi, M. P., Garciagarcia, F., Torres, F. and Cardona, G. (1996) *Phytochemistry* **41**, 753.
13. Linsmaier, E. M. and Skoog, F. (1965) *Physiol. Plant.* **18**, 100.