Tetrahedron Vol. 49, No. 26, pp. 5801-5804, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Ampelopsins F and G, Novel Bridged Plant Oligostilbenes from *Ampelopsis brevipedunculata* var. *hancei* Roots (Vitaceae)

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(*Received in Japan* 22 *February* 1993)

Abstract: Ampelopsins F and G, two novel oligostilbenes containing structurally unusual dibenzobicyclo[3.2.1]octadiene system have been isolated from the roots of *Ampelopsis brevipedunculata* var. *hancei* and their structures established on the basis of spectroscopic evidence.

INTRODUCTION

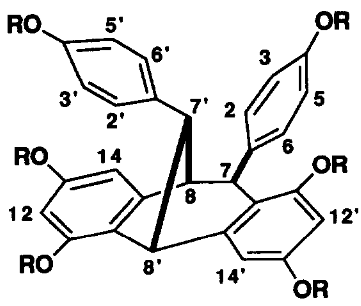
Since ancient times, Oriental medicines have been employing a variety of crude drugs to cure serious liver diseases such as hepatitis and liver cirrhosis. Some of the plants belonging to the family Vitaceae are reputed as the most effective crude drugs in the treatment of liver disorders, but little work on the plants has been done. In the programme to confirm the liver protective activity and to clarify the active principles of the drugs, it was found that methanol extracts of the Vitaceaeous plants, *Ampelopsis brevipedunculata*, *A. brevipedunculata* var. *hancei* and *Vitis coignetiae* exhibited significant anti-hepatotoxic activity in the assay using primary cultured rat hepatocytes injured by carbon tetrachloride or D-galactosamine.[1] Thus, we focused our attention on the constituents of biologically active fractions of their extracts and isolated a number of oligostilbenes, in which two-four resveratrol molecules are oxidatively condensed in a variety of manners.[2-4] An extension of our study of the biologically active fractions of *A. brevipedunculata* var. *hancei* roots led to the isolation of two new oxidative oligostilbenes termed as ampelopsins F and G.

RESULTS AND DISCUSSION

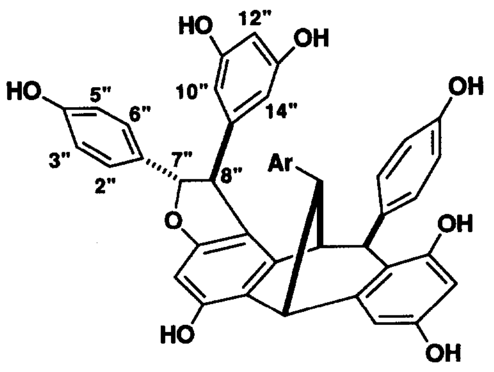
The molecular formula, $C_{28}H_{22}O_6$ indicated for ampelopsin F (1), m.p. 223°C, $[\alpha]_D$ +14.0°, was established by high-resolution EI-mass spectrum (*m/z*: 454.1420 [M$^+$]) and analysis of NMR spectra. The ^{1}H

Table 1. ^1H and ^{13}C NMR data of ampelopsins F (**1**) and G (**2**) (500 and 125 MHz, respectively, in acetone-d_6).

Position No.	Ampelopsin F (1)		Ampelopsin G (2)	
1		138.7 (s)		138.5 (s)
2,6	6.97 (2H d, J=7.5)	130.3 (d)	6.99 (2H d, J=7.5)	130.4 (d)
3,5	6.63 (2H d, J=7.5)	116.0 (d)	6.66 (2H d, J=7.5)	116.1 (d)
4		156.4 (s)		156.5 (s)
7	4.07 (1H brs)	47.5 (d)	4.15 (1H brd, J=1.0)	45.4 (d)
8	3.23 (1H brs)	58.5 (d)	3.15 (1H brs)	52.4 (d)
9		147.7 (s)		146.3 (s)
10		128.2 (s)		129.3 (s)
11		153.4 (s)		153.7 (s)
12	5.94 (1H d, J=2.5)	102.3 (d)	6.10 (1H s)	96.6 (d)
13		158.8 (s)		163.6 (s)
14	6.39 (1H d, J=2.5)	104.6 (d)		119.2 (s)
1'		135.7 (s)		134.9 (s)
2',6'	6.66 (2H d, J=7.5)	129.6 (d)	6.70 (2H d, J=8.5)	128.5 (d)
3',5'	6.44 (2H d, J=7.5)	115.9 (d)	6.60 (2H d, J=8.5)	116.0 (d)
4'		156.4 (s)		156.5 (s)
7'	3.52 (1H brs)	50.8 (d)	3.53 (1H brs)	51.0 (d)
8'	4.01 (1H brs)	50.1 (d)	4.09 (1H brs)	50.9 (d)
9'		147.9 (s)		148.2 (s)
10'		113.8 (s)		113.7 (s)
11'		158.1 (s)		158.3 (s)
12'	6.03 (1H d, J=2.5)	102.3 (d)	6.18 (1H d, J=2.0)	102.4 (d)
13'		157.3 (s)		157.7 (s)
14'	6.32 (1H d, J=2.5)	106.1 (d)	6.48 (1H d, J=2.0)	106.2 (d)
1"				133.8 (s)
2",6"			7.24 (2H d, J=8.5)	130.0 (d)
3",5"			6.84 (2H d, J=8.5)	116.5 (d)
4"				158.6 (s)
7"			5.60 (1H d, J=8.0)	94.6 (d)
8"			4.55 (1H d, J=8.0)	58.1 (d)
9"				142.9 (s)
10",14"			6.54 (2H d, J=2.0)	108.5 (d)
11",13"				160.4 (s)
12"			6.35 (1H t, J=2.0)	102.8 (d)



1: R=H
1a: R=Ac



2 (Ar: 4-hydroxylphenyl)

NMR spectrum of **1** exhibited *ortho*-coupled aromatic hydrogen signals at δ 6.44, 6.63, 6.66 and 6.97 (2H each d, J=7.5 Hz), *meta*-coupled aromatic hydrogen signals at δ 5.94, 6.03, 6.32 and 6.39 (1H each d, J=2.5 Hz) and four aliphatic methine hydrogen signals at δ 3.23, 3.52, 4.01 and 4.07 (1H each brs) (Table 1). Acetylation of **1** with acetic anhydride and pyridine afforded a hexaacetate (**1a**) (HREI-MS *m/z*: 706.2032 [M$^+$]). The ^{1}H NMR signals for the four methine hydrogens of **1a** resonated at δ 3.49, 3.86, 4.22 and 4.28, which were essentially the same as those of the original compound, pointing out that **1** bears six phenolic hydroxyls, and this was substantiated by the ^{13}C NMR spectrum which showed the absence of oxymethine carbons in the molecule. These findings, in conjunction with the molecular formula of **1** and the fact that a number of oligostilbenes were isolated from the same source, spoke that **1** is a member of oxidative resveratrol dimer. Double resonance experiments in the ^{1}H NMR spectrum of **1** showed that the methine hydrogens at δ 3.23, 3.52, 4.01 and 4.07 were long-range coupled with the aromatic hydrogens at δ 6.39, 6.66, 6.32 and 6.97, respectively. Moreover, the methine hydrogen signal at δ 3.23 displayed W-letter type coupling with the other methine hydrogen at δ 4.01. The accumulated data and the biogenetic consideration indicated the structure **1** bearing a dibenzobicyclo[3.2.1]octadiene system which is an unusual carbon skeleton in natural products, and this was confirmed by analysis of ^{1}H-^{13}C long-range couplings through 2σ and 3σ bonds observed in 2D ^{1}H-^{13}C correlation spectrum of **1** as follows: the C-1 carbon at δ 138.7 was spin-coupled with the H-7 and H-8 at δ 4.07 and 3.23 through 2σ and 3σ bonds, and the C-1' signal at δ 135.7 exhibited correlations with the H-7', H-8 and H-8' signals at δ 3.52, 3.23 and 4.01.

Small values of the vicinal coupling constants of the four methine hydrogens indicated all the dihedral angles of the vicinal hydrogens to be near to 90°, leading to the conclusion that the relative stereochemistry of ampelopsin F is represented by the formula **1**. A comparative study in the ^{1}H NMR spectrum of the four possible stereoisomers for ampelopsin F unambiguously verified the configuration of ampelopsin F.[5]

Like ampelopsin F (**1**), ampelopsin G (**2**), m.p. 225°C, $[\alpha]_D$ +32.0°, $C_{42}H_{32}O_9$ (HRFAB-MS *m/z*: 680.2043 [M]$^+$) showed characteristic methine hydrogen signals at δ 3.15, 3.53, 4.09 and 4.15 for dibenzobicyclo[3.2.1]octadiene moiety in its ^{1}H NMR spectrum. The ^{1}H NMR spectrum of **2** also revealed four sets of *ortho*-coupled aromatic hydrogen signals at δ 6.60, 6.66, 6.70 and 6.99, a pair of *meta*-coupled aromatic hydrogen signals at δ 6.18 and 6.48, and an isolated aromatic hydrogen signal at δ 6.10 instead of two pairs of *meta*-coupled aromatic hydrogen signals discernible in the ^{1}H NMR spectrum of **1**. In addition to these, double resonance experiments in the ^{1}H NMR spectrum of **2** displayed the presence of a dihydrobenzofuran moiety bearing 3,5-dihydroxyphenyl and 4-hydroxyphenyl group at C-8" and C-7". Moreover, in double resonance experiments, separate irradiations of the methine hydrogens at δ 3.53, 4.09 and 4.15 sharpened the aromatic hydrogen signals at δ 6.70, 6.48 and 6.99, respectively, while, irradiation at the frequency of the broad singlet signal at δ 3.15 caused no change in the aromatic hydrogen signals. This observation clearly indicated that additional resveratrol molecule is oxidatively condensed with ampelopsin F (**1**) at C-13 hydroxyl and C-14 to form the dihydrobenzofuran moiety.

The presence of NOEs between H-7" – H-10"(14") and H-8" – H-2"(6") reveals the *trans*-configuration of the C-7" and C-8" substituents in ampelopsin G (**2**). Also, distinct NOEs between H-7 – H-8" and H-8 – H-10"(14") clearly shows the β-configuration of the C-8" aryl group.

The absolute stereochemistry assigned to the two oligostilbenes is remained to be solved.

To the best of our knowledge, ampelopsins F (**1**) and G (**2**) are the first members of a new series of oligostilbenes bearing dibenzobicyclo[3.2.1]octadiene system.

EXPERIMENTAL

General Procedure. Melting points were determined on a Yanagimoto micromelting point hot stage type apparatus and are uncorrected. IR spectra were recorded as KBr on a SHIMADZU IR-408 spectrometer. UV spectra were recorded on a SHIMADZU UV-260 spectrometer in MeOH. High resolution EI and FAB-MS were measured on JEOL JMS-DX 303 and JEOL LMS-SX 102A mass spectrometers, respectively. Optical rotations were determined on a JASCO DIP-360 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM FX-500 spectrometer using tetramethylsilane as an internal standard. Coupling constants are in hertz. Multiplicity: s, singlet; brs, broad singlet; d, doublet; brd, broad doublet; t, triplet.

Isolation of Ampelopsins F and G. Dried roots of *A. brevipedunculata* var. *hancei* collected in Taiwan (5 kg), were extracted with MeOH (10 l x 3) at room temperature. The filtrate was concentrated under reduced pressure to yield an extract (225 g) which was partitioned between AcOEt and water. The AcOEt solubles (35 g) were repeatedly chromatographed on a silica gel column using $CHCl_3$-MeOH as eluting solvents. HPLC (column: Tosoh TSK gel ODS-120A: 30 cm x 2.15 cm I.D.; solvent: CH_3CN-water (25:75); flow rate: 4 ml/min) of the $CHCl_3$-MeOH (9:1) eluting fractions (5.8 g) yielded ampelopsins F (**1**) and G (**2**) (400 mg and 10 mg, respectively), ampelopsin F (**1**): pale brownish powder, m.p. 223°, $[\alpha]_D$ +14.0° (*c* 1.98, MeOH); UV λmax nm (log ε): 281 (3.89); IR νmax cm^{-1}: 3300, 1610, 1510, 1460; ^{1}H and ^{13}C NMR (Table 1); high-resolution EI-MS for $C_{28}H_{22}O_6$, obsd *m/z*: 454.1420 [M$^+$], calcd *m/z* 454.1417, ampelopsin G (**2**): pale brownish powder, m.p. 225°, $[\alpha]_D$ +32.0° (*c* 0.23, MeOH); UV λmax nm (log ε): 284 (4.95); IR νmax cm^{-1}: 3450, 1610, 1515, 1455; ^{1}H and ^{13}C NMR (see Table 1); HRFAB-MS (nitrobenzyl alcohol+glycerol matrix) for $C_{42}H_{32}O_9$, obsd *m/z*: 680.2043 [M]$^+$, calcd *m/z* 680.2046.

Acetylation of Ampelopsin F. To a solution of ampelopsin F (**1**) (5 mg) in pyridine (0.5 ml) acetic anhydride (1 ml) was added, the reaction mixture was kept at room temperature for 24 hr, evaporated under reduced pressure and the residue was chromatographed over silica gel to give ampelopsin F hexaacetate (**1a**) (3 mg) as colorless powder, HREI-MS for $C_{40}H_{34}O_{12}$, obsd *m/z* 706.2032 [M$^+$], calcd *m/z* 706.2050, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.03, 2.20, 2.24, 2.26, 2.27, 2.33 (3H each s), 3.49, 3.86, 4.22, 4.28 (1H each brs), 6.46, 6.56 (1H each d, J=2.5 Hz), 6.77, 6.83 (2H each d, J=7.5 Hz), 6.87 (1H d, J=2.5 Hz), 6.99 (2H d, J=7.5 Hz), 7.13 (1H d, J=2.5 Hz), 7.36 (2H d, J=7.5 Hz).

Acknowledgment – Financial assistance from The Japan Association of Chemistry is gratefully acknowledged by Y. O.

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