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AMPELOPSINS A, B AND C, NEW OLIGOSTILBENES OF AMPELOPSIS BREVIPEDUNCULATA VAR. HANCEI

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Abstract— Three novel oligostilbenes, ampelopsins A, B and C, have been isolated from Ampelopsis brevipedunculata var. hancei, and their structures were determined by means of spectroscopic evidence.

Ampelopsis brevipedunculata (Maxim.) Trautv. and its variety, A. brevipedunculata (Maxim.) Trautv. var. hancei Rehder (Vitaceae) are used as an anti-inflammatory in the treatment of hepatitis and nephritis. Biological studies showed that both alcoholic and water decoctions of the fruits, leaves, stems and roots of A. brevipedunculata had an inhibitory action of collagen synthesis of liver cells and anti-fatty liver action.[2] In our continuing effort to discover anti-hepatotoxic constituents from Oriental medicinal plants, we observed that methanol extracts of both plants exhibited strong activity at a dose of 1 mg/ml in the carbon tetrachloride- and D-galactosamine-induced cytotoxicity model systems employing primary cultured rat hepatocytes.[3] Further, ethyl acetate solubles of the methanol extract of A. brevipedunculata var. hancei were also anti-hepatotoxic in the assay. Fractionation of the solubles led to the isolation of three new oligostilbenes named ampelopsins A, B and C.

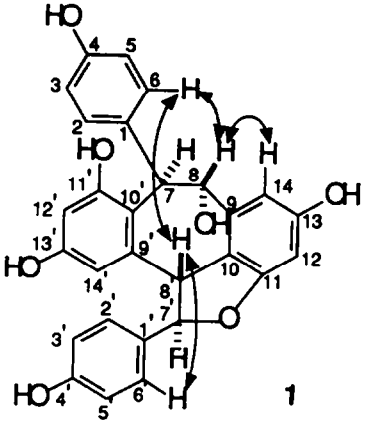
Ampelopsin A, m.p. 185-186°C, $[\alpha]_D$ +167°, was established to have the molecular formula $C_{28}H_{22}O_7$ on the basis of FAB-MS (m/z: 471 [MH^+]) and its ^{13}C NMR spectrum which showed signals for twenty-eight carbons. The ^{13}C NMR signals were assigned to four sp^3 and twenty-four sp^2 carbons, and out of the latters, six at δ 155.8 – 159.9 were found to bind with oxygen atoms. These spectral data, along with a UV absorption band at 283 nm (log ε 3.86) and IR spectral bands at 3400 (hydroxyl), 1600, 1515 and 1450 cm^{-1} (aromatic), spoke that ampelopsin A is an oxidative dimer of resveratrol. Methylation of ampelopsin A with dimethyl sulfate and potassium carbonate in acetone yielded a pentamethyl ether **1a** which afforded a monoacetyl derivative **1b** (MS m/z: 522 [M^+-AcOH]) after treatment with acetic anhydride in pyridine, suggesting that ampelopsin A bears five phenolic and one aliphatic hydroxyl groups. Thus, the remaining oxygen was as-

^{1}H NMR Data of Ampelopsins A, B and C (500 MHz, in acetone-d_6).

	Ampelopsin A	Ampelopsin B	Ampelopsin C
H-2,6	6.90 (d, J=8.3)	6.95 (d, J=8.5)	7.20 (d, J=8.5)
H-3,5	6.65 (d, J=8.3)	6.66 (d, J=8.5)	6.70 (d, J=8.5)
H-7	5.45 (d, J=5.0)	5.23 (t, J=4.0)	5.29 (d, J=3.5)
H-8	5.42 (brs)	3.20 (dd, J=18.0, 4.0)	3.67 (brd, J=12.0)
		3.60 (dd, J=18.0, 4.0)	
H-12	6.16 (d, J=2.3)	6.07 (d, J=2.0)	6.17 (s)
H-14	6.62 (d, J=2.3)	6.35 (d, J=2.0)	
H-2',6'	7.12 (d, J=8.3)	7.11 (d, J=8.5)	7.28 (d, J=8.5)
H-3',5'	6.78 (d, J=8.3)	6.78 (d, J=8.5)	6.82 (d, J=8.5)
H-7'	5.77 (d, J=11.7)	5.74 (d, J=11.5)	5.85 (d, J=12.0)
H-8'	4.17 (d, J=11.7)	4.19 (d, J=11.5)	4.48 (d, J=12.0)
H-12'	6.43 (d, J=2.3)	6.45 (d, J=2.0)	6.37 (d, J=2.0)
H-14'	6.24 (d, J=2.3)	6.24 (d, J=2.0)	6.18 (brs)
H-2",6"			7.03 (d, J=8.5)
H-3",5"			6.75 (d, J=8.5)
H-7"			4.26 (d, J=9.5)
H-8"			3.78 (dd, J=12.0, 9.5)
H-10",14"			6.22 (d, J=2.0)
H-12"			6.20 (t, J=2.0)

signed to be present as an ether linkage in the molecule.

Double resonance experiments carried out in the ^{1}H NMR spectrum of ampelopsin A indicated the presence of four sets of ortho-coupled hydrogens (δ 6.65, 6.90; 6.78, 7.12 (2H each d, J=8.3 Hz)), two sets of meta-coupled hydrogens (δ 6.16, 6.62; 6.24, 6.43 (1H each d, J=2.3 Hz)) as well as two sets of mutually coupled aliphatic methine hydrogens (δ 4.17, 5.77 (1H each d, J=11.7 Hz); 5.42 (1H brs), 5.45 (1H d, J=5.0 Hz)). The broad singlet signal at δ 5.42 was clearly changed to doublet (J=5.0 Hz) by the addition of D_2O, pointing out the presence of a secondary alcohol, which was further substantiated by a downfield shift to δ 6.66 in the ^{1}H NMR spectrum of the monoacetate **1b**. For the unambiguous assignment of the four methine hydrogen signals, two dimensional ^{1}H-^{13}C shift correlation spectrum of ampelopsin A was measured. In this way, it showed the following cross peaks between the aliphatic ^{1}H and ^{13}C NMR signals at δ 4.17 – 49.4, 5.45 – 43.7, 5.42 – 71.2 and 5.77 – 88.3, which implied that the former and latter two pairs were due to the benzylic hydrogens and oxymethine hydrogens, respectively. In order to settle the connectivities of the aromatic and aliphatic moieties, long range benzylic couplings in the double resonance experiments were analyzed. Thus, the methine hydrogen signal at δ 4.17, 5.42, 5.45 and 5.77 were found coupled with the aromatic hydrogen signals at δ 6.24, 6.62, 6.90 and 7.12, respectively. These accumulated data allowed us to propose the plain structure **1** for ampelopsin A, which can be well accounted for on the biogenetic ground.



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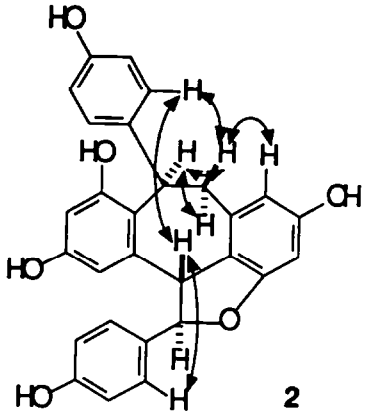

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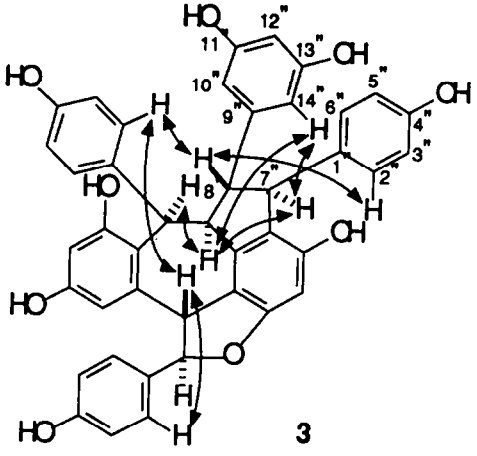

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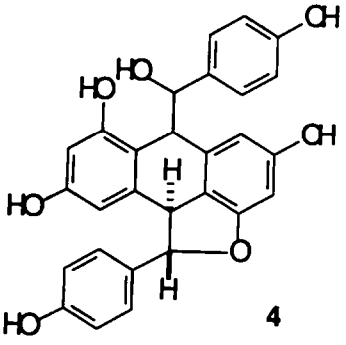

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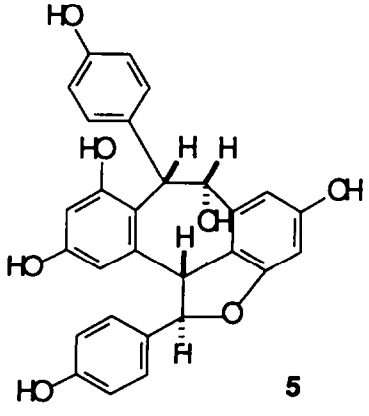

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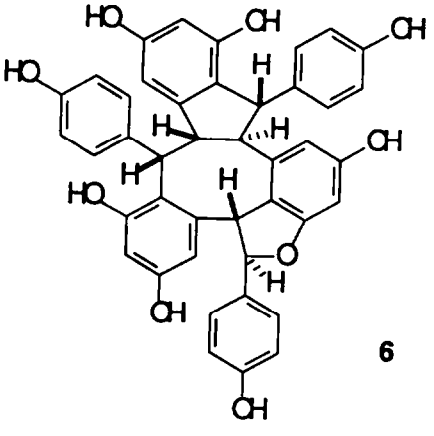

6

⟷ : NOE

Ampelopsin B, m.p. 170-171°, $[\alpha]_D$ +123°, showed a mass ion peak at m/z 455 [MH^+], which was different from that of ampelopsin A by 16 mass unit. This, along with the ^1H and ^{13}C NMR spectra, indicated its molecular formula $C_{28}H_{22}O_6$. From the similarity of the UV and IR spectra of ampelopsin B to those of ampelopsin A, the former was thought to be a congener of the latter. The ^1H and ^{13}C NMR spectra of ampelopsin B displayed the presence of a methylene group (δ 3.20 and 3.60 (1H each dd, J=18.0 and 4.0 Hz) and δ 34.3 (t)), instead of a carbinyl part as present in ampelopsin A. Moreover, the ^1H NMR spectrum of ampelopsin B exhibited similar long range couplings to those of ampelopsin A, indicating that ampelopsin B is an 8-dehydroxy derivative **2** of ampelopsin A.

Ampelopsin C, m.p. 268-269°, $[\alpha]_D$ +24°, had the molecular formula $C_{42}H_{32}O_9$ (FAB-MS: m/z 681 [MH^+]), suggesting it to be an oxidative trimer of resveratrol. The ^1H NMR spectrum of ampelopsin C showed signals for four sets of ortho-coupled hydrogens (δ 6.70, 7.20; 6.82, 7.28 (2H each d, J=8.5 Hz)), one set of meta-coupled hydrogens (δ 6.18 (1H brs), 6.37 (1H d, J=2.0 Hz)), two methine hydrogens of a dihydrobenzofuran group (δ 4.48 and 5.85 (1H each d, J=12.0 Hz)) which resembled those of ampelopsins A and B. While, in the ^1H NMR spectrum of ampelopsin C, the meta-coupled ^1H NMR signals assignable to H-12 and H-14 of ampelopsins A and B were not discernible, and instead a singlet signal was observed at δ 6.17. In addition to these, the ^1H NMR spectrum of ampelopsin C showed the following signals: δ 6.75 and 7.03 (2H each d, J=8.5 Hz) for ortho-coupled hydrogens, δ 6.20 (1H t, J=2.0 Hz) and 6.22 (2H d, J=2.0 Hz) for AX_2 type aromatic hydrogens, and δ 3.67 (1H brd, J=12.0 Hz), 3.78 (1H dd, J=12.0 and 9.5 Hz), 4.26 (1H d, J=9.5 Hz) and 5.29 (1H d, J=3.5 Hz) for four aliphatic methine hydrogens, the last four of which were found to be mutually coupled each other as indicated by double resonance experiments. Moreover, the double doublet signal at δ 3.78 showed long range coupling with the AX_2 type signal at δ 6.22, and two other methine hydrogen signals at δ 4.26 and 5.29 also had long range couplings with the ortho-coupled hydrogen signals at δ 7.03 and 7.20, respectively, demonstrating the structure **3** for ampelopsin C.

For the clarification of the relative stereochemistry of these three ampelopsins, detailed NOE studies were examined. In this way, all the ampelopsins showed significant NOE's between H-2'(6') and H-8', suggesting the trans orientation of the two methine hydrogens on the dihydrobenzofuran moiety. Irradiation of the H-2(6) signal enhanced the H-8' methine hydrogen signal, which can be observed only when the C-7 aryl group is situated cis to H-8'. The α-configuration of the C-8 hydroxyl group in ampelopsin A was deduced by NOE's between H-2(6) – H-8 and H-14 – H-8. Regarding the remaining stereochemistry at C-8, 7" and 8" in ampelopsin C, they were determined as indicated in structure **3** by the following NOE's: H-8 – H-10"(14"), H-8 – H-7", H-8" – H-2(6), H-8" – H-2"(6") and H-7" – H-10"(14").

It should be noted that the reported ^1H and ^{13}C NMR data of gnetin G (**4**) isolated previouly from Welwitschia mirabilis[4] closely resembled those of ampelopsin A (**1**). However, ampelopsin A (**1**) seems to be a C-7 stereoisomer of balanocarpol (**5**) isolated from Balanocarpus zeylanicus (Hopea brevipetiolaris) and Hopea jucunda.[5]

Unfortunately, ambiguous decoupling data for gnetin G (**4**) could not be suited for ampelopsin A (**1**). Moreover, the spectral data of distichol (**6**) from Shorea disticha[6,7] were very similar to those of ampelopsin C (**3**) except for $[\alpha]_D$. No clear ^1H NMR data are available for distichol (**6**), which might establish the relationship between this compound and ampelopsin C (**3**).

Some of resveratrol derivatives exhibit anti-fungal, antileukemic and liver-protective activities.[8] It is of value to investigate pharmacological activity of these compounds.

Experimental

Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a SHIMADZU IR-408 spectrometer, ^1H and ^{13}C NMR spectra on a JEOL JNM FX-100 and FX-500 spectrometers (TMS as internal standard). FAB and low resolutin EIMS were determined with JEOL DX-303 and HITACHI M-52 spectrometers, respectively.

Isolatin of Ampelopsins A (1), B (2) and C (3): Dried roots of A. brevipedunculata var. hancei (5 kg) were extracted with MeOH (10 l x 3) at room temperature to give the extract (225 g). The MeOH extract (225 g) was partitioned with AcOEt (10 l) and water (5 l) to yield AcOEt and water solubles (40 and 185 g, respectively). The AcOEt solubles (35 g) were chromatographed over silica gel (150 g) and the column was eluted with the increasing polarity of $CHCl_3$-MeOH mixture. The repeated silica gel chromatography of the $CHCl_3$-MeOH (9:1)-eluting fraction (5.8 g) using benzene-EtOAc as a solvent yielded ampelopsin A (500 mg) as colorless powder. The silica gel chromatography of the $CHCl_3$-MeOH (92.5:7.5) and (8:2)-eluting fractions (5.9 and 5.1 g, respectively) followed by 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) afforded ampelopsins B (**2**) and C (**3**) (10 and 300 mg, respectively) as colorless powders.

Ampelopsin A (**1**): m.p. 185-186°, $[\alpha]_D$ +167° (c 2.12, MeOH); FAB-MS m/z: 471 $[MH^+]$, 453 $[M^+-H_2O]$; UV (MeOH) λ_{max} nm (log ε): 283 (3.86); IR (KBr) ν_{max} cm^{-1}: 3300, 1605, 1515, 1450; ^1H NMR: in Table 1; ^{13}C NMR (125 MHz, acetone-d_6) δ: 43.7 (d, C-7), 49.4 (d, C-8'), 71.2 (d, C-8), 88.3 (d, C-7'), 97.2 (d, C-12), 101.6 (d, C-12'), 105.4 (d, C-14'), 110.4 (d, C-14), 115.4 (d, C-3,5), 115.9 (d, 3',5'), 118.1 (s, C-10), 128.6 (d, C-2,6), 129.8 (d, C-2',6'), 130.6 (s, C-1), 132.3 (s, C-1'), 139.8 (s, C-9), 142.8 (s, C-9'), 155.8 (s, C-11'), 157.0 (s, C-4'), 158.2 (s, C-4), 158.6 (s, C-13), 159.9 (s, C-11).

Ampelopsin B (**2**): m.p. 170-171°, $[\alpha]_D$ +123° (c 0.93, MeOH); FAB-MS m/z: 455 $[MH^+]$; UV (MeOH) λ_{max} nm (log ε): 281 (3.73); IR ν_{max} cm^{-1}: 3400, 1610, 1515, 1450; ^1H NMR: in Table 1; ^{13}C NMR (125 MHz, acetone-d_6) δ: 34.3 (t), 36.4 (d), 49.8 (d), 88.8 (d), 96.2 (d), 102.0 (d), 106.0 (d), 109.5 (d), 116.1 (d x 2C), 116.5 (d x 2C), 119.5 (s), 123.3 (s), 129.0 (d x 2C), 130.5 (d x 2C), 131.5 (s), 135.2 (s), 138.6 (s), 143.0 (s), 156.5 (s), 157.1 (s), 157.7 (s), 159.0 (s), 159.3 (s), 160.9 (s).

Ampelopsin C (3): m.p. 268-269°, $[\alpha]_D$ +24° (c 1.04, MeOH); FAB-MS m/z: 681 [MH^+]; UV (MeOH) λ_{max} nm (log ε): 282 (4.03); IR (KBr) ν_{max} cm^{-1}: 3400, 1610, 1515, 1450; ^1H NMR: in Table 1; ^{13}C NMR (25 MHz, acetone-d_6) δ: 37.4 (d), 48.7 (d), 52.3 (d), 57.1 (d), 61.9 (d), 90.4 (d), 96.5 (d), 101.6 (d x 2C), 105.8 (d), 107.3 (d x 2C), 115.4 (d x 2C), 115.7 (s), 115.7 (d x 2C), 116.0 (d x 2C), 120.9 (s), 124.6 (s), 129.8 (d x 2C), 130.0 (d x 2C), 130.3 (d x 2C), 130.6 (s), 132.6 (s), 133.2 (s), 141.4 (s), 143.9 (s), 146.7 (s), 154.4 (s), 155.5 (s), 155.6 (s), 156.4 (s x 2C), 158.3 (s), 158.9 (s x 2C), 159.2 (s).

Methylation of ampelopsin A (1): To a solution of ampelopsin A (1) (30 mg) in acetone (7 ml), dimethyl sulfate (0.7 ml) and anhydrous potassium carbonate (70 mg) were added. The reaction mixture was refluxed at 80°C for 6 hr and, after evaporation of solvent, it was chromatographed over silica gel to give ampelopsin A pentamethyl ether **1a** (15 mg) as colorless powder; ^1H NMR (500 MHz, $CDCl_3$) δ: 3.66, 3.67, 3.72, 3.75, 3.83 (3H s), 4.11 (1H d, J=11.7 Hz), 5.40 (1H dd, J=5.2, 5.0 Hz), 5.53 (1H d, J=5.0 Hz), 5.78 (1H d, J=11.7 Hz), 6.22 (1H brs), 6.43 (1H d, J=2.3 Hz), 6.59 (2H d, J=2.3 Hz), 6.62, 6.76, 6.82, 7.10 (2H each d, J=8.3 Hz).

Acetylation of ampelopsin A pentamethyl ether (1a): Ampelopsin A pentamethyl ether (**1a**) (5 mg) was dissolved in pyridine (0.5 ml) and acetic anhydride (1 ml) was added. The reaction mixture was kept at room temperature for 24 hr, evaporated under reduced pressure and chromatographed over silica gel to give the monoacetate **1b** (3 mg) as colorless powder, EIMS m/z: 522 [M^+-AcOH], ^1H NMR (500 MHz, $CDCl_3$) δ: 1.90, 3.66, 3.67, 3.72, 3.75, 3.83 (3H each s), 4.14 (1H d, J=11.7 Hz), 5.52 (1H d, J=5.2 Hz), 5.82 (1H d, J=11.7 Hz), 6.22 (1H bes), 6.28, 6.38, 6.45 (1H each d, J=2.3 Hz), 6.62 (2H d, J=8.3 Hz), 6.66 (1H d, J=5.2 Hz), 6.76, 6.84, 7.10 (2H d, J=8.3 Hz).

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