Vitisin A and *cis*-Vitisin A, Strongly Hepatotoxic Plant Oligostilbenes from *Vitis coignetiae* (Vitaceae)

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The methanolic extract of one of the common Vitaceaeous plants, *Vitis coignetiae*, showed marked prevention against injuries of primary cultured rat liver cells induced by carbon tetrachloride and D-galactosamine. Activity-guided fractionation of the extract resulted in the isolation of not only an antihepatotoxic stilbene derivative, ε-viniferin, but also novel oligostilbenes, vitisin A and its stereoisomer *cis*-vitisin A as a mixture. The structures of the oligostilbenes have been determined on the basis of spectroscopic evidence, especially by 2D NMR methods such as HMBC spectra of degradative products. The mixture of vitisin A and *cis*-vitisin A was found to cause marked liver lesions in mice.

There are a variety of crude drugs which are reputed to be effective for liver diseases in China and Japan. During the course of our effort to search for antihepatotoxic compounds from Oriental crude drugs, our laboratory observed that methanolic extracts of some Vitaceaeous plants such as *Ampelopsis brevipedunculata* Trautv., *A. brevipedunculata* Trautv. var. *hancei* Rehder, and *Vitis coignetiae* Pulliat et Planch exhibited marked antihepatotoxic activity at a dose of 1 mg/mL in carbon tetrachloride and D-galactosamine-induced cytotoxicity model systems employing primary cultured rat hepatocytes.[1] Phytochemical studies on this family revealed that they contain mostly oxidative oligomers of resveratrol.[2] In continuation of our work on Vitaceaeous plants, we focused our attention on biologically active constituents of the methanolic extract of *Vitis coignetiae* bark. Ethyl acetate solubles of the methanolic extract were also antihepatotoxic in the assay. Fractionation of the ethyl acetate solubles led to the isolation of a mixture of two novel oligostilbenes named vitisin A and *cis*-vitisin A, in addition to an antihepatotoxic constituent, ε-viniferin,[3] by monitoring the activity. The ratio of these two oligostilbenes was estimated at approximately 3:2 by the ^{1}H NMR spectrum.

The mixture of vitisin A and *cis*-vitisin A showed a molecular ion at *m/z* 907 [MH$^+$] in the FAB mass spectrum. In the ^{1}H and ^{13}C NMR spectra of the mixture, signals due to the major constituent, vitisin A, were analyzed. Thus, the ^{13}C NMR spectrum displayed signals for 56 carbons which were classified as fifty aromatic and olefinic carbons (=CO × 12, =C- × 12 and =CH- × 26) and six aliphatic carbons (>CHO × 2 and >CH × 4). These spectral data suggested that vitisin A is an oxidative tetramer of resveratrol, with the molecular formula $C_{56}H_{42}O_{12}$. The ^{1}H NMR spectrum indicated the presence of six sets of ortho-coupled aromatic hydrogens (δ 6.68, 7.06; 6.80, 7.17; 6.86, 7.22 (each 2 H, d, *J* = 8.5 Hz)), three sets of meta-coupled aromatic hydrogens (δ 6.06, 6.27; 6.08, 6.11; 6.28, 6.55 (each 1 H, d, *J* = 2.0 Hz)), AX_2-type meta-coupled aromatic hydrogens (δ 6.19 (2 H, d, *J* = 2.0 Hz) and 6.24 (1 H, t, *J* = 2.0 Hz)), and 1,2,4-trisubstituted aromatic hydrogens (δ 6.12 (1 H, d, *J* = 2.0 Hz), 6.71 (1 H, d, *J* = 8.5 Hz), and 6.89 (1 H, dd, *J* = 8.5 and 2.0 Hz)), as well as three sets of mutually coupled aliphatic methine hydrogens (δ 4.26, 5.91 (each 1 H, d, *J* = 10.5 Hz); 4.43, 5.38 (each 1 H, d, *J* = 5.0 Hz); 5.41, 5.50 (each 1 H, d, *J* = 3.0 Hz)). In addition, the ^{1}H NMR spectrum also exhibited a signal at δ 6.41 (2 H, brs) for olefinic hydrogens present in the resveratrol unit.

Due to a resemblance of the ^{1}H NMR spectrum of *cis*-vitisin A to that of vitisin A, they are thought to be close relatives. Further, significant difference of their olefinic hydrogen signals (*cis*-vitisin A: δ 5.83; vitisin A: δ 6.41) suggested that *cis*-vitisin A is an isomer of vitisin A with respect to configuration around the double bond present in the molecule, and this was substantiated by catalytic hydrogenation of the mixture to afford a single dihydro derivative (**3**) (FAB MS: *m/z* 909 [MH$^+$]; ^{1}H NMR: δ 1.95-2.05 (4 H, brs)).

In order to assign ^{1}H NMR signals of the oxymethine hydrogens in vitisin A, the two-dimensional ^{1}H-^{13}C shift correlation spectrum (2D ^{1}H-^{13}C COSY) of the dihydro derivative **3** was measured. The spectrum showed signals for six aliphatic methine hydrogens at δ 4.28, 5.88 (each 1 H, d, *J* = 10.5 Hz), 4.26, 5.37 (each 1 H, d, *J* = 5.0 Hz), and 5.33, 5.53 (each 1 H, d, *J* = 3.5 Hz), which had the cross peaks to those for carbons at δ 57.6, 88.9, 50.0, 94.2, 41.5, and 41.5, respectively. A comparison of the ^{1}H and ^{13}C NMR spectra of vitisin A to those of the dihydro derivative **3** indicated that the ^{1}H NMR signals of vitisin A at δ 4.26, 5.91, 4.43, 5.38, 5.41, and 5.50 corresponded to the ^{13}C NMR signals at δ 57.4, 88.8, 49.8, 94.1, 41.0, and 41.6, respectively, clearly demonstrating the two oxymethine hydrogens at δ 5.91 and 5.38. Moreover, the detailed decoupling studies showed that these two oxymethine hydrogens at δ 5.38 and 5.91 were found to couple with the ortho-coupled aromatic hydrogens at δ 7.17 and 7.22, respectively, which, along with the assumption that vitisin A is oxidatively biosynthesized from resveratrol, implied the presence of two dihydrobenzofuran units in the molecule.

The 2D ^{1}H-^{13}C COSY spectrum of the dihydro derivative **3** also showed the following correlation of the ^{1}H and ^{13}C NMR signals assigned to the trisubstituted benzene ring: δ 5.95 (1 H, brs)-132.0, 6.28 (1 H, dd, *J* = 8.5 and 2.0 Hz)-127.4, and 6.58 (1 H, d, *J* = 8.5 Hz)-115.0. Although the ^{1}H and ^{13}C NMR data suggested the different patterns,

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Table I. ^{1}H NMR Spectra of Vitisin A (1), *cis*-Vitisin A (2), and Their Derivatives (3, 6, and 7)[a]

position no.	1	2[b]	3	6[c]	7[d]
2 (6)	7.06 (d, 8.5)	7.06 (d, 8.5)	7.07 (d, 8.5)		7.00 (d, 8.5)
3 (5)	6.68 (d, 8.5)	6.69 (d, 8.5)	6.68 (d, 8.5)		6.66 (d, 8.5)
7	5.41 (d, 3.0)	5.41 (d, 3.0)	5.33 (d, 3.5)		5.33 (d, 4.0)
8	5.50 (d, 3.0)	5.54 (d, 3.0)	5.53 (d, 3.5)		5.44 (d, 4.0)
12	6.11 (d, 2.0)	6.23 (d, 2.0)[e]	6.24 (d, 2.0)		6.22 (d, 2.0)
14	6.08 (d, 2.0)	5.98 (brs)[e]	6.09 (d, 2.0)[f]		6.10 (d, 2.0)
2′ (6′)	7.22 (d, 8.5)	7.13 (d, 8.5)	7.18 (d, 8.5)		7.12 (d, 8.5)
3′ (5′)	6.86 (d, 8.5)	6.87 (d, 8.5)	6.86 (d, 8.5)		6.76 (d, 8.5)
7′	5.91 (d, 10.5)	5.85 (d, 10.5)	5.88 (d, 10.5)		5.88 (d, 12.0)
8′	4.26 (d, 10.5)	4.25 (d, 10.5)	4.28 (d, 10.5)		4.18 (d, 12.0)
12′	6.28 (d, 2.0)	6.30 (d, 2.0)[e]	6.09 (d, 2.0)[f]		5.93 (d, 2.0)
14′	6.55 (d, 2.0)	6.55 (d, 2.0)	6.30 (d, 2.0)		6.17 (d, 2.0)
2″	6.12 (d, 2.0)	6.20 (d, 2.0)[e]	5.95 (brs)		6.49 (brd, 2.0)
5″	6.71 (d, 8.5)	6.56 (d, 8.5)	6.58 (d, 8.5)		6.89 (d, 8.5)
6″	6.89 (dd, 8.5, 2.0)	6.69 (dd, 8.5, 2.0)	6.28 (dd, 8.5, 2.0)		7.59 (dd, 8.5, 2.0)
7″	6.41 (brs)	5.83 (brs)	1.95–2.05 (brs)		9.32 (s)
8″	6.41 (brs)	5.83 (brs)	1.95–2.05 (brs)	9.85 (s)	
12″	6.06 (d, 2.0)	6.14 (d, 2.0)[e]	6.12 (brs)[f]	6.78 (d, 2.0)	
14″	6.27 (d, 2.0)	6.24 (d, 2.0)[e]	6.12 (brs)[f]	6.96 (d, 2.0)	
2‴ (6‴)	7.17 (d, 8.5)	7.13 (d, 8.5)	7.17 (d, 8.5)	7.23 (d, 8.5)	
3‴ (5‴)	6.80 (d, 8.5)	6.78 (d, 8.5)	6.79 (d, 8.5)	6.89 (d, 8.5)	
7‴	5.38 (d, 5.0)	5.29 (d, 5.0)	5.37 (d, 5.0)	5.58 (d, 5.0)	
8‴	4.43 (d, 5.0)	4.09 (d, 5.0)	4.26 (d, 5.0)	4.79 (d, 5.0)	
10‴ (14‴)	6.19 (d, 2.0)	6.05 (d, 2.0)	6.19 (d, 2.0)	6.27 (d, 2.0)	
12‴	6.24 (t, 2.0)	6.22 (t, 2.0)	6.27 (t, 2.0)	6.34 (t, 2.0)	

[a] Spectra were measured in acetone-d_6 (1, 2, and 3) and $CDCl_3$ (6 and 7) at 500 MHz. Coupling constants are given in Hz. Assignments were confirmed by decoupling experiments. [b] Absorptions for 2 taken from a spectrum of a 3:2 mixture of 1 and 2. [c] OMe: 3.73 (2 × OMe), 3.81, 3.87. [d] OMe: 3.12, 3.56, 3.63, 3.69, 3.71, 3.95. [e,f] Assignments may be reversed.

Table II. ^{13}C NMR Spectra of Vitisin A (1) and Their Derivatives (3, 6, and 7)[a]

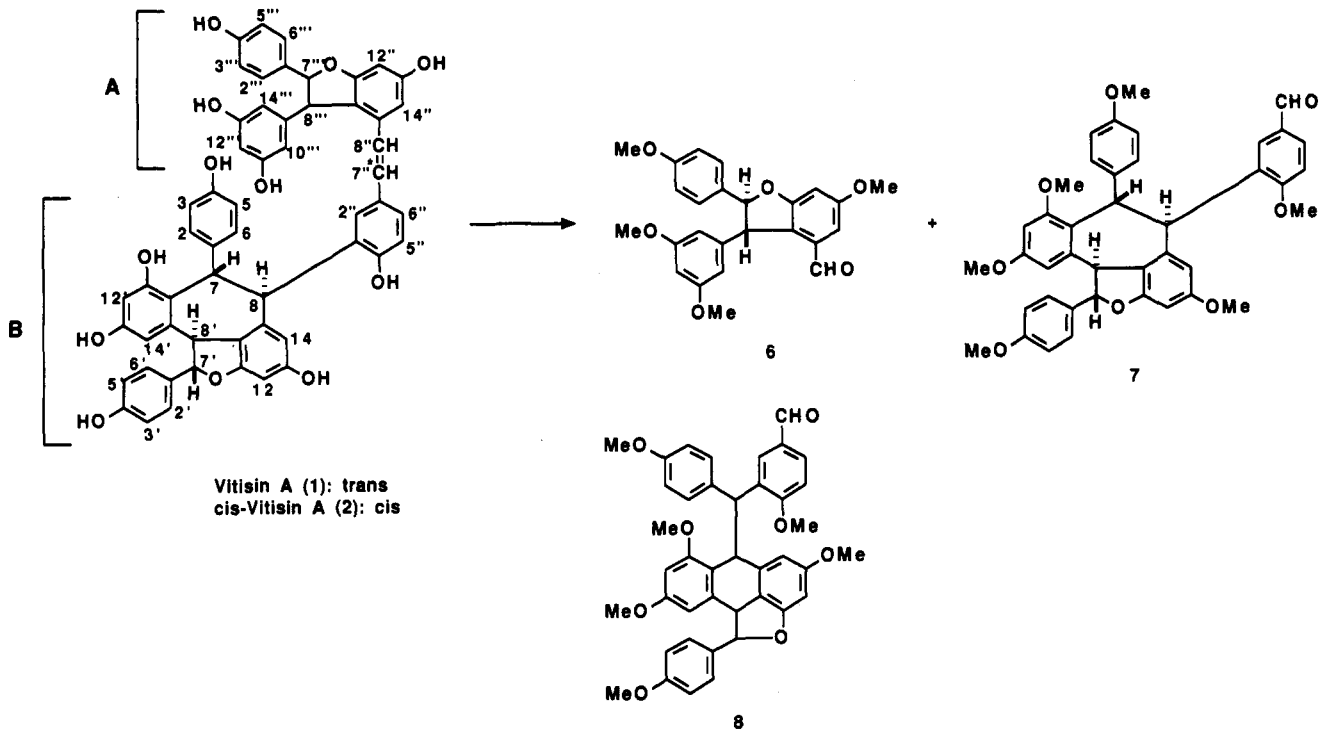
position no.	1	3[b]	6[c]	7[d]	position no.	1	3[b]	6[c]	7[d]
1	135.7 (s)	135.9 (s)		134.8 (s)	1″	129.2 (s)	133.0 (s)		127.8 (s)
2 (6)	129.2 (d)	129.3 (d)		128.0 (d)	2″	132.7 (d)	132.0 (d)		134.0 (d)
3 (5)	115.8 (d)	115.8 (d)		113.7 (d)	3″	136.9 (s)	134.6 (s)		129.1 (s)
4	155.5 (s)[e]	153.3 (s)[f]		157.9 (s)	4″	160.0 (s)[e]	160.2 (s)[f]		161.6 (s)
7	41.0 (d)	41.5 (d)		39.5 (d)	5″	115.8 (d)	115.0 (d)		109.4 (d)
8	41.6 (d)	41.5 (d)		41.0 (d)	6″	124.1 (d)	127.4 (d)		128.2 (d)
9	141.5 (s)	141.8 (s)		138.5 (s)	7″	131.4 (d)	36.8 (t)[g]		191.4 (s)
10	120.5 (s)	120.7 (s)		121.0 (s)	8″	123.0 (d)	37.3 (t)[g]	190.4 (s)	
11	157.2 (s)[e]	157.5 (s)[f]		159.5 (s)	9″	134.2 (s)	142.7 (s)	133.1 (s)	
12	96.4 (d)	95.8 (d)		94.8 (d)	10″	119.4 (s)	120.4 (s)	124.0 (s)	
13	159.1 (s)[e]	159.1 (s)[f]		160.9 (s)	11″	162.8 (s)[e]	162.3 (s)[f]	162.4 (s)	
14	110.4 (d)	110.7 (d)		108.1 (d)	12″	101.3 (d)	101.4 (d)	102.2 (d)	
1′	131.3 (s)	131.5 (s)		130.5 (s)	13″	159.8 (s)[e]	159.7 (s)[f]	161.5 (s)	
2′ (6′)	128.3 (d)	128.5 (d)		129.2 (d)	14″	105.3 (d)	110.0 (d)	106.5 (d)	
3′ (5′)	116.5 (d)	116.7 (d)		114.2 (d)	1‴	133.1 (s)	132.7 (s)	133.1 (s)	
4′	158.5 (s)[e]	158.7 (s)[f]		160.0 (s)	2‴ (6‴)	130.4 (d)	130.5 (d)	127.0 (d)	
7′	88.8 (d)	88.9 (d)		87.9 (d)	3‴ (5‴)	116.4 (d)	116.4 (d)	114.2 (d)	
8′	57.4 (d)	57.6 (d)		48.8 (d)	4‴	158.8 (s)[e]	159.0 (s)[f]	159.8 (s)	
9′	142.6 (s)	142.1 (s)		140.9 (s)	7‴	94.1 (d)	94.2 (d)	93.9 (d)	
10′	120.6 (s)	120.8 (s)		122.0 (s)	8‴	49.8 (d)	50.0 (d)	56.0 (d)	
11′	158.3 (s)[e]	158.3 (s)[f]		159.0 (s)	9‴	147.6 (s)	148.0 (s)	145.9 (s)	
12′	96.9 (d)	96.4 (d)		103.2 (d)	10‴ (14‴)	107.2 (d)	107.5 (d)	105.8 (d)	
13′	156.2 (s)[e]	156.4 (s)[f]		159.0 (s)	11‴ (13‴)	160.6 (s)	160.7 (s)	161.3 (s)	
14′	104.9 (d)	105.3 (d)		95.5 (d)	12‴	102.5 (d)	102.5 (d)	98.8 (d)	

[a] Spectra were measured in acetone-d_6 (1 and 3) and $CDCl_3$ (6 and 7) at 125 MHz. [b] Assignments were confirmed by 2D ^{1}H–^{13}C COSY spectrum. [c] Assignments were confirmed by HMBC spectrum. OMe: 55.5 (3 × OMe), 56.0. [d] Assignments were confirmed by HMQC and HMBC spectra. OMe: 55.2, 55.3 (3 × OMe), 55.4, 56.0. [e–g] Assignments may be reversed.

1-oxy-3,4- and 1-oxy-2,4-disubstitutions, respectively, the long-range coupling between the methylene hydrogen at δ 1.95–2.05 and the meta-coupled aromatic hydrogen at δ 5.95 supported the latter substitution, for which the unusual resonance positions of the ^{1}H NMR signals at δ 5.95 and 6.28 might be due to the shielding effects of spatially close aryl groups.

Methylation of the mixture with dimethyl sulfate and potassium carbonate in acetone yielded a mixture of two decamethyl ethers (4, 5) (FD MS: m/z 1046 [M$^+$]) which was oxidized with ozone to give two degradative products (6, 7). The ^{1}H NMR spectrum of the compound 6 (EI MS: m/z 420 [M$^+$]) exhibited the presence of an aldehyde hydrogen at δ 9.85 as well as two sets of ortho-coupled hydrogens (δ 6.89, 7.23 (each 2 H, d, J = 8.5 Hz)), meta-coupled hydrogens (δ 6.78, 6.96 (each 1 H, d, J = 2.0 Hz)), AX_2 type meta-coupled hydrogens (δ 6.27 (2 H, d, J = 2.0 Hz), 6.34 (1 H, t, J = 2.0 Hz)), and methine hydrogens of a dihydrobenzofuran group (δ 4.79, 5.58 (each 1 H, d, J = 5.0 Hz)). Moreover, saturation of the methoxyl group at δ 3.81 enhanced the integrations of the H-12″ and H-14″ meta-coupled aromatic hydrogen signals at δ 6.78 and 6.96. Distinct NOE's were detected between H-8‴ and H-2‴-(6‴) and H-7‴ and H-10‴(14‴), demonstrating the trans-orientation of the hydrogens of dihydrobenzofuran moiety. These observations, in connection with the biogenetic consideration, clarified the structure of the compound 6 including the relative stereochemistry.

Scheme I



Vitisin A (1): trans
cis-Vitisin A (2): cis

The compound 7 (FD MS: m/z 658 [M^+]) also showed an aldehyde hydrogen signal (δ 9.32) and those for four sets of ortho-coupled aromatic hydrogens (δ 6.66, 7.00 (each 2 H, d, J = 8.5 Hz); 6.76, 7.12 (each 2 H, d, J = 8.5 Hz)), two sets of meta-coupled aromatic hydrogens (δ 5.93, 6.17 (each 1 H, d, J = 2.0 Hz); 6.10, 6.22 (each 1 H, d, J = 2.0 Hz)), and 1,2,4-trisubstituted benzene ring hydrogens (δ 6.49 (1 H, brd, J = 2.0 Hz), 6.89 (1 H, d, J = 8.5 Hz), and 7.59 (1 H, dd, J = 8.5 and 2.0 Hz)) in its ^{1}H NMR spectrum. In addition, the H-7 and H-7′ methine hydrogens resonated at δ 5.33 (1 H, d, J = 4.0 Hz) and 5.88 (1 H, d, J = 12.0 Hz), which were coupled with the H-2(6) and H-2′(6′) ortho-coupled aromatic hydrogens at δ 7.00 and 7.12, respectively, and the other two methine hydrogens (H-8 and H-8′) at δ 5.44 (1 H, d, J = 4.0 Hz) and 4.18 (1 H, d, J = 12.0 Hz) were coupled with the H-14 and H-14′ meta-coupled aromatic hydrogens at δ 6.10 and 6.17. These spectral data allowed us to have two possible structures 7 and 8 for the compound. Unfortunately, the long-range coupling between the hydrogen (H-2″) on the benzene ring bearing a formyl group and the benzylic hydrogen (H-7 or H-8) which is crucial to differentiate these two possible structures was ambiguous. After the assignment of the ^{13}C NMR signals of the compound 7 by HMQC spectrum, the HMBC spectrum was analyzed, which gave three bond couplings between the signals at δ 5.33 (H-7)–128.0 (C-2(6)), 5.44(H-8)–134.0 (C-2″), 7.00 (H-2(6))–39.5 (C-7), clearly demonstrating the structure 7 for the compound. The relative stereochemistry of the compound 7 was deduced by NOE studies. In this way, a significant NOE was observed between H-2′ (6′) and H-8′ in the difference NOE spectrum, suggesting the trans-orientation of the two methine hydrogens (H-7′ and H-8′) on the dihydrobenzofuran moiety. Irradiation of the H-2 (6) at δ 7.00 enhanced the H-8′ methine hydrogen signal at δ 4.18, which can only be possible when the C-7 aryl group is situated cis to H-8′. The β-orientation of the C-8 aryl group was deduced by NOE's of H-2 (6)–H-8 and H-14–H-8. Thus, the degradation products 6 and 7 established the structures of vitisin A (1) and *cis*-vitisin A (2) except the relative stereochemistry between the A and B units and the absolute configurations.

When the vitisin A and *cis*-vitisin A mixture was intraperitoneally administered to mice at a dose of 30 mg/kg, it increased serum glutamic–pyruvic transaminase (GPT) values approximately 200 times from the control. Moreover, severe liver damage in the mice was observed by morphological investigations.

Vitisin A and *cis*-vitisin A molecules have a number of interesting features. They are the first examples of a class of compounds in which a resveratrol molecule is condensed at C-3 of the other resveratrol molecule. They are new natural hepatotoxins, but are constituents of an Oriental crude drug used in the treatment of liver disease.

Experimental Section

UV and IR spectra were recorded on SHIMADZU UV-260 and SHIMADZU IR-408 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM FX-500 spectrometer (TMS as internal standard). FAB, FD, and EI mass spectra were determined with JEOL DX-303 spectrometer.

Isolation of the Mixture of Vitisin A and *cis*-Vitisin A. Dried stems of *Vitis coignetiae* (25.5 kg) were extracted with MeOH (75 L × 3) at room temperature to give the extract (850 g). The MeOH extract (850 g) was partitioned with AcOEt (3 L) and water (3 L) to yield AcOEt (730 g) and water solubles. The AcOEt solubles (210 g) were chromatographed over silica gel (1 kg), and the column was eluted with *n*-hexane–AcOEt mixtures. The *n*-hexane–AcOEt (2:8)-eluting fraction (25.0 g) was chromatographed over silica gel (150 g), and the column was eluted with increasing polarity of $CHCl_3$–MeOH mixtures. A repeated silica gel chromatography of the $CHCl_3$–MeOH (9:1)-eluting fraction followed by HPLC (column: Tosoh TSK gel ODS-120A: 30 × 2.15 cm i.d.; solvent: CH_3CN–water (72.5:27.5); flow rate: 3 mL/min) afforded a mixture of vitisin A and *cis*-vitisin A (150 mg).

Vitisin A and *cis*-vitisin A: amorphous powder, FAB MS m/z: 907 [MH^+]; UV (MeOH) λ_{max} nm (log ϵ): 283 (4.40), 320 (4.22); IR (Nujol) ν_{max} cm^{-1}: 3160 (OH), 1600, 1510, 1450 (aromatic); ^{1}H and ^{13}C NMR (Tables I and II, respectively).

Catalytic Hydrogenation of Vitisin A and *cis*-Vitisin A. A mixture of vitisin A and *cis*-vitisin A (70 mg) and PtO_2 (15 mg) in MeOH (10 mL) was shaken under H_2 for 24 h at room temperature, and the reaction mixture, after filtration, was chromatographed over silica gel to yield a dihydro derivative (3) (42 mg); FAB MS *m/z*: 909 [MH^+]; ^1H and ^{13}C NMR (see Tables I and II).

Methylation Followed by Ozonolysis of Vitisin A and *cis*-Vitisin A. To a solution of vitisin A and *cis*-vitisin A mixture (80 mg) in acetone (10 mL) were added dimethyl sulfate (1.0 mL) and anhydrous potassium carbonate (200 mg). The reaction mixture was refluxed at 80 °C for 12 h, and after evaporation of solvent, it was chromatographed over silica gel to yield a mixture of the decamethyl ethers 4 and 5 (27 mg) (FD MS *m/z*: 1046 [M^+]). The solution of the mixture (27 mg) in methylene chloride (10 mL) at 0 °C was treated with an ozone-saturated methylene chloride solution (10 mL), and excess dimethyl sulfide was added to the resulting mixture. After evaporation of solvent, the residue was chromatographed over silica gel to afford two compounds (6 and 7). 6 EI MS *m/z*: 420 [M^+]. ^1H and ^{13}C NMR (refer to Tables I and II). 7. FD MS *m/z*: 658 [M^+]. ^1H and ^{13}C NMR (Tables I and II).

Biological Activity. Seven Std:ddY mice (6 weeks) were employed per group. The sample was suspended in physiological saline solution containing 3% gum arabic and injected intraperitoneally to normal mice. Blood was drawn from the orbital sinus by microhepatocrit tubes at 24 h after the sample administration. Serum glutamic–pyruvic transaminase (GPT) activities were measured according to the method of Karmen[4] using an autoanalyzer.

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