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Novel flavanol derivatives from grape seeds

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Abstract—Viniferone A, an oxidative derivative of catechin, together with viniferone B and C, presumably the oxidative derivatives of epicatechin, were isolated from grape seeds. Their structures were elucidated by spectroscopic methods. The X-ray structure of viniferone A is presented.

Grape seed proanthocyanidins (GSP) are natural antioxidants, which possess a broad spectrum of pharmacological, therapeutic and chemoprotective properties against free radicals and oxidative stress.[1] GSP are composed of monomeric flavan-3-ols including (+)-catechin, (−)-epicatechin and their galloylated derivatives,[2,3] oligomeric procyanidins[4] and polymeric proanthocyanidins.[5] In our continuing study on polyphenols from natural products,[6] three novel flavanol derivatives, viniferone A, together with viniferone B and C were obtained from grape seeds and they were considered as possible oxidative derivatives of (+)-catechin and (−)-epicatechin.

Grape seeds (10.52 kg) were collected from a winery. The 70% acetone extracts (462.9 g) was re-extracted with ethyl acetate. The ethyl acetate extracts (153.5 g) was chromatographed on hydrophobic resin DIAION® HP20 with increasing amounts of MeOH in H_2O. The fractions eluted with MeOH–H_2O (20:80; v/v) comprised of monomeric proanthocyanidins, which was re-chromatographed on Toyopearl™ HW-40F using 10% MeOH in H_2O to afford **1** (40 mg),[7] **2** (64 mg)[8] and **3** (22 mg)[9] (Fig. 1). Their molecular formulas were determined to be $C_{15}H_{14}O_8$ by HRESI-MS at m/z (M+H) 323.0689, 323.0694, 323.0680, respectively, with the degree of unsaturation being 9.

Compound **1** was obtained as pale yellow lamellar crystals, compound **2** as white acicular crystals, whereas compound **3** formed white granular crystals. The ^{1}H NMR and ^{13}C NMR (CD_3OD) spectra of all three compounds closely resembled each other (Tables 1 and 2), and were similar to those of (+)-catechin or (−)-epicatechin,[10] which were also obtained and identified in our work. The *meta*-coupled aromatic proton signals in

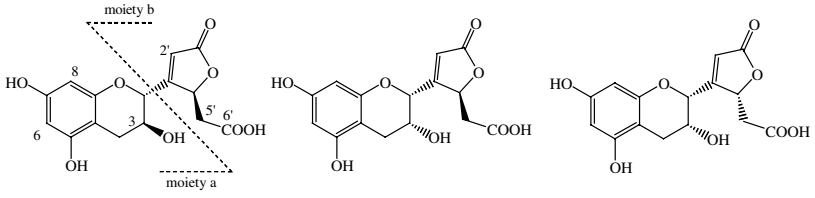

Figure 1. Structures of compounds **1**, **2** and **3**.

Keywords: *Vitis vinifera* L; Grape seeds; Viniferones.
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Table 1. NMR data of compound **1** (600 MHz for ^{1}H and 150 MHz for ^{13}C, δ, ppm (J, Hz), CD_3OD)

Position	δC	δH	^{1}H–^{1}H COSY	HMBC(H → C)	NOESY
2	76.0	4.61 dd (8.4, 1.2)	H-3, H4′	C-4, C-3, C-2′, C-4′, C-3′, C-9	H_b-4, H-5′, H-3, H-2′, H-4′
3	67.0	3.87–3.92 m	H-4, H-2	C-3′, C-10	H-4, H-2, H-4′
4	28.9	a: 3.02 dd (16.0, 5.5)	H_b-4, H-3	C-5, C-9, C-10, C-3, C-2	H_b-4, H-3
		b: 2.53 dd (16.0, 9.0)	H_a-4, H-3	C-5, C-9, C-10, C-3, C-2	H_a-4, H-3, H-2
5	156.6				
6	96.0	5.98 d (2.8)	H-8	C-5, C-10, C-8, C-7	
7	157.1				
8	94.5	5.92 d (2.8)	H-6	C-7, C-10, C-6	
9	154.5				
10	99.4				
1′	173.4				
2′	117.0	6.19 br s	H-4′, H-2	C-4′, C-1′, C-3′, C-2	H-3, H-2, H-4′
3′	171.0				
4′	80.7	5.73–5.75 m	H-5′, H-2′	C-5′, C-3′, C-6′, C-4′	H-5′, H-3, H-2
5′	37.4	a: 3.13 dd (16.3, 5.5)	H_b-5′, H-4′	C-6′, C-3′, C-4′	H_b-5′, H-3, H-2, H-4′
		b: 2.58 dd (16.3, 8.5)	H_a-5′, H-4′	C-6′, C-4′	H_a-5′, H-4′, H-2
6′	171.8				

Table 2. NMR data of **2** and **3** (600 MHz for ^{1}H and 150 MHz for ^{13}C, δ, ppm (J, Hz), CD_3OD)

Position	**2**			**3**		
	δC	δH	NOESY	δC	δH	NOESY
2	76.2	4.95 br s	H_a-4, H-5′, H-3, H-2′, H-4′	76.2	4.95 br s	H_a-4, H_a-5′, H-3, H-2′, H-4′
3	66.1	4.28–4.31 m	H-4, H-2, H-4′	66.0	4.34–4.36 m	H-4, H-2
4	28.5	a: 2.85 dd (16.0, 5.0)	H_b-4, H-3, H-2	28.5	a: 2.86 dd (16.0, 5.0)	H_b-4, H-3, H-2
		b: 2.52 dd (16.0, 5.0)	H_a-4, H-3		b: 2.57 (overlapped with H_b-5′)	H_a-4, H-3
5	158.4			158.4		
6	97.4	5.95 d (3.0)		97.4	5.95 d (3.0)	
7	158.6			158.4		
8	96.1	5.90 d (3.0)		96.1	5.91 d (3.0)	
9	155.9			156.0		
10	100.2			100.2		
1′	174.7			175.0		
2′	119.1	6.05 br s	H-3, H-2, H-4′	118.7	6.07 br s	H-3, H-2
3′	172.1			172.6		
4′	81.2	5.59–5.61 m	H-5′, H-3, H-2	81.9	5.61–5.63 m	H-5′, H-3, H-2
5′	39.0	a: 3.12 dd (16.0, 3.4)	H_b-5′, H-2, H-4′	40.6	a: 2.98 dd (16.0, 4.5)	H_b-5′, H-2, H-4′
		b: 2.60 dd (16.0, 8.3)	H_a-5′, H-4′, H-2		b: 2.60 (overlapped with H_b-4)	H_a-5′, H-4′
6′	173.4			174.9		

1 at δ 5.98 (1H, d, $J = 2.8$ Hz, H-6) and 5.92 (1H, d, $J = 2.8$ Hz, H-8) and the characteristic signals due to the C-ring protons of flavan-3-ols at δ 4.61 (1H, dd, $J = 8.4$, 1.2 Hz, H-2), 3.87–3.92 (1H, m, H-3), 3.02 (1H, dd, $J = 16.0$, 5.5 Hz, H_a-4) and 2.53 (1H, dd, $J = 16.0$, 9.0 Hz, H_b-4) in ^{1}H NMR revealed the presence of moiety a.[11] This moiety was also confirmed by ^{1}H–^{1}H COSY and HMBC (Table 1, Fig. 2). The absence of any catechol B-ring signals, and instead, the presence of additional four sp^2 carbons at δ 171.8 (s), 171.0 (s), 173.4 (s) and 117.0 (d) and two sp^3 carbons at δ 80.7 (d), 37.4 (t) suggested that compounds **1** was a derivative of catechin with B-ring alteration (b moiety).

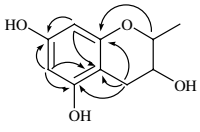


Figure 2. Selected HMBC correlations for moiety a of **1**.

The b-moiety structure was determined to be an α,β-unsaturated lactone by the low field chemical shift of C-4′ at δ 80.7,[12] and the long range correlation between the proton at δ 5.73–5.75 (1H, m, H-4′) and the carbonyl at δ 173.4 (C-1′) as determined by HMBC. The tri-substituted double bond was deduced from the olefinic proton signal at δ 6.19 (1H, br s, H-2′) and assignment of the carbon signals made by HMQC, which was confirmed by HMBC. The occurrence of one carboxy methyl group was decided by the methylene signal at δ 3.13 (1H, dd, $J = 16.3$, 5.5 Hz, H_a-5′) and 2.58 (1H, dd, $J = 16.3$, 8.5 Hz, H_b-5′) in ^{1}H NMR and one carboxyl at δ 171.8 in ^{13}C NMR. Its linkage was determined by the coupling correlation between H-4′ and H-5′ in ^{1}H–^{1}H

COSY as well as the long range correlation between H-4′ and C-3′, C-5′, H-5′ and C-6′, C-4′ C-3′ as determined by HMBC. Thus the structure of b-moiety was assigned to be 4-carboxymethyl-γ-butenolide-3-yl.

The 2,3-*trans* stereochemistry was decided by the coupling constant ($J = 8.4$ Hz) for H-2.[13] The high-amplitude negative Cotton effect in the diagnostic wavelength region of the CD spectrum (λ_{ext} 232 nm, $\Delta\varepsilon$ −2.56) defined the absolute configuration at C-2 as *R*.[14] The stereostructure of **1** was determined by X-ray diffraction analysis[15] (Fig. 3). Consequently, **1** was unambiguously determined to be (2*R*,3*S*,4′*S*)-2-(4-carboxymethyl-γ-butenolide-3-yl)-3,5,7-trihydroxychroman and named as viniferone A.

Since the structure of **1** was closely related to (+)-catechin, one of the main ingredients of grape seeds, a biogenetic route for **1** is proposed (Scheme 1). The oxidation mechanism is similar to the biogenetic pathway of psiguarin from eugenigrandin A,[16] and the configuration at the C-2, C-3 of catechin remained unchanged during enzyme-catalyzed oxidation.

The same methods were used to determine the structures of **2** and **3**. Both **2** and **3** have ^{1}H NMR spectra similar to that of **1** (Fig. 4). The singlet signal at δ 4.95 (1H, br s, H-2) indicated the 2,3-*cis* configuration, and suggested that **2** and **3** were oxidative derivatives of epicatechin. The same 2*R*-configuration depicted in structure **2** and **3** was

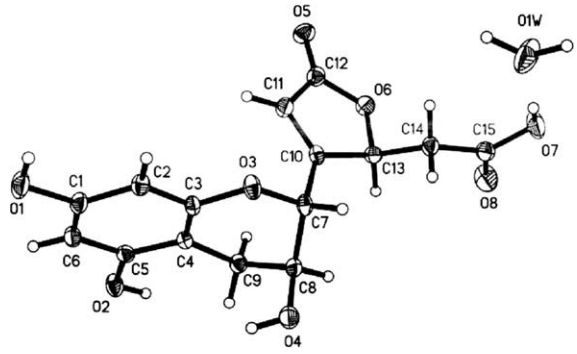
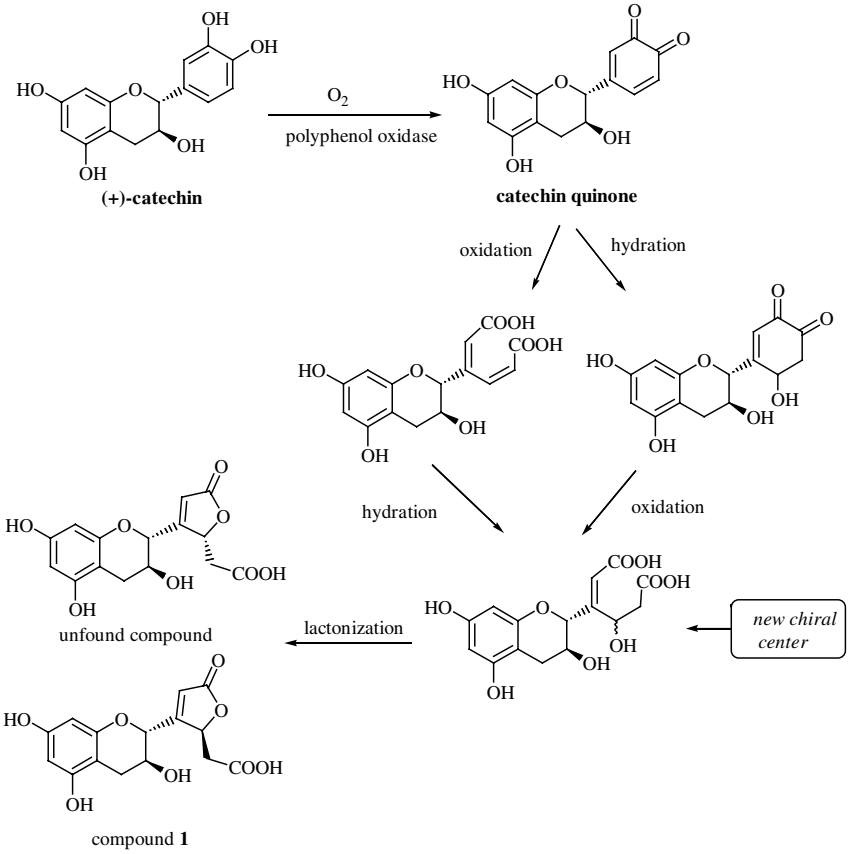


Figure 3. ORTEP structure of **1**.

based on the negative Cotton effect in the 220–240 regions of their CD spectra. Accordingly, **2** and **3** were determined to be stereoisomers at C-4′ position of the b-moiety. The comparison between the ^{1}H NMR data of **2** and **1** revealed **2** to have the same configuration at C-4′ with that of **1**, based on their identical coupling patterns in ^{1}H NMR of the b-moiety. On the contrary, the signal of H_a-5′ had an up-field shift of 0.14 ppm in the ^{1}H NMR spectra of **3**, when compared with that of **1** or **2** (Table 2, Fig. 4). In addition, the NOE correlations between H-2 and two protons of H-5′ for **1** and **2**, as well as the NOE correlation between H-2 and H_a-5′ at lower field for **3** in their respective NOESY spectra (Table 2), also confirmed this conclusion. On the basis of above analysis, compound **2** was tentatively determined as (2*R*,3*R*,4′*S*)-



Scheme 1. Possible biogenesis of **1** from (+)-catechin.

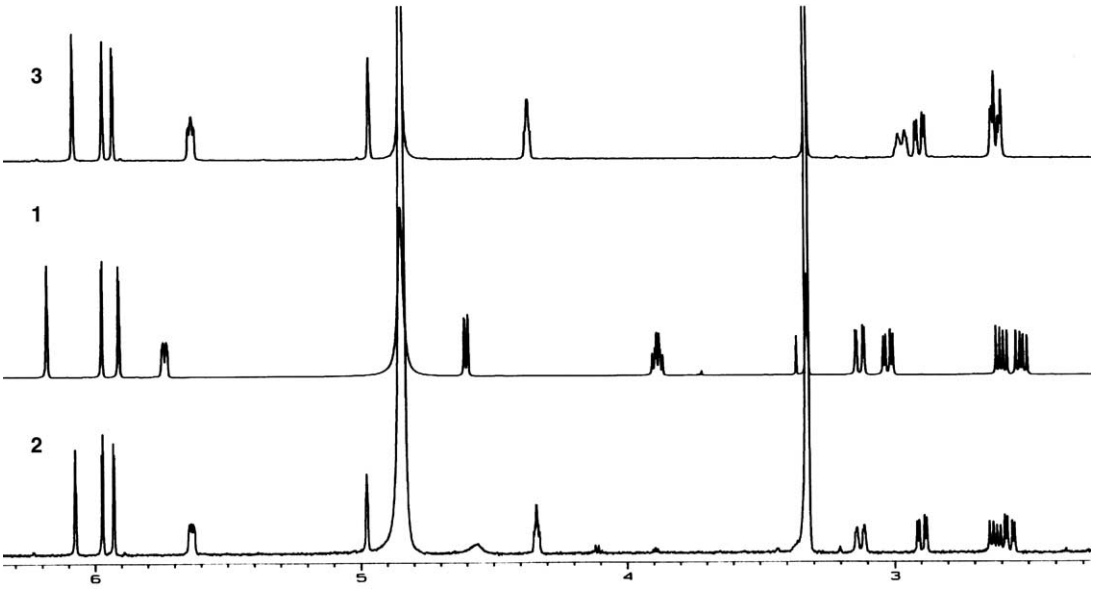


Figure 4. ^{1}H NMR spectra of **1**, **2** and **3**.

2-(4-carboxymethyl-γ-butenolide-3-yl)-3,5,7-trihydroxychroman and was named as viniferone B, while **3** was assigned (2*R*,3*R*,4′*R*)-2-(4-carboxymethyl-γ-butenolide-3-yl)-3,5,7-trihydroxychroman and was named as viniferone C. Both **2** and **3** can be considered as oxidative derivatives from another main constitution of grape seeds (−)-epicatechin arising from a biogenetic pathway analogous to that of viniferone A from (+)-catechin.

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7. Yellow lamellar crystal (MeOH–H_2O), $R_f = 0.34$ [TLC in $CHCl_3$–MeOH–HCOOH (15:1:0.1), blue spot stained with 1% $FeCl_3$–$K_3Fe(CN)_6$], mp 199.1–201.6 °C, $[\alpha]_D^{20}$ −22 (*c* 0.5, MeOH) CD (MeOH, *c* 4E–4 g/mL): $\Delta\varepsilon_{232\,nm} - 2.56$, $\Delta\varepsilon_{280\,nm} + 0.695$.
8. White acicular crystal (MeOH–H_2O), $R_f = 0.4$ [TLC in $CHCl_3$–MeOH–HCOOH (15:1:0.1), blue spot stained with 1% $FeCl_3$–$K_3Fe(CN)_6$], mp 225.6–227 °C, $[\alpha]_D^{20}$ −50 (*c* 0.5, MeOH), CD (MeOH, *c* 4E–4 g/mL): $\Delta\varepsilon_{214\,nm} - 4.879$, $\Delta\varepsilon_{228\,nm} - 2.195$.
9. White granular crystal (MeOH–H_2O), $R_f = 0.26$ [TLC in $CHCl_3$–MeOH–HCOOH (15:1:0.1), blue spot stained with 1% $FeCl_3$–$K_3Fe(CN)_6$], mp unobtained because of decompose, $[\alpha]_D^{20}$ −45 (*c* 0.5, MeOH), CD (MeOH, *c* 4E–4 g/mL): $\Delta\varepsilon_{225\,nm} - 4.39$, $\Delta\varepsilon_{282\,nm} + 0.356$.
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15. X-ray crystal structure analysis of **1**: crystal data: $C_{15}H_{14}O_8 \cdot H_2O$, monoclinic, $P2_1$, $a = 5.3527$ (7), $b = 15.024$ (2), $c = 8.9880$ (13) Å, $\beta = 96.697$ (10)°, $V = 717.89$ (17) Å^3, $Z = 2$, crystal size: $0.38 \times 0.36 \times 0.10$ mm. A total of 2319 unique reflections were collected using graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) on a Bruker-P4 diffractometer. The structure was solved by direct methods (SIR-97) refined by full matrix least squares techniques based on F^2 to give $R = 0.0422$, $wR2 = 0.0988$. Additional crystallographic details, CCDC213913 (atomic coordinates and equivalent isotropic displacement coefficients) have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.
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