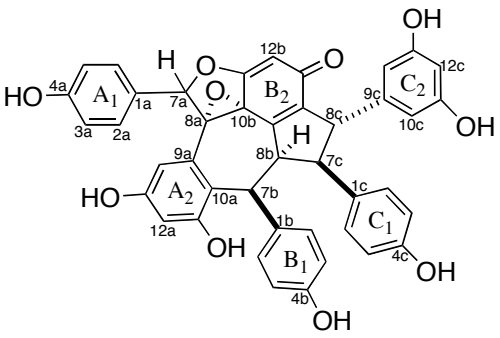
HETEROCYCLES, Vol. 68, No. 8, 2006, pp. 1617 - 1630. © The Japan Institute of Heterocyclic Chemistry
Received, 19th April, 2006, Accepted, 7th June, 2006, Published online, 8th June, 2006. COM-06-10769

STILBENOIDS WITH ONE EPOXY GROUP FROM *COTYLELOBIUM LANCEOLATUM*

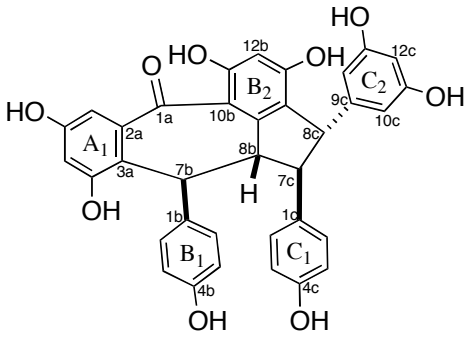
Tetsuro Ito, [a,*] Zulfiqar Ali,[b] Toshiyuki Tanaka, [a] Ken-ichi Nakaya, [a] Jin Murata, [c] Dedy Darnaedi, [d] and Munekazu Iinuma[b]

a) Gifu Prefectural Institute of Health and Environmental Sciences, 1-1 Naka-fudogaoka, Kakamigahara 504-0838, Japan (*Corresponding author. e-mail: ito-tetsuro@pref.gifu.lg.jp)
b) Department of Pharmacognosy, Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan
c) Botanical Gardens, Koishikawa, Graduate School of Science, University of Tokyo, 3-7-1 Hakusan, Bunkyo-Ku, Tokyo, 112-0001, Japan
d) Herbarium Bogoriense, The Indonesian Institute of Science, Research and Development Center for Biology, J1 Ir. Juanda 18, Bogor 16122, Indonesia

Abstract – A phytochemical investigation of an acetone extract of *Cotylelobium lanceolatum* stem resulted in the isolation of two orange pigments—two stilbene trimer derivatives, cotylelophenols D and E and an artifact derivative, cotylelophenol F. The structure and relative configuration were confirmed by 1D and 2D NMR spectral data. Cotylelophenols D and E are the first examples of stilbene oligomers bearing an epoxy group. The biogenetic relationships between the isolates are also discussed.



1 H-7a = β
2 H-7a = α



3

INTRODUCTION

In the course of our research directed towards the isolation and identification of bioactive polyphenols in plants, we previously reported the structural variety of the resveratrol oligomers from plants belonging to the family Dipterocarpaceae.[1–8] Resveratrol and its oligomers have been drawn to our attention because of their multifunctional bioactivity, e.g., chemoprevention of cancers[9] and activation of the human SIRT1 enzyme.[10] The active interest in resveratrol oligomers of this family led us to the current phytochemical study of the stem of *Cotylelobium lanceolatum* (Dipterocarpaceae). In our previous studies of chemical constituents in this material, some new structures of resveratrol oligomers were characterized.[1] Further, detailed examination of the acetone extract yielded two novel resveratrol trimers, cotylelophenols D (**1**) and E (**2**), and an artifact derivative, cotylelophenol F (**3**). The structure of isolates (**1–3**) were elucidated by means of 2D NMR techniques such as ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, and heteronuclear multiple-bond correlation (HMBC), and the stereostructures were proposed by analysis of the NOESY spectra. The possible biogenetic pathways concerning the resveratrol oligomers have been proposed.

RESULTS AND DISCUSSION

Cotylelophenol D (**1**) ($[\alpha]_D^{25}$ + 336º) and E (**2**) ($[\alpha]_D^{25}$ + 389º) were purified from an acetone-soluble part of the stem of *C. lanceolatum* by column chromatography over silica gel, ODS, Sephadex LH-20 and preparative TLC. An artifact derivative, cotylelophenol F (**3**) ($[\alpha]_D^{25}$ + 323º), was also isolated from the extract.

Cotylelophenol D (**1**), obtained as an orange solid, showed a weak-positive reaction with the Gibbs reagent. The composition of **1** was deduced to be $C_{42}H_{30}O_{10}$ from the pseudo-molecular ion peak of $[M-H]^-$ at *m/z* 693.1768 in the HR–FAB–MS spectrum and the ^{13}C NMR spectrum which showed 42 carbon signals. A band in the IR spectrum at 1670 cm^{-1} and a signal in the ^{13}C NMR spectrum (δ_C 183.8) showed the presence of an α,β-unsaturated carbonyl group (C-13b) in the molecule. The ^1H and ^{13}C NMR spectral data for **1** (Table 1) together with ^1H–^1H COSY, ^{13}C–^1H COSY, and HMBC spectra [Figure 1

Table 1. NMR Data for Cotylelophenols D (**1**) and E (**2**)[a]

position	**1**		**2**	
	δH	δc	δH	δc
1a		126.6		127.3
2a(6a)	7.72 (*d*, *J* = 8.6)	131.5	7.30 (*d*, *J* = 8.6)	130.4
3a(5a)	7.00 (*d*, *J* = 8.6)	116.0	6.71 (*d*, *J* = 8.6)	115.7
4a		159.1		158.6
7a	5.89 (*s*)	89.3	6.46 (*s*)	91.5
8a		73.0		76.8
9a		131.0		131.1 [c]
10a		123.3		123.7
11a		156.7		157.2
12a	6.44 (*d*, *J* = 2.4)	104.6	6.34 (*d*, *J* = 2.4)	104.7
13a		156.2		156.4 [d]
14a	6.34 (*d*, *J* = 2.4)	111.3	6.83 (*d*, *J* = 2.4)	113.4
1b		130.3		130.6
2b(6b)	6.53 (*d*, *J* = 8.6)	131.4	6.45 (*d*, *J* = 8.6)	132.0
3b(5b)	6.55 (*d*, *J* = 8.6)	114.4	6.49 (*d*, *J* = 8.6)	114.5
4b		159.9 [b]		156.4 [d]
7b	5.19 (*d*, *J* = 2.7)	40.9	4.99 (*d*, *J* = 2.7)	40.8
8b	3.91 (*dt*, *J* = 9.4, 2.7)	52.9	3.92 (*dt*, *J* = 9.4, 2.7)	52.3
9b		149.3		149.5
10b		64.2		66.0
11b		170.3		171.7
12b	5.65 (*s*)	105.2	5.83 (*s*)	105.8
13b		183.8		183.9
14b		146.6		146.9 [e]
1c		128.7		128.9
2c(6c)	7.25 (*d*, *J* = 8.6)	130.5	7.09 (*d*, *J* = 8.6)	131.1 [c]
3c(5c)	6.87 (*d*, *J* = 8.6)	115.1	6.78 (*d*, *J* = 8.6)	115.3
4c		156.0 [b]		156.5
7c	4.00 (*t*, *J* = 9.4)	56.6	3.98 (*t*, *J* = 9.4)	57.4
8c	3.76 (*dd*, *J* = 9.4, 2.7)	52.0	3.83 (*dd*, *J* = 9.4, 2.7)	53.1
9c		146.4		146.9 [e]
10c(14c)	6.25 (*d*, *J* = 2.0)	106.9	6.19 (*d*, *J* = 2.0)	107.2
11c(13c)		158.4		158.8
12c	6.10 (*t*, *J* = 2.0)	100.9	6.09 (*t*, *J* = 2.0)	101.3

a : Measured in CD_3COCD_3 at 300 MHz (^1H NMR) and 75 MHz (^{13}C NMR); δ in ppm, *J* in Hz.
b : Interchangeable. c–e : Overlapping.

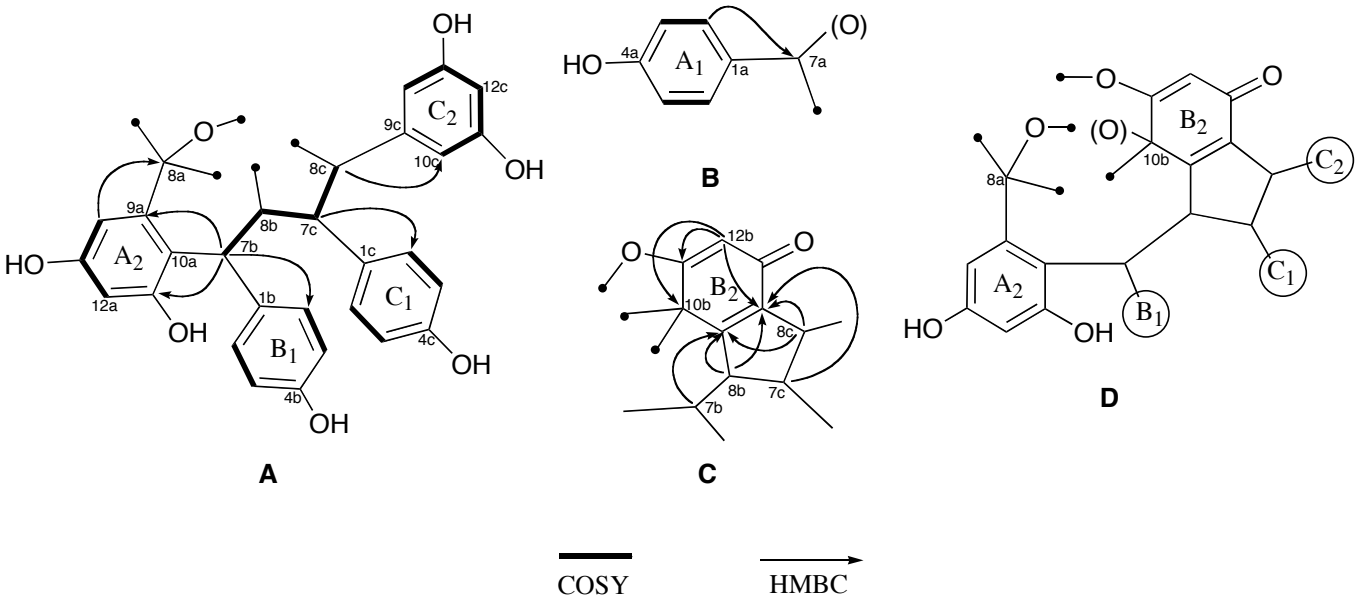


Figure 1 Selected correlations in 2D NMR in the partial structures (A–D) of **1**

(selected) and Table 2 (total)] indicated the presence of five oxygenated aromatic rings, which form three 4-hydroxyphenyl groups (rings A_1–C_1), a 1,2,3,5-tetrasubstituted benzene ring (ring A_2), and a 3,5-dihydroxyphenyl group (ring C_2). The spectrum also exhibited a sequence of four aliphatic protons in the following order—H-7b/H-8b/H-7c/H-8c. Two isolated proton signals (H-7a and H-12b) were further observed. In the HMBC spectrum (Figure 1), significant ^3J correlations were observed between H-7a/C-2a(6a), H-7b/C-11a,H-7b/C-2b(6b), H-7c/C-2c(6c), and H-8c/C-10c(14c), which supported the connections between C-7a/C-1a, C-7b/C-10a, C-7b/C-1b, C-7c/C-1c and C-8c/C-9c, respectively (Figure 1, partial structures (**A** and **B**)). The remaining ring system and the connectivity in the molecule were determined as follows. The presence of a six-membered ring system was apparent from the signals of C-9b–C-14b in the ^{13}C NMR spectrum (Table 1). The ring was composed of four quaternary sp^2 carbons [δ_C 149.3, 170.3, 183.8 (carbonyl), 146.6], one oxygenated quaternary carbon (δ_C 64.2), and a protonated sp^2 carbon (δ_C 105.2), which suggested that the ring formed a cyclohexa-2,5-dienone ring (ring B_2). The ring system was supported by the cross peaks of H-12b/C-10b, H-12b/C11b, and H-12b/C14b (Figure 1, partial structure **C**). Two carbon atoms (C-8a and C-10b) were identified to be quaternary. The important correlations of HMBC measurements for the fused cyclic system of the ring B_2 were as follows, H-7b/C-9b, H-8b/C-14b, and H-8c/C-9b for the connection of four carbons of C-8b, C-9b, C-14b, and C-8c in this order. The established partial structures of **B** and **D** accounted for 25 of the 28 required

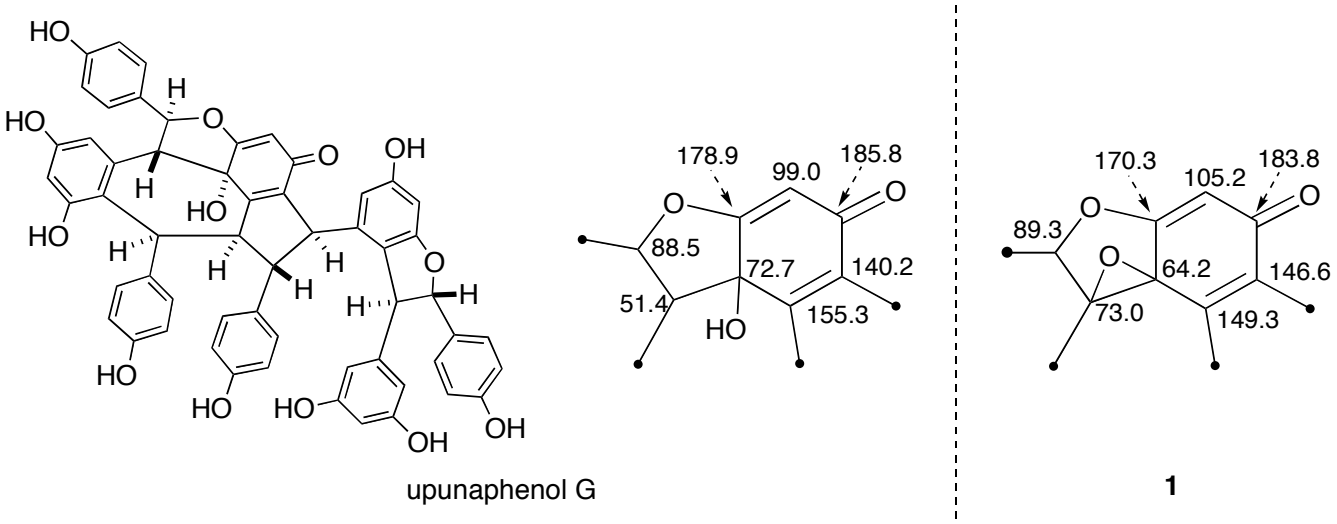


Figure 2 Structure (upunaphenol G) and ^{13}C-NMR data of dihydrobenzofuranone moiety (upunaphenol G and **1**)

degrees of unsaturation, which suggested that the formation of three rings was required. Although no long-range correlation between H-7a/C-8a and/or H-7a/C-10b was observed, the bonds C-7a–C-8a and C-8a–C-10b were assumed after consideration of the molecular skeleton. The remaining two oxygen atoms could be allotted to ether linkages, forming a 3,6-Dioxa-bicyclo[3.1.0]hexane ring including an epoxyl group. Subsequently, the planar structure of cotylelophenol F was concluded to be **1**. The structure is composed of three resveratrols A–C [(resveratrol A: ring A_1–7a–8a–ring A_2)] by the six rings (A_1–C_1 and A_2–C_2) and the six sp^3 carbon units. The carbons, C-9b–C-14b, in the ^{13}C NMR spectrum were observed at δ_C 149.3, 64.2, 170.3, 105.2, 183.8, and 146.6, and were assigned to a cyclohexa-2,5-dienone ring. Similar patterns in the same partial structure were also observed in upunaphenol F (δ_C 155.3, 72.7, 178.9, 99.0. 185.8, 140.2),[3] where the carbon atom (C-10b) was substituted by a hydroxyl group instead of an epoxyl group (Figure 2). In the case of **1**, C-9b–C-12b and C-14b were shifted upfield by $\Delta\delta$ 6.0–8.6. The neighboring epoxy group could be responsible for these shifts. In the four proton sequence (H-7b–H-8c), the protons H-8b and H-8c were observed as *dt* (J = 9.4, 2.7, 2.7 Hz) and *dd* (J = 9.4, 2.7 Hz), respectively. The protons display a long-range coupling via 4J (2.7 Hz), which causes the respective signals, because their original splitting patterns were *dd* (J = 9.4, 2.7 Hz) and *d* (J = 9.4 Hz).

The stereostructure of **1** was determined from the results of the NOESY experiment [Figure 3 (selected) and Table 2 (total)]. Clear cross peaks between H-7a/H-2b(6b) and H-2b(6b)/H-8c were observed, indicating that H-7a, ring B1, and H-8c are oriented on the same side of the reference plane (β-

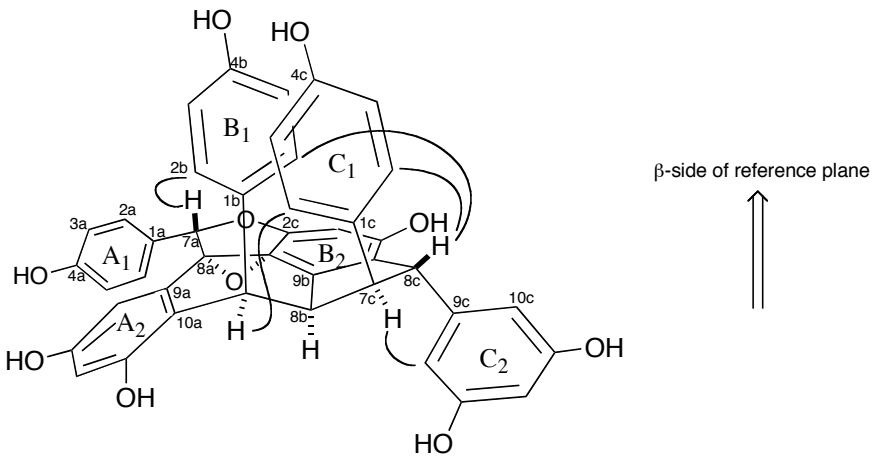


Figure 3 Selected NOEs observed in the NOESY experiment with **1**

orientation) and C-7b forms the flap of the envelope conformation in the cycloheptadiene ring (C-8a-C-9a-C-10a-C-7b-C-8b-C-9b-C-10b). The ring system and co-facial relationship between H-7a and the ring B1 allowed H-8b and the epoxy oxygen to be in α-orientation. When the proton (H-8b) was oriented in β-configuration, H-7a and the ring B_1 would be much more distant and the above correlation would not be observed. Distinct NOEs of H-7c/H-10c(14c) and H-2c(6c)/H-8c explained H-7c to be in α-orientation. Although the ring C_1 and H-7b in **1** are situated on the opposite side of the reference plane, both are located in near positions [NOE: H-2c(6c)/H-7b], which can be explained in the same manner as those in pauciflorol A.[4] Based on these results, the relative stereostructure of **1** was confirmed.

Cotylelophenol E (**2**) was obtained as an orange solid. The molecular formula of $C_{42}H_{30}O_{10}$ was established from the HR–FAB–MS spectrum ($[M-H]^-$ ion at *m/z* 693.1765). Analysis of the ^1H and ^{13}C NMR, ^1H–^1H COSY, ^{13}C–^1H COSY, and HMBC spectra (Tables 1 and 2) confirmed that **2** had the same planar structure as **1**. The relative stereostructure of **2** was determined from the NOESY spectrum. For a similar reason as described in **1**, the relative stereostructure of **2** was confirmed. The differences between **1** and **2** were attributed to the configuration of H-7a, which was situated in α configuration in **2**. The configuration was deduced from the NOE H-2a(6a)/H-2b(6b), which could only be observed when the rings A_1 and B_1 were located in a co-facial relationship (β configuration).

The ^1H- and ^{13}C-NMR spectral data of **2** was similar to those of **1** except for their resveratrol A unit. The conspicuous differences in their spectra were the δ_H values of H-2a(6a) and H-7a (Table 1), which can well support the view that their structural difference is due to the configuration of C-7c. The co-facial

epoxyl group would contribute to the downfield shift of the protons: H-2a(6a) and H-3a(5a) of **1** were observed lower (Δ 0.42 and 0.29) than those of **2** and H-7a of **2** was observed lower (Δ 0.57) than that of **1**. The neighboring epoxyl group also induced downfield shifts of the carbon signals: C-2a(6a) and C-3a(5a) of **1** were observed lower (Δ 1.1 and 0.3) than those of **2** and C-7a of **2** was observed lower (Δ 2.2) than that of **1**. The downfield inductive effect of an epoxyl group could be used as a tool for the positional determination of neighboring substituents in resveratrol oligomers.

Cotylelophenol F (**3**) was obtained as an orange solid. The molecular formula of $C_{35}H_{26}O_9$ was established from the HR–FAB–MS spectrum ($[M-H]^-$ ion at *m/z* 589.1509). The IR spectrum (1697 cm^{-1}) and ^{13}C NMR spectrum (δ_C 195.2) showed the presence of an α,β-unsaturated carbonyl group (C-1a) in the molecule. The presence of five oxygenated aromatic rings (rings A, B_1, B_2, C_1, and C_2) and a sequence of mutually coupled aliphatic protons (H-7b/H-8b/H-7c/H-8c) were exhibited from the analysis of ^1H and ^{13}C NMR spectral data of **3** (Table 3). In addition, the NMR spectral data disclosed the presence of seven phenolic hydroxyl groups (δ_H 7.83–8.77) and a chelated hydroxyl group (δ_H 13.60). Analysis of ^3J long-range correlations in the HMBC spectrum (Table 3) confirmed the connection of the partial structures to be **3**. Although the C–C bond (C-1a–C-10b) was not established from the data analysis, the connection is apparent, based on the molecular formula and the presence of the chelated hydroxyl group.

Table 2. HMBC and NOESY Spectral Data of **1** and **2**

psosition	**1**		**2**	
	HMBC	NOESY	HMBC	NOESY
2a(6a)	4a, 7a	14a, 7a	4a, 7a	14a, 7a
3a(5a)	1a, 4a		1a, 4a	
7a	1a, 2a(6a)	2a(6a), 14a, 2b(6b)	1a, 2a(6a)	2a(6a), 14a
12a	10a, 11a, 13a, 14a		10a, 11a, 13a, 14a	
14a	8a, 10a, 12a, 13a	2a(6a), 7a	8a, 10a, 12a, 13a	2a(6a), 7a
2b(6b)	4b, 7b	7a, 7b, 8c	4b, 7b	7b
3b(5b)	1b, 4b		1b, 4b	
7b	10a, 11a, 1b, 2b(6b), 8b, 9b, 7c	2b(6b), 2c(6c)	10a, 11a, 1b, 2b(6b), 8b, 9b	2b(6b), 2c(6c)
8b	1b, 7b, 9b, 14b, 8c		1b, 9b, 14b, 8c	2c(6c)
12b	10b, 11b, 14b		10b, 11b, 14b	
2c(6c)	4c, 7c	7b, 7c, 8c, 10c(14c)	4c, 7c	7b, 8b, 7c, 8c, 10c(14c)
3c(5c)	1c, 4c		1c, 4c	
7c	7b, 8b, 14b, 1c, 2c(6c), 8c, 9c	2c(6c), 10c(14c)	7b, 8b, 14b, 1c, 2c(6c), 8c, 9c	2c(6c), 10c(14c)
8c	9b, 14b, 1c, 7c, 9c, 10c(14c)	2b(6b), 2c(6c), 10c(14c)	9b, 14b, 1c, 7c, 9c, 10c(14c)	2c(6c), 10c(14c)
10c(14c)	8c, 11c(13c), 12c	2c(6c), 7c, 8c	8c, 11c(13c), 12c	2c(6c), 7c, 8c
12c	10c(14c), 11c(13c)		10c(14c), 11c(13c)	

The NOESY spectrum (Table 3) well explained the stereostructure of **3**, where the four-methine sequence

(H-7b–H-8c) was situated in a similar manner as in **1** and **2**. Previously, we reported the presence of resveratrol trimers, cotylelophenols A and B, which could be derived from pauciflorol A.[4] We suggested the plausible biogenetic pathways between them based on their co-occurrence and stereochemical similarity. Moreover, the present isolates (**1–3**) bear similarity in the partial structure drawn as **E** (Figure 4), which involves the four-methine sequence (H-7b–H-8c), and adds a further biogenetic relationship between them.

Thin layer chromatography of the crude fraction did not show the presence of **3**. When **1** and **2** were left dissolved in acetone-d_6 in a NMR tube at room temperature (rt), the spectrum gradually showed the production of **3** and *p*-hydroxybenzaldehyde. Cotylelophenol F (**3**) was considered an artifact.

Table 3 1D and 2D NMR Data for Cotylelophenol F (**3**)[a]

position	δH	δc	HMBC	NOESY
1a		195.2		
2a		140.4		
3a		127.8		
4a (OH)	8.51 (br. *s*)	155.2	3a, 4a, 5a	5a, 7b
5a	6.61 (*d*, *J* = 2.6)	108.0	3a, 4a, 6a, 7a	4a-OH, 6a-OH
6a (OH)	8.36 (br. *s*)	156.5	5a, 6a, 7a	5a, 7a
7a	7.40 (*d*, *J* = 2.6)	110.8	1a, 3a, 5a	6a-OH
1b		132.0		
2b(6b)	6.44 (*d*, *J* = 8.6)	131.4	4b, 7b	7b
3b(5b)	6.31 (*d*, *J* = 8.6)	114.4	1b, 4b	4b-OH
4b (OH)	7.83 (br. *s*)	155.7	3b(5b), 4b	3b(5b)
7b	4.93 (*d*, *J* = 2.6)	40.6	2a, 3a, 4a, 2b(6b), 8b, 9b, 7c	4a-OH, 2b(6b), 2c(6c)
8b	4.46 (*dd*, *J* = 9.3, 2.6)	54.9	3a, 1b, 9b, 10b, 14b, 8c	2c(6c)
9b		149.6		
10b		112.6		
11b (OH)	13.60 (*s*)	167.4	10b, 11b, 12b	12b
12b	6.28 (*s*)	102.6	10b, 11b, 13b, 14b	11b-OH, 13b-OH
13b (OH)	8.77 (br. *s*)	161.7	12b, 13b, 14b	12b
14b		124.5		
1c		131.5		
2c(6c)	7.12 (*d*, *J* = 8.6)	130.9	4c, 7c	7b, 8b, 7c, 8c, 10c(14c)
3c(5c)	6.68 (*d*, *J* = 8.6)	115.3	1c, 4c	4c-OH
4c (OH)	8.09 (br. *s*)	156.5	3c(5c), 4c	3c(5c)
7c	3.91 (*dd*, *J* = 9.3, 7.3)	58.9	9b, 14b, 1c, 9c	2c(6c), 10c(14c)
8c	4.30 (*d*, *J* = 7.3)	52.4	9b, 13b, 14b, 1c, 9c, 10c(14c)	2c(6c), 10c(14c)
9c		147.8		
10c(14c)	6.18 (*d*, *J* = 2.0)	106.8	8c, 11c(13c), 12c	2c(6c), 7c, 8c, 11c(13c)-OH
11c(13c) (OH)	7.95 (br. *s*)	159.1	10c(14c), 11c(13c), 12c	10c(14c), 12c
12c	6.12 (*t*, *J* = 2.0)	101.4	10c(14c), 11c(13c)	11c(13c)-OH

a : Measured in CD_3COCD_3 at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR); δ in ppm, J in Hz.

Cotylelophenol F (**3**) has a rare skeleton of a dibenzo[*a*,*d*]cyclohepten-5-one system. The other examples include hemsleyanol E[5] isolated from *Shorea hemsleyana* and parviflorol[6] from *Hopea parviflora*. When configurational similarities between them are considered, they could be products generated from (+)-balanocarpol[5] and hemsleyanol A,[7] respectively.

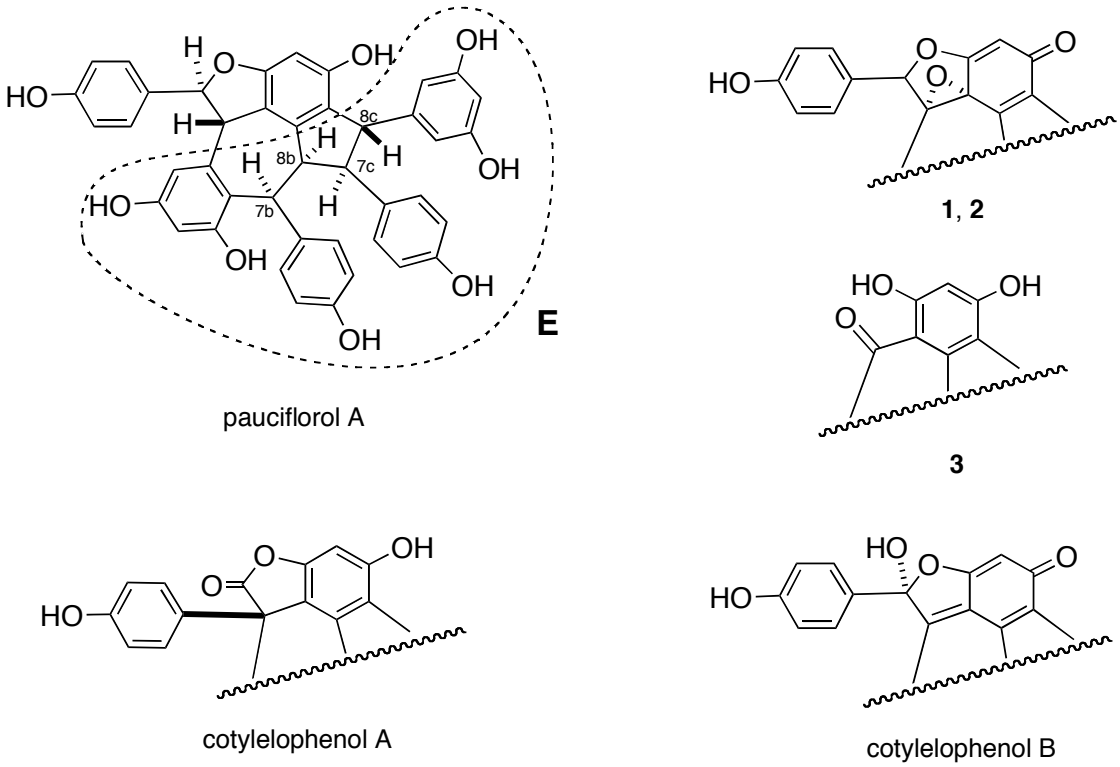
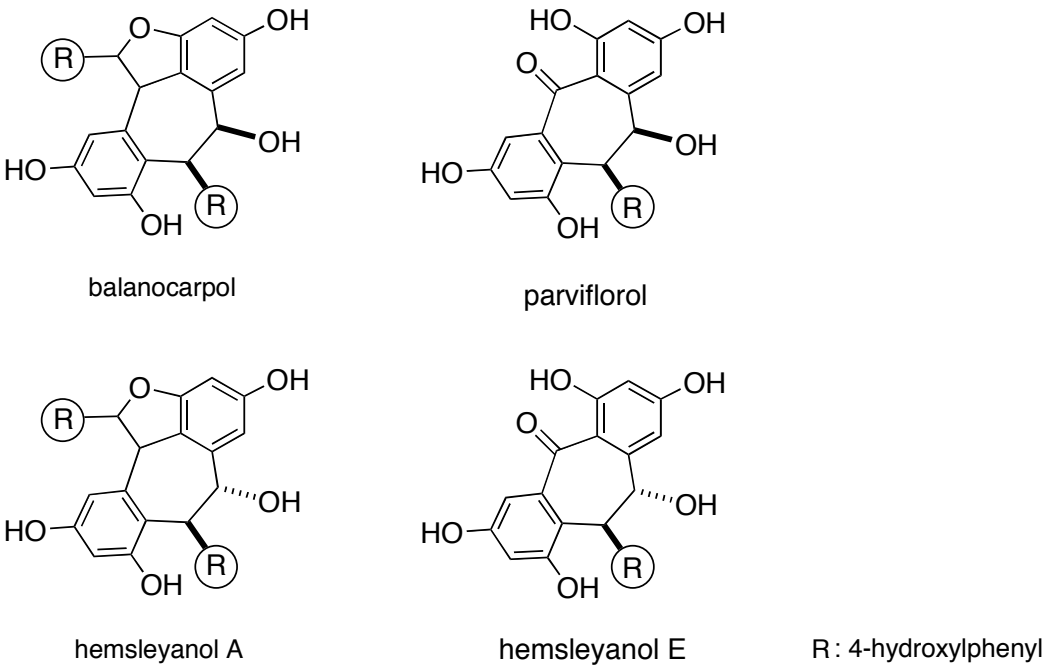
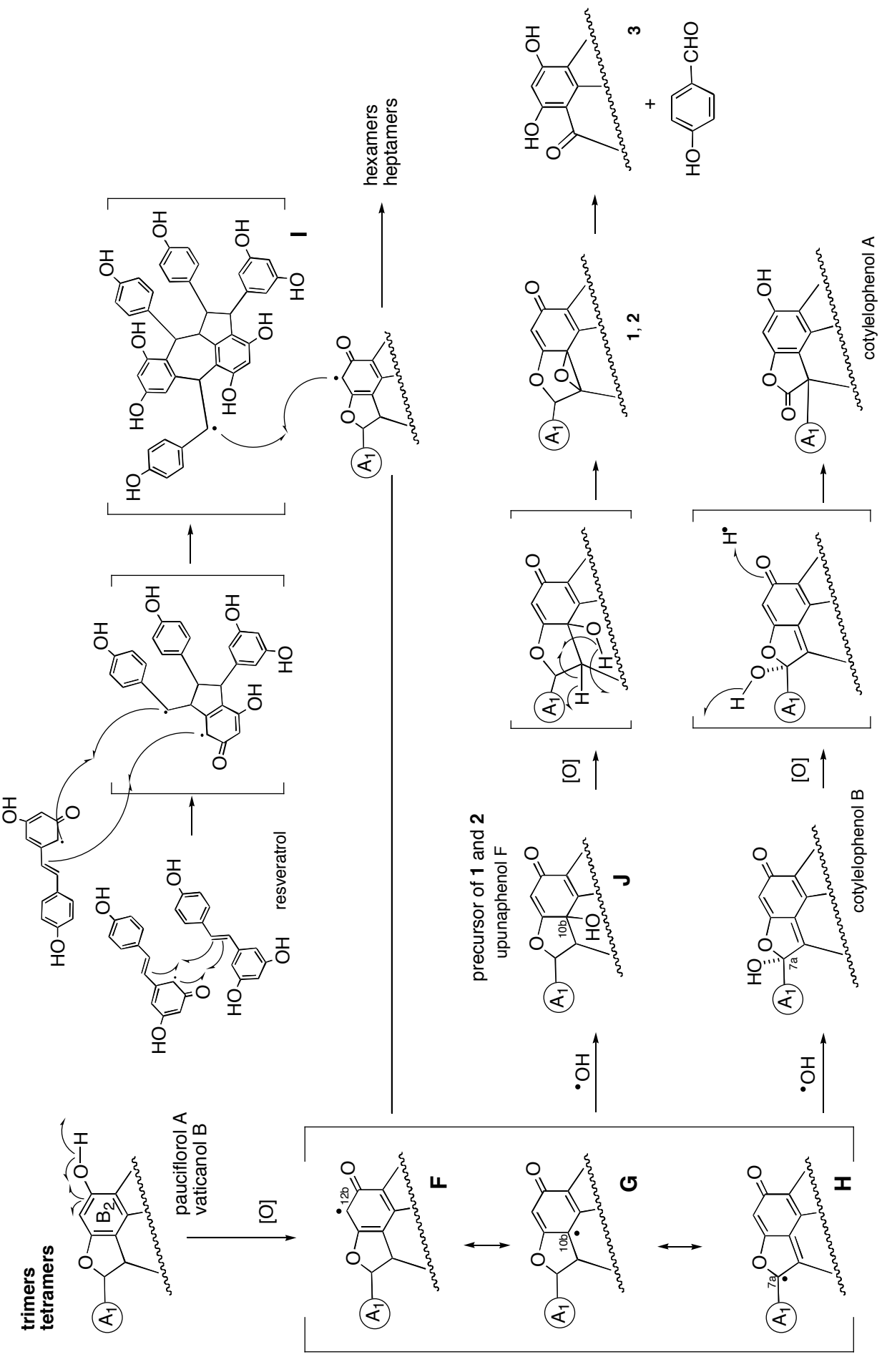


Figure 4 Structural similarity between pauciflorol A, cotylelophenols A, B, D (**1**) – F (**3**)





Scheme Plausible biogenetic pathways of resveratrol oligomers

The resveratrol oligomers are produced by successive oxidative couplings of a resveratrol. The explanation for the relationship between the isolates in the biogenetic pathway requires the definition of a radical precursor. A common intermediate is essential when the relationship between the structurally related isolates is considered. An example is a series of highly conjugated resveratrol oligomers, hexamers (vaticanols D, H and I) and a heptamer (vaticanol J),[2,8] all of which have a common trimeric unit derived from radical (**I**) (Scheme, designated as **E** in the literature 2). As a result, they have identical structure in each partial structure.

When the relationship between the resveratrol trimeric derivatives, cotylelophenols A, B, D, and E, and the artifact derivative, cotylelophenol F, is considered in the same way, pauciflorol A can be regarded as their common precursor by consideration of the stereostructural similarity in the methine sequence (Scheme). This would result in the generation of trimeric radicals (**F**–**H**), which would react individually. Their difference is in the position of each radical, where **F**, **G,** and **H** bear each radical on positions C-12b, C-10b and C-7a, respectively. Radical (**G**) reacts with a hydroxyl radical to afford a precursor (**J**) and then generates **1** and **2** after oxidation. The reductive elimination of the C_6–C_1 unit (ring A_1–C-7a) from **1** and **2** would give **3**. In the case of trimers (**1** and **2**), **J** is the hypothetical precursor and is not isolated from any of the materials. Conversely, the corresponding tetramer exists as an isolate, upunaphenol F, from *Upuna borneensis*.[3] Upunaphenol F can be produced from vaticanol B because all their stereo centers are identical. Radical (**H**) reacts with a hydroxyl radical to form cotylelophenol B and then generates cotylelophenol A after an intra-molecular oxidation–reduction reaction together with a rearrangement of the ring A_1 from C-7a to C-8a. Radical (**F**) would give a series of highly conjugated oligomers by coupling with **I**.

In the current context, only one trimeric precursor, pauciflorol A, and the radical products have been discussed. In fact, various stereoisomers exist in Dipterocarpaceaeous plants. For example, the trimers, vaticanols A and E, and pauciflorols B, were reported.[4] They would all generate radicals in the same manner and generate related derivatives, which would add further variation to the resveratrol oligomers.

EXPERIMENTAL

General method

The following instruments were used: FAB–MS spectra, JEOL JMS-DX-300 instrument; ^{1}H and ^{13}C NMR spectra, JEOL JNM LA-300 (TMS as internal standard); UV spectra, Shimadzu UV-2200 spectrophotometer (in MeOH); IR spectra, JASCO FT-IR 410 spectrometer (as KBr pellet); optical rotations, JASCO P-1020 polarimeter (in MeOH). The following adsorbents were used for purification: analytical and preparative TLC, Merck Kieselgel 60 F_{254} (0.25 mm), column chromatography, Pharmacia Fine Chemicals AB Sephadex LH-20 and Fuji Silysia Chemical Chromatorex.

Plant material

Cotylelobium lanceolatum, identified by one of the co-authors (D.D.), was cultivated at Bogor Botanical Garden, Bogor, Indonesia, where several leaves were collected in May 2000. A voucher specimen has been deposited in Gifu Prefectural Institute of Health and Environmental Sciences, Gifu, Japan.

Extraction and isolation

The dried and ground stem of *C. lanceolatum* (800 g) was successively extracted with acetone (3 L, 3×), MeOH (3 L, 3×) and 70% MeOH (3 L, 2×) at rt, and the extracts were evaporated: 70 g (acetone), 15 g (MeOH), and 14 g (70% MeOH). A part (65 g) of the acetone extract was subjected to CC (silica gel, $CHCl_3$/MeOH of increasing polarity): *Fractions 1–13*. *Fr. 4* ($CHCl_3$/MeOH 8:1; 1.2 g) was further subjected to CC (Sephadex LH-20*,* MeOH) to give the subfractions *Fr. 4a–4c*. Compounds (**1**) (48 mg), (**2**) (9 mg) and (**3**) (6 mg) were obtained from *Fr. 4c* after purification by CC (reversed-phase, 40% MeOH; then Sephadex LH-20*,* MeOH) and prep. TLC (EtOAc/$CHCl_3$/MeOH/H_2O 15:8:4:1).

Cotylelophenol D (1): An orange solid. $[\alpha]_D^{25}$ + 336° (*c* = 0.1, MeOH); UV λ_{max} (MeOH) (nm (log ε)): 300 (3.99), 271 (4.35), 225 (sh, 4.66), 212 (4.74); IR ν_{max} (KBr) 3310, 1670, 1613, 1595, 1514, 1455, 1363, 1241; Negative ion HR–FAB–MS: $[M–H]^-$ *m/z* 693.1768 (Calcd 693.1760 for $C_{42}H_{29}O_{10}$); Negative ion FAB–MS: $[M–H]^-$ *m/z* 693; The ^{1}H and ^{13}C NMR spectral data are listed in Table 1.

Cotylelophenol E (2): An orange solid. $[\alpha]_D^{25}$ + 389° (*c* = 0.1, MeOH); UV λ_{max} (MeOH) (nm (log ε)): 295 (4.10), 264 (4.32), 225 (sh, 4.69), 212 (4.77); IR ν_{max} (KBr) 3311, 1670, 1613, 1595, 1514, 1456,

1370, 1243; Negative ion HR–FAB–MS: $[M–H]^-$ *m/z* 693.1765 (Calcd 693.1760 for $C_{42}H_{29}O_{10}$); Negative ion FAB–MS: $[M–H]^-$ *m/z* 693; The ^1H and ^{13}C NMR spectral data are listed in Table 1.

Cotylelophenol F (3): An orange solid. $[\alpha]_D^{25}$ + 323° (*c* = 0.1, MeOH); UV λ_{max} (MeOH) (nm (log ε)): 319 (4.10), 283 (4.05), 225 (sh, 4.68), 214 (4.75); IR ν_{max} (KBr) 3336, 1697, 1608, 1513, 1456, 1315, 1235; Negative ion HR–FAB–MS: $[M–H]^-$ *m/z* 589.1509 (Calcd 589.1498 for $C_{35}H_{25}O_9$); Negative ion FAB–MS: $[M–H]^-$ *m/z* 589; The ^1H and ^{13}C NMR spectral data are listed in Table 3.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. Y. Doke of Gifu Prefectural Institute of Industrial Product Technology for technical support with NMR spectra during the course of these studies. They also express their appreciation to Mrs. M. Hosokawa and Mrs. M. Hayashi of Gifu Pharmaceutical University for measurement of FAB–MS spectra.

REFERENCES

1. T. Ito, Z. Ali, M. Furusawa, I. Iliya, T. Tanaka, K. Nakaya, J. Murata, D. Darnaedi, and M. Iinuma, *Chem. Pharm. Bull.*, 2006, **54**, 363.
2. T. Ito, Z. Ali, M. Furusawa, I. Iliya, T. Tanaka, K. Nakaya, J. Murata, D. Darnaedi, M. Oyama, and M. Iinuma, *Chem. Biodivers.*, 2005, **2**, 1200.
3. T. Ito, I. Iliya, T. Tanaka, K. Nakaya, Y. Akao, Y. Nozawa, J. Murata, D. Darnaedi, and M. Iinuma, *Heterocycles*, 2005, **65**, 173.
4. T. Ito, T. Tanaka, M. Iinuma, I. Iliya, K. Nakaya, Z. Ali, Y. Takahashi, R. Sawa, Y. Shirataki, J. Murata, and D. Darnaedi, *Tetrahedron*, 2003, **59**, 5347.
5. T. Tanaka, T. Ito, Y. Ido, T.-K. Son, K. Nakaya, M. Iinuma, M. Ohyama, and V. Chelladurai, *Phytochemistry*, 2000, **53**, 1015.
6. T. Tanaka, T. Ito, K. Nakaya, M. Iinuma, Y. Takahashi, H. Naganawa, and S. Riswan, *Heterocycles,* 2001, **55**, 729.

7. T. Ito, T. Tanaka, Y. Ido, K. Nakaya, M. Iinuma, and S. Riswan, *Chem. Pharm. Bull.*, 2000, **48**, 1001.

8. T. Ito, T. Tanaka, K. Nakaya, M. Iinuma, Y, Takahashi, H. Naganawa, M. Ohyama, Y. Nakanishi, K. F. Bastow, and K.-H. Lee, *Tetrahedron*, 2001, **57**, 7309.

9. M. Jang, L. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C. W. W. Beecher, H. H. S. Fong, N. R. Farnsworth, A. D. Kinghorn, R. G. Mehta, R. C. Moon, and J. M. Pezzuto, *Science*, 1997, **275**, 218.

10. M. T. Borra, B. C. Smith, and J. M. Denu, *J. Biol. Chem.*, 2005, **280**, 17187.