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Grandione, a New Heptacyclic Dimeric Diterpene from *Torreya grandis* Fort.

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Abstract: A new dimeric diterpenoid, grandione (**1**), has been isolated from *Torreya grandis* Fort. and its structure elucidated by spectroscopic and chemical analysis. Grandione is a heptacyclic rearranged abietane-type dimer. Dynamic HPLC (DHPLC) studies showed the presence of a tautomeric equilibrium in solution between **1** and its enolic form **1a**.

Dedicated to the memory of Prof. Giacomo Randazzo

Torreya grandis Fort. (Taxaceae), ornamental plant common in China and Japan,[1] is a large sized evergreen coniferous tree with dioecious flowers and drupe-like fruits with nut-seeds used as food in Japan. Our investigation regarded the chemical composition of the hard yellow wood of this tree. This resulted in the isolation of a new dimeric diterpene, grandione (**1**), whose structure is based on two rearranged abietane-type monomeric units. Dimeric diterpenes are of rare occurrence in nature,[2,3] most of them apparently arising as the results of a Diels-Alder type condensation of two monomeric moieties.[4] Grandione (**1**) appears to be particularly interesting because the two monomers are linked through two ether linkages, which, to the best of our knowledge, is an unprecedented feature among the dimeric diterpenes. We report herein on the isolation and characterization of this compound, as well as on its existence in a tautomeric equilibrium with the enolic form **1a**.

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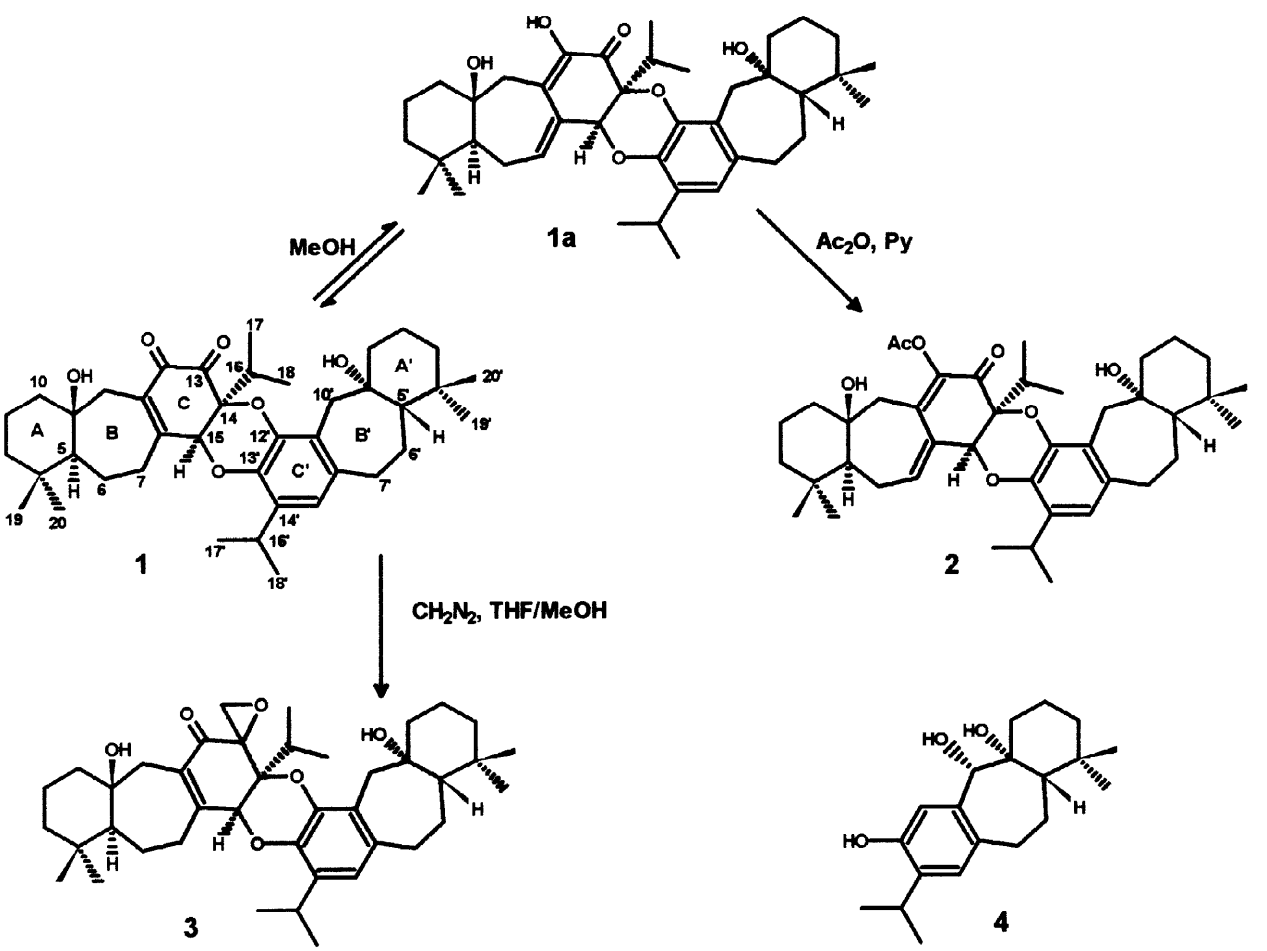
RESULTS AND DISCUSSION

The crude yellow grandione (**1**) (Scheme), isolated from a petroleum ether extract of the wood, was recrystallized from $CHCl_3$ to obtain yellow needle crystals (m.p. 247-248) with an $[\alpha]_D^{25}$ = +75 (c 0.03, $CHCl_3$). The purity of the sample was checked by RP-HPLC (Hypersil ODS, 3 μm, mobile phase MeOH/H_2O 9/1, v/v). HREIMS analysis (molecular ion at m/z 632.3968) gave a molecular composition of **1** as $C_{40}H_{56}O_6$ with 13 formal unsaturation degrees. ^{13}C NMR spectra of **1** contained, in the aliphatic sp^3 region, signals for 8 methyls, 12 methylenes, 5 methines (one of which was linked to oxygen), and 5 quaternary carbons (three of which were linked to oxygen). Furthermore, the low field sp^2 region showed signals for two carbonyls and four C-C double bonds (Table 1). These data confirmed the molecular formula and also indicated the heptacyclic nature of the molecule. The chemical shifts of the two carbonyl functions and the corresponding bands in the IR spectra (Table 1 and Experimental) suggested the two carbonyls to be part of a conjugated α-diketone.[5]



Scheme

Besides the pseudomolecular ion at m/z 633 $[M + H]^+$, the FAB MS of **1** showed peaks at m/z 615 $[M - H_2O + H]^+$ and 597 $[M - 2H_2O + H]^+$ indicating the presence of two free hydroxyl groups, which most likely had to be tertiary, as evidenced by the presence of the above mentioned C-O sp^3 quaternary carbon signals resonating at δ 70.8 and δ 71.0 (C-11' and C-11). This was confirmed by an IR band at 3517 cm^{-1} and by the fact that no acetylation reaction was observed by treatment of **1** with Ac_2O in pyridine (Scheme).

The terpenoidic nature of the compound was mostly deduced by the analysis of ^1H NMR spectrum, which contained the characteristic set of methyl signals. Furthermore, the presence of several related pairs of signals in both ^1H and ^{13}C NMR spectra together with the C-40 nature of the compound, led us to hypothesize a dimeric diterpenoid skeleton. This was also indicated by the LREIMS in which fragments at m/z 316 and 298 (see Experimental) related to the presence of a monomeric unit. The presence of only eight methyl signals (four singlets and four doublets in the ^1H NMR spectrum), and of two similar uncoupled diastereotopic methylene signals, were indicative of rearranged monomeric diterpene skeletons, in which one of the original methyl groups had been included in a ring between a pair of quaternary carbons. A detailed analysis of NMR data (Table 1) indicated the presence of two similar *gem*-dimethyl cyclohexane rings (A and A'), as depicted in formula, commonly found in diterpene compounds. The partial structures were then extended to the seven-membered rings (B and B') on the basis of detailed analysis of ^1H-^1H COSY and COLOC experiments. Long-range heterocorrelations reported in Table 2 allowed us to establish the A-B and A'-B' junctions and also to locate the two *gem*-dimethyls at C-4 and C-4' and the two tertiary hydroxyl groups at C-11 and C-11'. At this point a rearranged abietane-type diterpenoid skeleton could be hypothesized for each of the two monomeric units and thus the remaining part of the molecule (rings C and C') were assembled as shown in formula according to the remaining data in the ^1H-^1H COSY and COLOC spectra. Most relevant for one moiety were long-range couplings of H-10 with C-8 as well as C-9 and C-12, while H-15 correlated with C-8, as well as C-9, C-13 and C-14. On the other moiety H-10' gave a cross peak with C-12', H-15' correlated with C-13' and C-16', while H-17' and H-18' were both coupled with C-14'. These data complete the two monomeric units that must be linked to each other through C-14/C-15 and C-12'/C-13'. An analogous diterpene carbon skeleton has been first found in pisiferdiol (**4**, Scheme) isolated from *Chamaecypers pisifera*.[6]

Since no correlation data could be detected between the two monomeric units, further data required for structural characterization were acquired through a heteronuclear single frequency gated decoupling experiment. Irradiation at the frequency of the methine proton 15 (δ 4.60) transformed the broad double doublet (J 3.5 and 6.5 Hz) at δ 137.8 (C-13') into a sharp double doublet, due to the removal of the small *J* coupling between H-15 and C-13'. This coupling, most likely, was too small to be observed in the COLOC experiment. On the basis of this evidence we propose the linkage between the monomeric moieties as depicted in formula.

The relative configuration at the stereogenic centres C-5, C-11, C-5', C-11' was established by comparison with the reported NMR spectral data of pisiferdiol[6] and on the basis of a nOe among H-5 and H-7α

(δ 2.70) and H-10α (δ 2.30). The relative configuration at C-14 and C-15 was determined by nOe difference experiments. Irradiation at the frequency of methyls 17 and 18 and of the methine proton 16 caused enhancement of the H-15 proton signal. Finally nOe effects observed among H-7α and the isopropyl methyl groups CH_3-17 and CH_3-18, and between H-7β and H-15 allowed us to define tne relative stereostructure of the grandione skeleton as depicted in formula **1** (Scheme).

In a protic solvent grandione (**1**) gives rise to a tautomeric equilibrium with **1a**. Evidence on this matter was obtained chemically. Reaction with acetic anhydride after a pre-equilibration period (Experimental) afforded a single compound (**2**, Scheme) and tautomeric equilibrium, as expected, was no longer present. ^1H and ^{13}C NMR data for compound **2** are reported in Table 1 and 2. Reaction of **1** with CH_2N_2 also gave a single compound (**3**, Scheme), identified by spectroscopic data (see Experimental) as an oxirane derivative obtained through the insertion of a methylene group on the carbonyl at C-13. In addition, the equilibrium between **1** and **1a** was studied by high speed HPLC in free solution (off-column enolization) and by dynamic HPLC (DHPLC, on-column interconversion).

High-speed reverse phase chromatography (Hypersil ODS 3 μm, 50×4 mm ID column, MeOH/water 90/10 v/v eluent) of freshly prepared MeOH solutions of **1** clearly showed the presence of two species, that were identified as the keto and the enol forms (1^{st} and 2^{nd} eluted, respectively) by comparison of their on-line diode-array recorded UV spectra with those of pure **1** and **1a** (see Experimental). Moreover, the elution order observed on the ODS phase indicates that **1** is more polar than **1a**, a finding consistent with the known greater polarity of the diketo form of 1,3-diones compared to the intramolecularly H-bonded enol form.[7,8,9,10]

HPLC analysis of THF solutions of **1** showed the equilibrium to be shifted completely towards the keto form, the second peak being undetectable even after prolonged time at room temperature (Fig. 1); no additional peaks due to hemiacetal, acetal or hydrate formation were observed for MeOH or MeOH/water solutions of **1**. The existence of keto-enol isomerization during the HPLC separation process (on-column interconversion) was proved by dynamic-HPLC in the form of variable flow and variable temperature chromatography.[11] Changes in the eluent flow rate (ϕ) or in the column temperature (T_c) greatly affect the shape of the chromatographic peaks when the separation and interconversion processes occur at comparable rates: a set of peak coalescence-decoalescence phenomena and peak distortions are seen in chromatograms recorded at different ϕ or T_c. A slow exchange regime was found for **1/1a** at ϕ =1.5 ml/min and T_c = 25 °C, with two distinct, well resolved peaks for the two species. An intermediate exchange regime was observed at T_c = 55 °C and ϕ in the range 0.2-1.4 ml/min: under these conditions the two peaks, still recognizable in the *fast* HPLC chromatograms (ϕ between 1.4 and 1.0 ml/min) progressively coalesce and eventually collapse in a single broad peak at ϕ = 0.2 ml/min (Fig. 2). Similar peak deformations were observed at constant ϕ and variable T_c: the fast exchange regime, where a single, sharp averaged peak is present in the chromatogram was observed at T_c around 80 °C and ϕ = 1.0 ml/min.

Table 1. ^{13}C and ^{1}H NMR data of compounds **1** and **2**

	1			2		
C	**DEPT**	**^{13}C**	**^{1}H** (mult, *J* in Hz)	**DEPT**	**^{13}C**	**^{1}H** (mult, *J* in Hz)
1	CH_2	42.8	1.65	CH_2	43.5	1.65
2	CH_2	18.9	n.c.	CH_2	18.8	1.43, 1.85
3	CH_2	42.3	1.40	CH_2	42.0	1.35, 1.40
4	C	34.4	=	C	34.1	=
5	CH	58.3	1.30	CH	53.1	1.55
6	CH_2	24.0	1.80, 1.92	CH_2	26.6	2.53
7	CH_2	35.8	2.70, 2.80	CH	139.7	6.48 (bd)
8	C	140.8[a]	=	C	139.9	=
9	C	159.7[a]	=	C	131.4	=
10	CH_2	40.0	2.30, 3.10 (ds, 14.5)	CH_2	45.1	2.38, 2.80 (ds, 14.5)
11	C	71.0	=	C	73.1	=
12	C	186.2	=	C	139.8	=
13	C	191.5	=	C	189.7	=
14	C	86.3	=	C	82.3	=
15	CH	78.1	4.60 (s)	CH	82.2	4.65 (s)
16	CH	30.6	2.60 (m, 7)	CH	31.5	2.32 (m, 7)
17	CH_3	16.1[b]	1.00 (d, 7)	CH_3	16.5[a]	0.98 (d, 7)
18	CH_3	16.8[b]	1.10 (d, 7)	CH_3	17.0[a]	1.11 (d, 7)
19	CH_3	32.2	0.94 (s)	CH_3	21.5	0.88[a] (s)
20	CH_3	21.6	0.94 (s)	CH_3	31.9	0.99 (s)
1'	CH_3	42.6	1.65	CH_3	42.5	1.75
2'	CH_2	18.3	n.c.	CH_2	18.1	1.43, 1.85
3'	CH_2	42.3	1.40	CH_2	42.3	1.40
4'	C	34.4	=	C	34.3	=
5'	CH	58.1	1.30	CH	58.1	1.25
6'	CH_2	24.0	n.c.	CH_2	24.0	1.92
7'	CH_2	36.0	2.70, 2.80	CH_2	35.9	2.60, 2.70
8'	C	136.9	=	C	136.9	=
9'	C	121.5	=	C	120.8	=
10'	CH_2	40.3	2.35, 3.10 (ds, 14.5)	CH_2	40.1	2.36, 3.10 (ds, 14.5)
11'	C	70.8	=	C	70.7	=
12'	C	141.1	=	C	141.0	=
13'	C	137.8	=	C	137.5	=
14'	C	134.8	=	C	134.8	=
15'	CH	119.4	6.60 (s)	CH	118.1	6.55 (s)
16'	CH	27.7	3.27 (m, 7)	CH	27.5	3.25 (m, 7)
17'	CH_3	21.9[c]	1.30 (d, 7)	CH_3	21.8[b]	1.29 (d, 7)
18'	CH_3	22.5[c]	1.30 (d, 7)	CH_3	22.4[b]	1.31 (d, 7)
19'	CH_3	32.1	0.87 (s)	CH_3	21.5	0.92[a] (s)
20'	CH_3	21.6	0.92 (s)	CH_3	32.2	0.97 (s)
-$\underline{C}OOCH_3$				C	170.0	
-$COO\underline{C}H_3$				CH_3	20.3	2.25 (s)

[a,b,c] Signals of each column with the same superscript may be reversed.

Table 2. Long-range carbon-proton correlations (COLOC) of **1** and **2**.

	1	**2**		**1**	**2**
carbon	**protons**	**protons**	**Carbon**	**Protons**	**Protons**
1	10	2, 10	1'	10'	10'
2			2'	3'	
3	19, 20		3'	19', 20'	
4	3, 19, 20		4'	3', 19', 20'	
5	10		5'	10', 19'	
6			6'	17'	7'
7	5, 15		7'	15', 18'	15'
8	10, 15		8'	10'	10'
9	10, 15	10	9'	10', 15'	10', 15'
10			10'		
11		10	11'	10'	10'
12	10	10	12'	10'	10'
13	15	15	13'	15'	15'
14	15		14'	17', 18'	17', 18'
15			15'		
16			16'	15'	
17			17'		
18			18'		
19	5, 20		19'	20'	
20	5, 20		20'	17', 19'	
			-CO-OCH_3		-CO-OCH_3

In protic solvents the equilibrium is shifted towards the enol form; the equilibration reaction is slow enough below room temperature to be followed off-column by high-speed chromatography: thus, a THF solution of **1** was added via syringe to a cooled (0 ± 0.2 °C) reactor containing a solvent and the solution was periodically monitored by very fast (elution time < 3 min) HPLC.

In MeOH/THF 70/30 and MeOH/THF 30/70 the keto-enol interconversion follows first-order reversible kinetics, with enolization rate constants of 0.056 and 0.040 min^{-1}, respectively. The equilibrium constants measured after 10 half lives were 25.2 and 20.7 in the two solvent mixtures: from these figures the reverse ketonization rate constants were determined as 0.0022 and 0.0019 min^{-1}. In MeOH/H_2O 90/10 (the eluent used in the HPLC runs) at 0 ± 0.2 °C we found for the enolization rate constant a value of 0.037 min^{-1} which translates, neglecting entropy effects, to an half-life time at 25 °C for **1** of about 0.8 minutes. When the separation is carried out at T_c = 25 °C and ϕ 1.5 ml/min, the isomerization rate in the mobile phase exceeds the HPLC separation rate, and a single averaged peak should be observed for the two species. The presence of two distinct peaks under these conditions is a clear indication of a retarding effect of the stationary phase on the interconversion reaction. In fact,

as the two interconverting species travel along the column, they reside for a fraction of time in the stationary phase and the rate constants for the tautomerization reaction may differ from those observed in the bulk mobile phase.[11,12] Presumably, the apolar environment offered by the octadecyl chains of the stationary phase, and the reduced availability of protic molecules within it, are responsible for the observed rate depression.

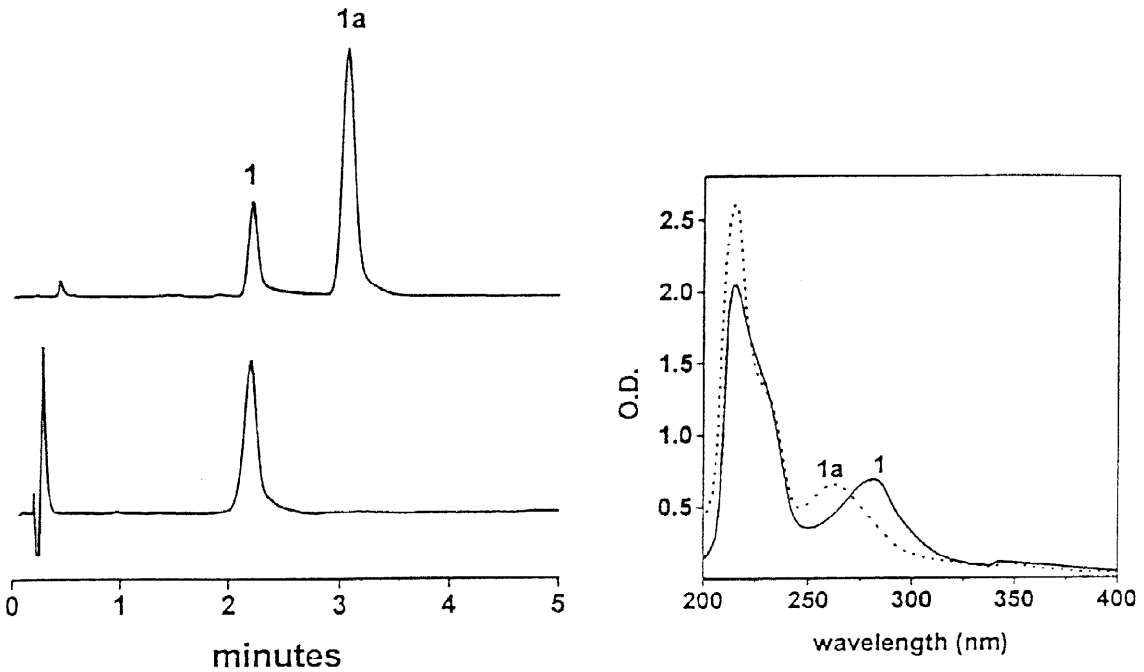


Figure 1. High speed HPLC of 1/1a. Column: 50×4 mm ID packed with Hypersil ODS 3μm; eluent: MeOH/H_2O 90/10 v/v; flow rate: 1.5 ml/min; temperature: 25 °C; UV detection at 210 nm. Bottom trace: THF solution; top trace: freshly prepared MeOH solution. Inset: UV spectra of pure **1** (THF solution) and **1a** (MeOH/THF 90/10).

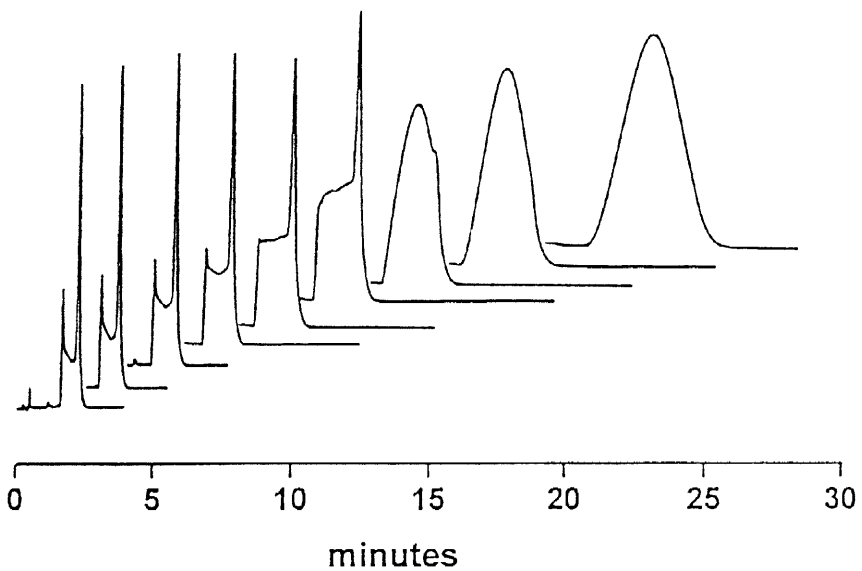


Figure 2. Dynamic HPLC of 1/1a . Column temperature: 55 °C; eluent flow rate, from bottom to top: 1.4, 1.2, 1.0, 0.8, 0.6, 0.5, 0.4, 0.3, 0.2 ml/min. The sample was dissolved and equilibrated in the eluent. Other conditions as in Figure 1.

EXPERIMENTAL

General Experimental Procedures

All NMR measurements were performed in $CDCl_3$ on Bruker spectrometers (AC-400; WM-250). ^1H and ^{13}C chemical shifts were referenced to the residual solvent signals ($\delta_H = 7.26$, $\delta_c = 77.0$). ^1H assignments were confirmed by decoupling, decoupling difference, and ^1H-^1H COSY experiments. The nOe's were determined by difference spectroscopy using a standard Bruker microprogram. The sample used for nOe measurements was previously degassed by gently bubbling Ar through the solution. The multiplicities of ^{13}C resonances were distinguished by DEPT experiments. ^1H-^{13}C connectivities were determined with a 2D HETCOR experiment, optimized for an average $^1J_{CH}$ of 130 Hz. Two and three-bond heteronuclear ^1H-^{13}C connectivities were determined by a 2D COLOC experiment, optimized for $^{2\text{-}3}J_{CH}$ of 6 Hz. Selective heteronuclear decoupling experiments were performed with the single frequency CW heteronuclear decoupling with power gating (SFDEC) microprogram, which is commercially available. Optical rotation was measured on a Perkin-Elmer 243-B polarimeter. Infrared spectrum (KBr) was recorded on a Perkin-Elmer 1600 spectrometer. UV spectra were recorded on a Beckmann DU-70 instrument. Mass spectra were obtained by electron impact on a Fisons VG ProSpec instrument. Melting point, which is uncorrected, was determined using a Bausch & Lomb microscope. HPLC separations were performed on a Waters 2690 Separation Module equipped with a Rheodyne 8125 injector, a Waters 996 photo diode array and Waters 410 refractive index detectors. Thin layer chromatography was carried out on 0.25 mm silica gel coated plates (Merck 60 F_{254}) using UV detection.

Extraction and Isolation

The wood of *Torreya grandis* Fort. was collected in China. Voucher specimens for this collection are maintained for botanical reference at the Institute of Botany, Academia Sinica, People's Republic of China (Prof. Yin Wan-fen). The air-dried material was extracted with petroleum ether. Removal of the solvent under reduced pressure provided an organic extract that was crystallized from $CHCl_3$ to obtain grandione (**1**).

Grandione (**1**): Needle yellow crystals, m.p. 247–248 °C. $[\alpha]_D^{25}$ +75 (*c* 0.03, $CHCl_3$). IR (KBr) ν_{max}: 3517, 2943, 1749, 1675, 1659, 1441, 1368, 1085 cm^{-1}; HREIMS: *m/z* found 632.3968, $C_{40}H_{56}O_6$ requires 632.4077. LREIMS: *m/z* 632 (M^+), 614 $(M-H_2O)^+$, 596 $(M-2H_2O)^+$, 316 $(M-C_{20}H_{28}O_3)^+$, 298 $(M-C_{20}H_{28}O_3-H_2O)^+$. ^1H and ^{13}C NMR spectra: see Table 1 and 2.

Enolization of Grandione (1) Followed by Acetylation to 2

Compound **1** (26 mg, 0.039 mmol) was dissolved in THF (2 ml) and MeOH (20 ml) and the solution stirred at room temp. for 24 h (following the complete transformation in **1a** by HPLC). The mixture was dried and Ac_2O (6 ml) and pyridine (200 µl) were added to the residue. The reaction was stirred at 90 °C for 12 h, and followed by HPLC (LiChrosorb Si60 10 µm, 250×4 mm ID column, CH_2Cl_2/AcOEt 98/2, flow rate 2.0 ml/min, UV detector at 254 nm). The reaction was then acidified (pH = 4) by adding a solution of HCl 0.1 N (10 ml), and a precipitate was observed. The mixture was extracted with $CHCl_3$ using an Extrelux^R (Merck, Darmstadt, Germany) column, and the eluate passed through a second Extrelux previously percolated with HCl 0.1 N. The solution was dried and the residue purified by semipreparative HPLC (Lichrosorb Si60 5µm, 250×10.0 mm ID column, CH_2Cl_2/AcOEt 98/2, flow rate 3.0 ml/min, RI detector) to give compound **2** (yield 98%).

Compound **2**: $[\alpha]_D^{25}$ +73 (*c* 0.03, $CHCl_3$). HREIMS: *m/z* found 674.4122, $C_{42}H_{58}O_7$ requires 674.4182. ^1H and ^{13}C NMR spectra: see **Table 1** and **2**.

Reaction of Grandione with Diazomethane

Compound **1** (43 mg, 0.068 mmol) was dissolved in THF (2.0 ml) and MeOH (10 ml). The equilibration was followed by HPLC (ODS 3μm, 50×4 mm ID column, MeOH/H_2O 9/1, T = 25 °C, flow rate 2.0 ml/min, UV detector at 254 and 220 nm). After 1 h, when **1** was completely converted to **1a**, an ethereal solution of CH_2N_2 (1 ml) (obtained from Diazald^R, Aldrich Chemical Co.) was added to the solution, and the reaction progress monitored by HPLC and TLC (Kieselgel G F_{254} plates, *n*-hexane /AcOEt 7/3). Solvent removal and crystallisation of the solid residue gave compound **3** (yield 96%).

Compound **3**: $[\alpha]_D^{25}$ +62 (*c* 0.03, $CHCl_3$). HREIMS: *m/z* found 646.4181, $C_{41}H_{58}O_6$ requires 646.4233; IR ν_{max}: 2948, 1669 (unsaturated ketone), 1443, 1339 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.63 (2 H, H_2-1), 1.41 and 1.84 (each 1 H, Ha-2 and Hb-2), 1.35 and 1.40 (each 1H, Ha-3 and Hb-3), 1.30 (1H, H-5), 1.80 and 1.91 (each 1 H, Ha-6 and Hb-6), 2.65 and 2.80 (each 1 H, Ha-7 and Hb-7), 2.41 and 3.24 (each 1 H, ds, *J* 14.5 Hz, Ha-10 and Hb-10), 4.43 (1 H, s, H-15), 2.43 (1 H, m, *J* 7 Hz, H-16), 1.20 (3 H, d, *J* 7 Hz, H_3-17), 1.00 (3 H, d, *J* 7 Hz, H_3-18), 0.93 (3 H, s, H_3-19), 0.93 (3 H, s, H_3-20), 2.51 (1 H, d, *J* 7 Hz, Ha-21), 2.62 (1 H, d, *J* 7 Hz, Hb-21), 1.65 (2 H, H_2-1'), 1.42 and 1.85 (each 1 H, Ha-2' and Hb-2'), 1.33 and 1.40 (each 1 H, Ha-3' and Hb-3'), 1.30 (1 H, H-5'), 1.90 and 1.93 (each 1 H, Ha-6' and Hb-6'), 2.65 and 2.80 (each 1 H, Ha-7' and Hb-7'), 2.22 and 3.10 (each 1 H, ds, *J* 14.5 Hz, Ha-10' and Hb10'), 6.62 (1 H, s, H-15'), 3.20 (1H, m, *J* 7 Hz, H-16'), 1.25 (3 H, d, *J* 7 Hz, H_3-17'), 1.21 (3 H, d, *J* 7 Hz, H_3-18'), 0.89 (3 H, s, H_3-19'), 0.93 (3 H, s, H_3-20'). ^{13}C NMR ($CDCl_3$) δ 42.9 (t, C-1), 18.9 (t, C-2), 42.4 (t, C-3), 34.4 (s, C-4), 58.3 (d, C-5), 24.2 (t, C-6), 35.9 (t, C-7), 142.8 (s, C-8), 154.5 (s, C-9), 39.7 (t, C-10), 70.9 (s, C-11), 193.4 (s, C-12), 61.9 (s, C-13), 77.2 (s, C-14), 77.3 (d, C-15), 33.7 (C-16), 17.5 (q, C-17), 17.8 (q, C-18), 21.7 (q, C-19), 32.2 (q, C-20), 49.8 (t, J_{CH} 160 Hz, C-21), 42.6 (t, C-1'), 18.3 (t, C-2'), 42.4 (t, C-3'), 34.4 (s, C-4), 58.1 (d, C-5), 24.2 (t, C-6'), 35.9 (t, C-7'), 121.7 (s, C-8'), 136.5 (s, C-9'), 40.4 (t, C-10'), 70.8 (s, C-11'), 139.6 (s, C-12'), 138.2 (s, C-13'), 134.3 (s, C-14'), 119.4 (d, C-15'), 27.7 (C-16'), 22.0 (q, C-17'), 22.4 (q, C-18'), 21.6 (q, C-19'), 32.2 (q, C-20').

Off-Column Enolization Reactions

UV spectra of the pure **1** and **1a** forms were obtained by dissolution of **1** (1.01×10^{-4} M) in THF and MeOH/THF 90/10, respectively. **1**: λ (ε) 210 (20257), 282 (6842), 342 (1109). **1a**: 210 (25823), 260 (6486), 342 (963). 25 μl of a THF solution of **1** was added via syringe to 1 ml of a cooled (0 ± 0.2 °C) reaction solvent (final concentration 1.6×10^{-3} M) and the composition of the mixture was analyzed by HPLC (Hypersil ODS 3μm, 50×4 mm ID column, MeOH/H_2O 90/10 as eluent, T_c 25 °C, eluent flow rate 1.5 ml/min, UV detection at 210 nm) as a function of time over at least 10 half lives.

The relative amounts of **1** and **1a** were determined by peak area integration at 210 nm; a correction factor was used for the different ε of the two species at that wavelength. Experimental data were fitted to the equation $\ln[(C-C_{eq})/(C-C_0)] = -[(K_{eq}+1)/K_{eq}]k_e\times t$ where C, C_{eq} and C_0 are concentration of **1** at time t, at the equilibrium and the initial concentration, respectively. Rate constants (±5%) are averaged values of three replicate kinetic runs.

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