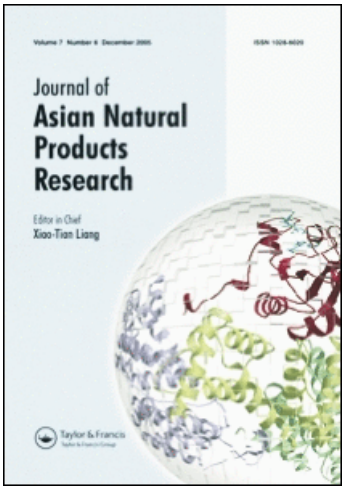

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NEW PRODUCTS FROM ALKALI FUSION OF GINKGOLIDES A AND B

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Alkali fusion of ginkgolides A and B has afforded five unexpected products **3–7**. Their structures were established from their spectral data and chemical reactions. They were evaluated for their *in vitro* activity to inhibit the platelet-activating factor-induced aggregation of rabbit platelets and show less potency than ginkgolides A and B.

Keywords: Ginkgolide analogs; Structure and activity relationship

INTRODUCTION

Platelet-activating factor (PAF) is a potent bioregulator which appears to play a key role in *acute inflammation*, *asthma*, *ischemic injury* and *tissue rejection* through its action at high affinity receptors ($EC_{50} \sim 10^{-10}$ M) [1]. Consequently, the development of PAF antagonists that are suitable for therapeutic use has assumed considerable importance. Among the known types of PAF antagonists, ginkgolides A and B are especially interesting because of its long medical history, its notable lack of toxicity and its total resistance to metabolism. Previously, Corey [2–4] has investigated a range of synthetic analogs to provide insights regarding the structural features of ginkgolides A and B that enhanced anti-PAF activity. In order to obtain

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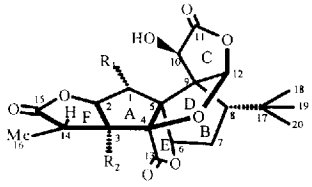
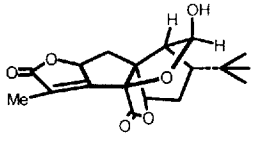
further insights for the structural requirements of the PAF receptor, we prepared new ginkgolide analogs lacking ring C (**3–9**) through alkali fusion and evaluated their *in vitro* activity to inhibit the PAF-induced aggregation of rabbit platelets.

RESULTS AND DISCUSSION

Treatment of ginkgolides A with 50% NaOH (aq.) at 160°C for 30 min furnished four pure compounds (**3–6**) after purification with Sil gel (hexane–acetone) and Sephadex LH-20 (chloroform–acetone) chromatography.



ginkgolide A (**1**) R_1=H, R_2=OH
ginkgolide B (**2**) R_1=R_2=OH



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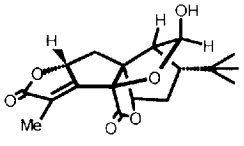

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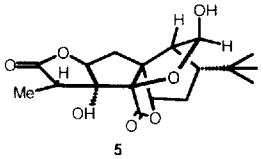

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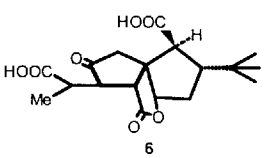

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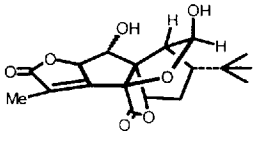

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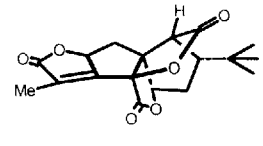

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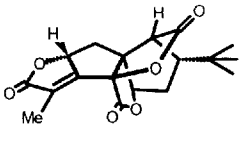

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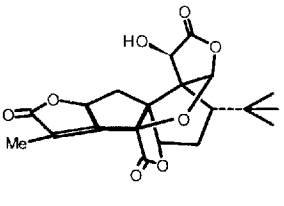

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Compound **3** had the molecular formula $C_{18}H_{22}O_6$, supported by its HREIMS (*m/z* $[M]^+$ 334.1411), indicating the loss of $C_2H_2O_3$ as compared with ginkgolide A (**1**) ($C_{20}H_{24}O_9$). The IR spectrum showed two lactonic carbonyl absorption at 1770 and 1760 cm^{-1}. Comparison of the NMR data of compound **3** with those of ginkgolide A showed that it was a mono-anhydro and lacking ring C ginkgolide analog, formed by loss of a two-carbon unit, which was identified in the form of oxalic aldehyde, from ginkgolide A. The assignment and connectivities of protons were derived from an ^1H–^1H COSY experiment. The singlet at δ 5.57 ppm (H-12) and the doublet at δ 2.33 ppm (H-9) indicated the dihedral angle between

H_{12}–C_{12}–C_9–H_9 is about 90°, namely, the absolute configuration at C_{12} is *S* as shown in **3**. This was confirmed by absence of a NOE between H-9 and H-12 in a 2D NOESY spectrum.

HREIMS (*m/z* $[M]^+$ 334.1411) of compound **4** showed that it had the same molecular formula ($C_{18}H_{22}O_6$) as compound **3**. Its NMR spectra were very similar to those of **3**. The only obvious difference was that the signal at δ 5.75 ppm (m, H-2) was located at lower field than the signal at δ 5.51 ppm (s, H-12). There were three positions that might be isomerized at C_{12}, C_2, and C_6 under these reactive conditions. The singlet at δ 5.51 ppm (H-12) and the doublet at δ 2.45 ppm (H-9) showed the *trans* arrangement between H-9 and H-12, namely, the absolute configuration at C_{12} is *S* as shown in **4**, and this was confirmed by two reactions. In one of the reactions, the compound **3** was stirred at room temperature for a week under acid condition and no other products were formed, thus this indicated that the hemiacetal structure at C_{12} was stable. In the other reaction, compounds **3** and **4** were oxidized to **8** and **9** which were different. These results indicated that compounds **3** and **4** were not isomerized at C_{12}. If isomerized at C_6, it was impossible to form the lactonic ring E in view of the characteristic backbone formed by rings A, B, and D. So it was only isomerized at C_2, which was confirmed by the 2D NOESY spectrum. NOES were observed between H-9 and H-1β, and between H-1β and H-2. The formation of **4** can be explained by the plausible mechanism proposed in Fig. 1.

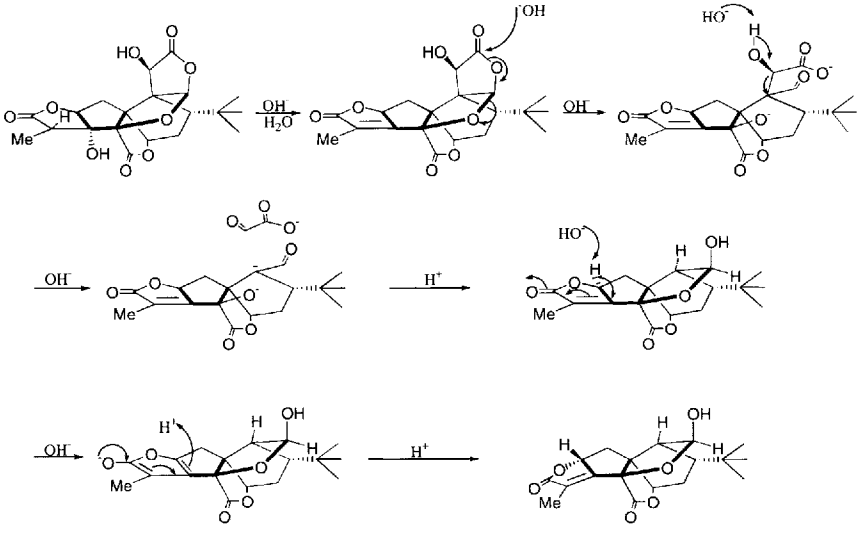
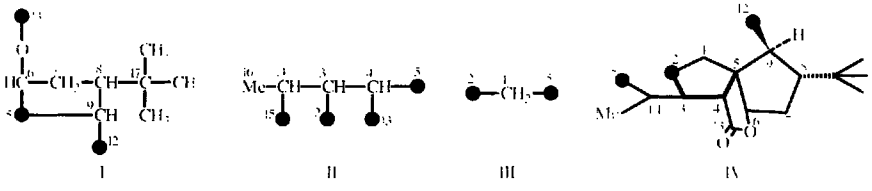


FIGURE 1 The plausible mechanism of formation of compound **4**.

Compound **5** gave $[M]^+$ *m/z* 352.1520 in the HREIMS, corresponding to the molecular formula of $C_{18}H_{24}O_7$. Comparison of its NMR data with those of compound **3** showed it retained 3-OH as shown in **5**.

Compound **6** was isolated as colorless crystals. The HREIMS spectrum showed $[M]^+$ 352.1506, which indicated the molecular formula of $C_{18}H_{24}O_7$. The IR spectrum indicated the presence of a carboxylic group (3000–2500 and 1760 cm^{-1}). The characteristic downfield proton signals for H-2 and H-12 were absent and only the signal for H-6 conserved, which showed the backbone of **6** had been much changed. There were a carbonyl carbon signal (δ 213.7 ppm, s) and three lactonic or carboxylic carbonyl carbon signals (δ 175.0, 175.0, 176.0 ppm, s). The ^1H–^1H COSY and HMQC spectra defined three structural fragments as shown in I, II, and III. According to the backbone of C-nor-ginkgolide analogs lacking ring C, we could deduce the possible partial structure IV ($C_{15}H_{22}O_3$). Based on the above three fragments, $C_3H_2O_4$, namely, –COOH × 2 and –CO × 1, was obtained from the molecular formula $C_{18}H_{24}O_7$ minus part structure IV ($C_{15}H_{22}O_3$). Therefore the planar structure of **6** was established as shown in **6**. The α configurations for H-3 and H-9 were established from a 2D NOESY spectrum. NOEs were observed between H-9 and *t*-Bu, H-4 and H-1β, H-1α and H-3. The configuration for H-14 has not been determined.



I II III IV

Treatment of ginkgolide B with 50% NaOH (aq.) at 160°C for 30 min provided a major compound **7**. The molecular formula of $C_{18}H_{22}O_7$ for compound **7** was determined by its HREIMS spectrum (*m/z* $[M]^+$ 350.1362). The chemical shift value (1.89 ppm) and doublet (J = 1.8 Hz) of C_{14}-Me indicated the lactonic ring F was α,β-unsaturated lactone. In its ^1H-NMR spectrum, two upfield proton signals for H-1 were eliminated and replaced by a downfield doublet signal (δ 3.99 ppm, d, J = 8.2 Hz) as compared with **3**, namely there was a hydroxy group substituted at C_1.

Biological Evaluation of Ginkgolide Analogs Lacking Ring C

Compounds **3–9** were evaluated as PAF antagonist *in vitro* using an assay involving rabbit platelets. We observed that ginkgolide analogs lacking ring

TABLE I *In vitro* biological evaluation of C-nor-ginkgolide analogs

Compound	*PAF-induced platelet aggregation* IC_{50}[a] (μM)	*Compound*	*PAF-induced platelet aggregation* IC_{50}[a] (μM)
Ginkgolide A (**1**)	0.410 (0.310–0.557)	**6**	90.2 (76.4–103.3)
Ginkgolide B (**2**)	0.128 (0.102–0.145)	**7**	50.2 (40.9–67.2)
3	86.7 (73.4–90.3)	**8**	80.4 (66.4–95.7)
4	54.0 (30.7–69.5)	**9**	40.9 (29.1–63.7)
5	44.8 (35.1–52.7)	**10**	17.8 (15.4–19.6)

[a]Concentration required to inhibit PAF-induced maximum aggregation by 50%. Parentheses contain 95% confidence limits.

C can drastically modify anti-PAF potency (Table I). Thus, compounds **1**, **10** to **5**, **3** were associated with a noticeable decrease in anti-PAF inhibition (> 100-fold for **5** and 4-fold for **3**). The stereochemistry at C_2 of ginkgolide analogs lacking ring C seems to affect their anti-PAF activities. Thus compounds **4** and **9** were found to be 1.6 and 2.2 times more potent than compounds **3** and **8**, respectively. The replacement of C_{12} hemiacetal group to ester group does not affect their anti-PAF activity.

EXPERIMENTAL SECTION

General Experimental Procedures

IR spectra were measured with a Perkin-Elmer 559B apparatus. NMR spectra were obtained on Bruker AMX-400 and GEMIM-300 spectrometer, using DMSO-d_6 as solvent and TMS as internal standard. Mass spectra were measured on a MAT-711 mass spectrometer. Combustion analyses were performed with a Carlo ErBa 1106 analyzer. C-18-PAF acether was purchased from Sigma Co. Ltd.

Plant Material

Ginkgolides A and B, isolated from Chinese medicinal herb *Ginkgo biloba* L., was used as starting material.

Alkali fusion of ginkgolide A Two grams of ginkgolides A was added to a stirred solution containing 10.0 g of sodium hydroxide in 10 ml of water. The mixture was kept stirring at 160–170°C for 30 min. The reaction mixture was cooled, diluted with H_2O (50 ml), adjusted to pH = 2 with diluted HCl. The product was extracted four times with ethyl acetate (50 ml). The combined organic layers were repeatedly chromatographed on sil gel (cyclohexane–acetone, 2 : 1) and on Sephadex LH-20 (chloroform–acetone, 2 : 1)

to provide compounds **3** (403 mg, 24.6%), **4** (102 mg, 6.2%), **5** (84 mg, 4.9%), **6** (38 mg, 2.2%).

Compound **3** Colorless crystals: IR (KBr) ν_{max} 3530, 3490, 1770, 1760 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 2.72 (1H, dd, J = 11.9, 7.3 Hz, H-1α), 1.77 (1H, dd, J = 11.6, 7.1 Hz, H-1β), 5.49 (1H, m, H-2), 4.96 (1H, d, J = 4.7 Hz, H-6), 1.99 (1H, dd, J = 14.2, 6.8 Hz, H-7β), 1.80 (1H, m, H-7α), 1.68 (1H, m, H-8), 2.33 (1H, d, J = 10.4 Hz, H-9), 5.57 (1H, s, H-12), 1.92 (3H, d, J = 1.9 Hz, H-16), 0.90 (9H, s, *t*-Bu); ^{13}C (DMSO-d$_6$, 100 MHz) δ 39.0 (C-1), 81.0 (C-2), 164.1 (C-3), 91.0 (C-4), 71.6 (C-5), 88.3 (C-6), 35.6 (C-7), 49.8 (C-8), 60.4 (C-9), 108.2 (C-12), 174.3 (C-13), 123.7 (C-14), 171.2 (C-15), 9.1 (C-16), 32.0 (C-17), 28.1 (C-*t*-Bu); EI-HRMS: *m/z* 334.1411, $C_{18}H_{22}O_6$ requires 334.1416; EIMS *m/z* 334 [M]$^+$, 319, 317, 301, 290, 259, 233; *anal.* C 65.18%, H 6.47%; calcd. for $C_{18}H_{22}O_6$, C 64.66%, H 6.63%.

Compound **4** Colorless crystals: IR (KBr) ν_{max} 3530, 3490, 1770, 1760 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 2.67 (1H, dd, J = 12.5, 8.3 Hz, H-1α), 1.89 (1H, m, H-1β), 5.75 (1H, m, H-2), 4.89 (1H, d, J = 3.7 Hz, H-6), 1.98 (1H, m, H-7β), 1.98 (1H, m, H-7α), 1.78 (1H, m, H-8), 2.45 (1H, d, J = 9.6 Hz, H-9), 5.51 (1H, s, H-12), 1.88 (3H, d, J = 2.4 Hz, H-16), 0.90 (9H, s, *t*-Bu); ^{13}C (DMSO-d$_6$, 100 MHz) δ 37.9 (C-1), 83.8 (C-2), 160.0 (C-3), 88.9 (C-4), 72.3 (C-5), 90.8 (C-6), 35.3 (C-7), 52.0 (C-8), 62.8 (C-9), 108.5 (C-12), 175.1 (C-13), 125.1 (C-14), 171.2 (C-15), 9.8 (C-16), 32.3 (C-17), 28.2 (C-*t*-Bu); EI-HRMS: *m/z* 334.1406, $C_{18}H_{22}O_6$ requires 334.1416; EIMS *m/z* 334 [M]$^+$, 316, 288, 243, 205, 188, 111, 57; *anal.* C 65.34%, H 6.53%; calcd. for $C_{18}H_{22}O_6$, C 64.66%, H 6.63%.

Compound **5** Colorless crystals: IR (KBr) ν_{max} 3520, 3430, 1780, 1740 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 2.67 (1H, dd, J = 13.6, 7.2 Hz, H-1α), 1.97 (1H, m, H-1β), 4.78 (1H, m, H-2), 4.73 (1H, d, J = 3.5 Hz, H-6), 1.97 (1H, m, H-7β), 1.75 (1H, ddd, J = 13.5, 5.8, 4.2 Hz, H-7α), 1.65 (1H, m, H-8), 2.23 (1H, d, J = 10.1 Hz, H-9), 5.41 (1H, s, H-12), 3.41 (1H, q, J = 7.1 Hz, H-14), 1.08 (3H, d, J = 7.1 Hz, H-16), 0.87 (9H, s, *t*-Bu); ^{13}C (DMSO-d$_6$, 100 MHz) δ 38.3 (C-1), 86.0 (C-2), 87.2 (C-3), 97.8 (C-4), 67.3 (C-5), 88.7 (C-6), 36.3 (C-7), 50.4 (C-8), 60.4 (C-9), 108.7 (C-12), 172.8 (C-13), 40.4 (C-14), 178.0 (C-15), 8.5 (C-16), 31.9 (C-17), 28.1 (C-*t*-Bu); EI-HRMS: *m/z* 352.1520, $C_{18}H_{24}O_7$ requires 352.1522; EIMS *m/z* 352 [M]$^+$, 334, 316, 295, 288, 223, 187, 168, 57; *anal.* C 62.03%, H 6.43%; calcd. for $C_{18}H_{24}O_7$, C 61.35%, H 6.86%.

Compound **6** Colorless crystals: IR (KBr) ν_{max} 3442–2500, 1747, 1707 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 2.80 (1H, d, J = 13.8 Hz, H-1α), 2.56 (1H, d, J = 13.9 Hz, H-1β), 2.76 (1H, m, H-3), 2.85 (1H, d, J = 9.4 Hz, H-4), 4.76 (1H, d, J = 3.6 Hz, H-6), 2.00 (1H, dd, J = 14.1, 6.0 Hz,

H-7β), 1.74 (1H, ddd, $J = 13.9, 6.0, 4.0$ Hz, H-7α), 2.36 (1H, m, H-8), 2.64 (1H, d, $J = 10.8$ Hz, H-9), 3.06 (1H, m, H-14), 1.19 (3H, d, $J = 4.7$ Hz, H-16), 0.85 (9H, s, *t*-Bu); ^{13}C (DMSO-d_6, 100 MHz) δ 48.0 (C-1), 213.7 (C-2), 54.2 (C-3), 49.2 (C-4), 55.7 (C-5), 88.6 (C-6), 31.9 (C-7), 51.6 (C-8), 54.2 (C-9), 175.0 (C-12), 176.0 (C-13), 39.7 (C-14), 175.0 (C-15), 14.8 (C-16), 31.9 (C-17), 28.0 (C-*t*-Bu); EI-HRMS: *m/z* 352.1506, $C_{18}H_{24}O_7$ requires 352.1522; EIMS *m/z* 352 $[M]^+$, 334, 308, 277, 233, 205, 107, 57; *anal.* C 60.84%, H 6.23%; calcd. for $C_{18}H_{24}O_7$, C 61.35%, H 6.86%.

Compound **7** Alkali fusion of ginkgolide B (500 mg), using the procedure described for the synthesis of **3–6**, which afforded **7** (67 mg, 16.2%). Colorless crystals: IR (KBr) ν_{max} 3444, 1763, 1707 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.99 (1H, d, $J = 8.2$ Hz, H-1β), 5.36 (1H, dd, $J = 8.4, 1.9$ Hz, H-2), 5.25 (1H, d, $J = 4.5$ Hz, H-6), 2.03 (1H, dd, $J = 13.1, 4.7$ Hz, H-7β), 1.62 (1H, m, H-7α), 1.62 (1H, m, H-8), 2.37 (1H, d, $J = 10.0$ Hz, H-9), 5.58 (1H, s, H-12), 1.89 (3H, d, $J = 1.8$ Hz, H-16), 0.90 (9H, s, *t*-Bu); ^{13}C (DMSO-d_6, 100 MHz) δ 77.6 (C-1), 82.8 (C-2), 159.3 (C-3), 91.2 (C-4), 75.3 (C-5), 86.4 (C-6), 36.5 (C-7), 49.5 (C-8), 59.4 (C-9), 108.6 (C-12), 174.8 (C-13), 124.7 (C-14), 171.3 (C-15), 9.4 (C-16), 32.1 (C-17), 28.3 (C-*t*-Bu); EI-HRMS: *m/z* 350.1367, $C_{18}H_{22}O_7$ requires 350.1365; EIMS *m/z* 350 $[M]^+$, 332, 303, 277, 232, 203, 181, 125, 107, 57; *anal.* C 61.04%, H 6.23%; calcd. for $C_{18}H_{22}O_7$, C 61.71%, H 6.33%.

Compound **8** Fifty mg of **3** was dissolved in 2 ml of acetone. To this solution, Jone's reagent (0.1 ml) was added and the mixture was stirred for 8 h at room temperature. Then isopropyl alcohol was added dropwise until the excess chromic acid was destroyed, and the suspension was filtered and the filter cake was washed with 5 ml of acetone. The filtrate was evaporated under reduce pressure. The residue was dissolved with 3 ml of EtOAc and 3 ml of H_2O. The aqueous layer was washed with additional EtOAc (2×3 ml). The combined organic layers were then washed with H_2O and brine, and after drying over sodium sulfate, the solution was filtered and evaporated to yield the crude product which was subjected to sil gel chromatography (cyclohexane : acetone 2 : 1), affording **8** (47.3 mg, 95.2%). Colorless crystals: IR (KBr) ν_{max} 3500, 1797, 1782, 1765 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.70 (1H, dd, $J = 12.2, 7.1$ Hz, H-1α), 1.99 (1H, dd, $J = 11.8, 7.3$ Hz, H-1β), 5.58 (1H, m, H-2), 5.08 (1H, d, $J = 4.5$ Hz, H-6), 2.12 (1H, dd, $J = 14.7, 6.5$ Hz, H-7β), 2.06 (1H, m, H-7α), 1.92 (1H, m, H-8), 3.10 (1H, d, $J = 8.1$ Hz, H-9), 1.94 (3H, d, $J = 1.8$ Hz, H-16), 0.92 (9H, s, *t*-Bu); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 39.1 (C-1), 80.2 (C-2), 159.6 (C-3), 85.7 (C-4), 67.8 (C-5), 88.8 (C-6), 36.3 (C-7), 51.8 (C-8), 53.2 (C-9), 175.9 (C-12), 174.1 (C-13), 126.2 (C-14), 169.0 (C-15), 9.6 (C-16), 32.7 (C-17), 27.9

(C-*t*-Bu); EI-HRMS: *m/z* 332.1267, $C_{18}H_{20}O_6$ requires 332.1260; EIMS *m/z* 332 $[M]^+$, 317, 299, 185, 157, 57; *anal.* C 66.21%, H 6.34%; calcd. for $C_{18}H_{20}O_6$, C 65.05%, H 6.07%.

Compound **9** Oxidation of **4** (50 mg), was carried out with the procedure described for the synthesis of **8**, which afforded **9** (45.2 mg, 90.9%). Colorless crystals: IR (KBr) ν_{max} 3442, 1795, 1778, 1765 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.63 (1H, dd, $J = 12.3, 8.0$ Hz, H-1α), 2.06 (1H, m, H-1β), 5.58 (1H, m, H-2), 5.05 (1H, d, $J = 3.9$ Hz, H-6), 2.30 (1H, dd, $J = 12.3$, 8.0 Hz, H-7β), 1.93 (1H, m, H-7α), 2.06 (1H, m, H-8), 3.35 (1H, d, $J =$ 7.7 Hz, H-9), 1.92 (3H, d, $J = 2.2$ Hz, H-16), 0.93 (9H, s, *t*-Bu); EI-HRMS: *m/z* 332.1253, $C_{18}H_{20}O_6$ requires 332.1260; EIMS *m/z* 332 $[M]^+$, 314, 286, 185, 109, 57; *anal.* C 64.79%, H 6.29%; calcd. for $C_{18}H_{20}O_6$, C 65.05%, H 6.07%.

Biological method: inhibition of platelet aggregation in vitro Platelet aggregation studies were done by the method of Born [5]. Blood was collected in 3.9% sodium citrate (1 vol/9 vol of blood) by cardiac puncture from male New Zealand rabbits (2–2.5 kg body weight). Platelet-rich plasma (PRP) was prepared by centrifuging the blood at 250*g* for 10 min at 4°C. The PRP was diluted with platelet-poor plasma obtained by further centrifuging at 3000*g* for 10 min. The platelet number was adjusted to 3.5×10^5 cells/mm^3. Platelet aggregation was induced by C-18-PAF (1.5×10^{-8} M) and measured with a dual-channel aggreometer Chrono-log 560 instrument. Activity is expressed as the IC_{50} value, i.e., the concentration required to inhibit platelet aggregatory response by 50%. The values shown in the tables were calculated by linear regression from a single experimental curve with no less than four data points, each point being the mean of the percentage inhibition at a given concentration obtained from three independent experiments.

References

[1] D.J. Hanahan. *Ann. Rev. Biochem.* 1986, **55**, 483.
[2] E.J. Corey and A.V. Gavai. *Tetrahedron Lett.* 1989, **30**, 6959–6962.
[3] E.J. Corey and K. Kamiyama. *Tetrahedron Lett.* 1990, **31**, 3995–3998.
[4] E.J. Corey and K.S. Rao. *Tetrahedron Lett.* 1991, **32**, 4623–4626.
[5] G.V.R. Born. *Nature* (London) 1962, **194**, 927–930.