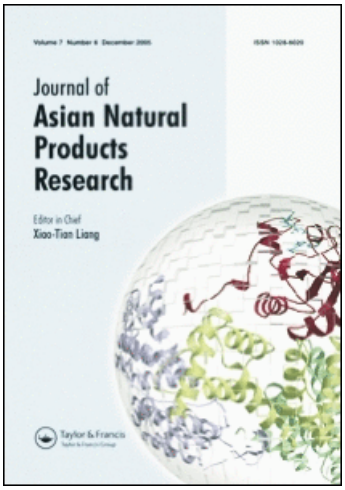

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Guo, Chun-Jun , Ren, Yi , Qin, Yin-Lin and Zhang, Qian(2008) 'The synthesis and biological evaluation of 10-*O*-dialkylaminoethyl ginkgolide B as platelet-activating factor antagonist', Journal of Asian Natural Products Research, 10: 10, 989 — 992
To link to this Article: DOI: 10.1080/10286020802240376
URL: http://dx.doi.org/10.1080/10286020802240376

Journal of Asian Natural Products Research
Vol. 10, No. 10, October 2008, 989–992

The synthesis and biological evaluation of 10-*O*-dialkylaminoethyl ginkgolide B as platelet-activating factor antagonist

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(*Received 27 February 2008; final version received 6 May 2008*)

Four nitrogen-containing derivatives of ginkgolide B were synthesized to improve the physical–chemical properties and bioavailability of ginkgolide B. The reaction was accomplished with the nitrogen atom as neighboring group participating in the replacement reaction. All of the four compounds were proved to have excellent inhibiting effect on rabbit platelet aggregation induced by platelet-activating factor which is as well as ginkgolide B.

Keywords: ginkgolide B; nitrogen-containing groups; anti-PAF; participation of neighboring nitrogen atom

1. Introduction

The chemical structure of ginkgolide B possesses a unique 20-carbon cage molecule incorporating a *tert*-butyl group, in which six five-membered rings A–F consist of a spiro[4.4]nonane system including three lactonic rings and a tetrahydrofuran ring with three hydroxyl groups on C_1, C_3, and C_{10}, respectively (Figure 1) [1]. It is an important component of the extract from the leaves of *Ginkgo biloba* and exhibits the most efficient anti-platelet-activating factor (PAF) activity among all the ginkgolides (including A, B, C, J, and M) [2–4]. In addition, due to its strong activity, low toxicity and long using history, it has been applied to treat cardiovascular disease in clinic.

However, disadvantages such as the poor aqueous solubility and low bioavailability limit the applications of ginkgolide B. Herein we hope to introduce some nitrogen-containing groups into the scaffold of ginkgolide B and those groups can be converted into various salts to improve the physical and chemical properties, especially the solubility and bioavailability of the molecule.

2. Results and discussion

Among the three hydroxyl groups in ginkgolide B, the nucleophilic reactivity of 10-OH is the highest owing to the adjacent carbonyl group. Although Hu and his colleagues [5] had synthesized a series of 1-, or 10-*O*-methylginkgolide B and 10-*O*-benzylginkgolide B using methyl iodide and various substituted benzyl bromides as halogenated reagents, respectively, in our research the reactions could not result in the corresponding products when a weaker reactive alkyl halide, such as ethyl bromide, was used under the same condition.

In this study, we introduced some dialkylaminoethyl groups to ginkgolide B using substituted aminoethyl chlorides as electrophiles to provide four 10-*O*-ethers. This reaction was carried out by refluxing a

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802240376
http://www.informaworld.com

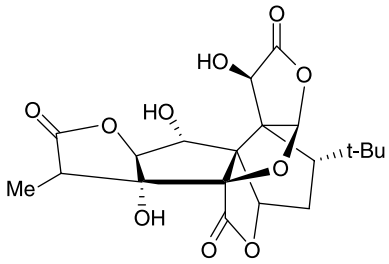
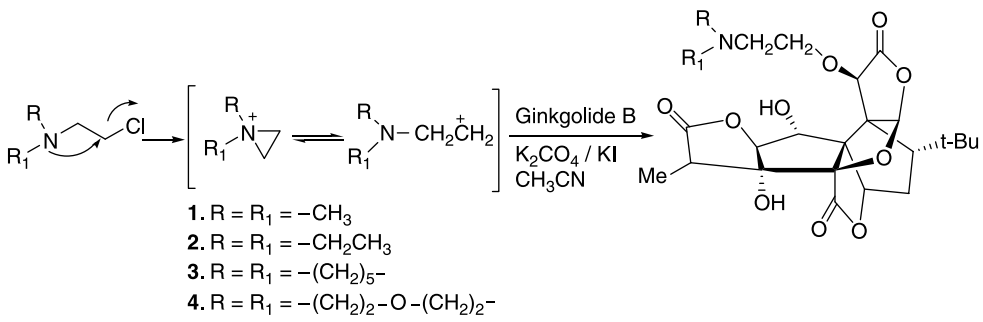


Figure 1. The structure of ginkgolide B.

mixture of ginkgolide B with substituted aminoethyl chloride in acetonitrile for 1 h in the presence of K_2CO_3 and KI. The products were purified *via* column chromatography and the average yield is 40–50%. Compared with ethyl bromide, substituted aminoethyl chloride had a much higher reaction rate because of the participation of the neighbouring nitrogen atom in the substitution reaction. In this procedure, the substituted aminoethyl chloride was first transformed into a tricyclic ammonium similar to intra-molecular nucleophilic substitution in basic condition. The formed quaternary ammonium strengthened the activity of the carbon atoms in the tricyclic intermediate or could be transformed into the corresponding carbon cation, which then reacted with 10-OH of ginkgolide B to form the aimed compounds: 10-*O*-(*N*,*N*-dimethylaminoethyl)ginkgolide B (**1**), 10-*O*-(*N*,*N*-diethylaminoethyl)ginkgolide B (**2**), 10-*O*-(1-piperidinylethyl)ginkgolide B (**3**), and 10-*O*-(4-morpholinylethyl)ginkgolide B (**4**) (Scheme 1).

The chemical structures of compounds **1**–**4** were identified by ^{1}H-NMR, MS, and HR-MS. The reactive position of ginkgolide B was determined by comparing the ^{1}H-NMR spectra of ginkgolide B and its ethers. In the ^{1}H-NMR spectrum of ginkgolide B (DMSO-d_6, 400 MHz), we assigned the coupling signals at δ 5.00 and 7.46 ($J = 5.5$ Hz) to 10-H and 10-OH, respectively [6]. When an ether was obtained, the spectrum showed that the signal at δ 7.46 disappeared and the one at δ 5.00 became a single signal, namely the hydrogen atom of 10-OH was replaced by the dialkylaminoethyl group. Meanwhile a downfield shift for the 1-OH signal from δ 4.90 to about 7.00 indicated that there existed a hydrogen bond between the hydrogen atom of 1-OH and the nitrogen atom of the introduced group.

The anti-PAF activities of compounds **1**–**4** were evaluated on rabbit platelet aggregation induced by PAF, with ginkgolide B as the reference compound. The bioassay data are shown in Table 1 and suggested that all the compounds had significant activities as PAF antagonists. Among them, **1** and **2** were as active as ginkgolide B and **4** was more active than ginkgolide B. The fact that **4,** which was derived with 4-morpholinylethyl group, is more active than ginkgolide B and promotes us to synthesize more derivatives for investigating whether an oxygen-containing



Scheme 1. The synthesis of 10-*O*-dialkylaminoethyl ginkgolide B and the structures of **1**–**4.**

Table 1. *In vitro* biological evaluation of 10-*O*-dialkylaminoethyl ginkgolide B (**1**–**4**).

Compounds	R, R_1	PAF-induced platelet aggregation IC_{50} (μM)
Ginkgolide B		0.051
1	$R = R_1 = -CH_3$	0.057
2	$R = R_1 = -CH_2CH_3$	0.070
3	$R = R_1 = -(CH_2)_5-$	0.152
4	$R = R_1 = -(CH_2)_2O(CH_2)_2-$	0.031

group at this position could be favored for the anti-PAF activity. 10-*O*-Dimethylaminoethyl ginkgolide B (**1**) had been converted into its hydrochloride, mesylate, and acetyl salicylate, respectively. The X-ray single crystal diffraction of mesylate shows that it is a complex with $2H_2O$ and it merits the appropriate aqueous solubility. Further bioactivity evaluations both *in vitro* and *in vivo* are in progress.

3. Experimental

3.1 General experimental procedures

Melting points were measured in open capillary tubes and are uncorrected. Optical rotations were measured on a JASCO P-1020 automatic polarimeter. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured on a VARIAN Mercury plus 400 apparatus using TMS as internal standard. Mass spectra (MS) were obtained on a HP5989A mass spectrometer and HRMS were obtained on a WATERS-Micromass GCT mass spectrometer. All the chemicals were of analytical grade.

3.2 Reagents and synthesis procedures

A mixture of ginkgolide B (600 mg, 1.41 mmol), substituted aminoethyl chloride (2.12 mmol), K_2CO_3 (2.33 g, 16.90 mmol), and KI (235 mg, 1.41 mmol) in 40 ml acetonitrile was stirred and heated at 100°C for 1 h. After cooling, the solid was removed by filtration and the filtrate was concentrated *in vacuo* to give an oil. The crude product was purified via chromatography (silica gel H, CH_2Cl_2-MeOH 8:1) to give the aimed products.

3.2.1 *10*-O-*(*N,N-*dimethylaminoethyl) ginkgolide B (**1**)*

Yield 46.2%; mp 158–160°C; $[\alpha]_D^{23}$ −20.8 (*c* 0.04, $CHCl_3$); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 1.03 (s, 9H, *t*-Bu), 1.08 (d, 3H, $J = 7.0$ Hz, 14-Me), 1.74 (dd, 1H, $J_1 = 14.1$ Hz, $J_2 = 4.3$ Hz, 8-H), 1.85 (ddd, 1H, $J_1 = 14.1$ Hz, $J_2 = 13.7$ Hz, $J_3 = 4.3$ Hz, 7α-H), 2.14 (dd, 1H, $J_1 = 13.7$ Hz, $J_2 = 3.9$ Hz, 7β-H), 2.18 (s, 6H, $-N(CH_3)_2$), 2.29, 2.62, 3.56, 4.38 (m × 4, 1H × 4, $-NCH_2CH_2O-$), 2.82 (q, 1H, $J = 7.0$ Hz, 14-H), 4.08 (d, 1H, $J = 7.8$ Hz, 1 -H), 4.55 (d, 1H, $J = 7.8$ Hz, 2-H), 5.14 (s, 1H, 10-H), 5.31 (d, 1H, $J = 3.9$ Hz, 6-H), 6.14 (s, 1H, 12-H), 6.40 (s, 1H, 3-OH), 7.22 (s, 1H, 1-OH); MS *m/z* (%): 495 (M^+, 2.49), 282 (100); HR-MS *m/z* 495.2097 $[M]^+$ (calcd for $C_{24}H_{33}NO_{10}$, 495.2104).

3.2.2 *10*-O-*(*N,N-*diethylaminoethyl)ginkgolide B (**2**)*

Yield 37.4%; mp 145–152°C; $[\alpha]_D^{23}$ −34.6 (*c* 0.01, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.03 (t, 6H, $J = 7.0$ Hz, $-N(CH_2CH_3)_2$), 1.08 (s, 9H, *t*-Bu), 1.29 (d, 3H, $J = 7.0$ Hz, 14-Me), 1.91 (dd, 1H, $J_1 = 14.1$ Hz, $J_2 = 3.9$ Hz, 8-H), 1.97 (dt, 1H, $J_1 = 14.1$ Hz, $J_2 = 4.3$ Hz, 7α-H), 2.26 (dd, 1H, $J_1 = 12.1$ Hz, $J_2 = 3.9$ Hz, 7β-H), 2.47, 2.71 (m × 2, 6H $(CH_3CH_2)_2NCH_2$), 2.89 (s, 1H, 3-OH), 3.05 (q, 1H, $J = 7.0$ Hz, 14-H), 3.52, 4.65 (dt, 2H, $J_1 = 9.4$ Hz, $J_2 = 2.7$ Hz, $-NCH_2CH_2O-$),

4.23 (d, 1H, $J = 7.8$ Hz, 1-H), 4.55 (d, 1H, $J = 7.8$ Hz, 2-H), 4.73 (s, 1H, 10-H), 5.51 (d, 1H, $J = 3.9$ Hz, 6-H), 5.92 (s, 1H, 12-H), 7.52 (br s, 1H, 1-OH); MS *m/z* (%): 523 (M^+, 1.50), 100 ($(CH_3CH_2)_2NCH_2CH_2^+$, 8.57), 86 ($(CH_3CH_2)_2NCH_2^+$, 100); HR-MS *m/z* 523.2411 $[M]^+$ (calcd for $C_{26}H_{37}NO_{10}$, 523.2417).

3.2.3 *10*-O-*(1-piperidinylethyl)ginkgolide B (**3**)*

Yield 45.9%; mp 178°C; $[\alpha]_D^{23}$ −22.1 (*c* 0.02, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.10 (s, 9H, *t*-Bu), 1.31 (d, 3H, $J = 7.0$ Hz, 14-Me), 1.48 (m, 2H, —$CH_2CH_2CH_2CH_2CH_2$—), 1.61 m, 4H, —$CH_2CH_2CH_2CH_2CH_2$—), 1.93 (dd, 1H, $J_1 = 14.3$ Hz, $J_2 = 4.1$ Hz, 8-H), 2.02 (dt, 1H, $J_1 = 13.3$ Hz, $J_2 = 4.1$ Hz, 7α-H), 2.28 (dd, 1H, $J_1 = 12.9$ Hz, $J_2 = 4.1$ Hz, 7β-H), 2.37, 2.70 (m × 2, 6H, —$CH_2CH_2CH_2CH_2CH_2$—, —NCH_2CH_2O—), 2.88 (s, 1H, 3-OH), 3.07 (q, 1H, $J = 7.0$ Hz, 14-H), 3.53, 4.71 (m × 2, 2H, —NCH_2CH_2O—), 4.25 (d, 1H, $J = 7.6$ Hz, 1-H), 4.59 (d, 1H, $J = 7.6$ Hz, 2-H), 4.74 (s, 1H, 10-H), 5.55 (d, 1H, $J = 3.9$ Hz, 6-H), 5.95 (s, 1H, 12-H), 7.60 (bs, 1H, 1-OH); MS *m/z* (%): 535 (M^+, 0.84), 128 (5.36), 112 (6.46), 98 (100); HR-MS *m/z* 535.2436 $[M]^+$ (calcd for $C_{27}H_{37}NO_{10}$, 535.2417).

3.2.4 *10*-O-*(4-morpholinylethyl)ginkgolide B (**4**)*

Yield 47.9%; mp 290–294°C; $[\alpha]_D^{23}$ −14.9 (*c* 0.01, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.10 (s, 9H, *t*-Bu), 1.31 (d, 3H, $J = 7.1$ Hz, 14-Me), 1.93 (dd, 1H, $J_1 = 14.3$ Hz, $J_2 = 3.7$ Hz, 8-H), 2.00 (dt, 1H, $J_1 = 12.7$ Hz, $J_2 = 4.3$ Hz, 7α-H), 2.28 (dd, 1H, $J_1 = 12.1$ Hz, $J_2 = 3.5$ Hz, 7β-H), 2.43, 2.75 (m × 2, 6H, $N(CH_2—)_3$), 3.05 (q, 1H, $J = 7.1$ Hz, 14-H), 3.13 (s, 1H, 3-OH), 3.57, 4.71 (dt, 2H, $J_1 = 9.6$ Hz, $J_2 = 2.5$ Hz, —NCH_2CH_2O—), 3.77 (m, 4H, —CH_2OCH_2—), 4.23 (d, 1H, $J = 7.6$ Hz, 1-H), 4.60 (d, 1H, $J = 7.6$ Hz, 2-H), 4.76 (s, 1H, 10-H), 5.51 (d, 1H, $J = 3.5$ Hz, 6-H), 5.95 (s, 1H, 12-H), 7.09 (bs, 1H, 1-OH); MS *m/z* (%): 537(M^+, 0.94), 130 (3.41), 114 (7.66), 100 (100); HR-MS *m/z* 537.2229 $[M]^+$ (calcd for $C_{26}H_{35}NO_{11}$, 537.2210).

Acknowledgements

This work was partially supported by the Undergraduate Student Scientific and Innovation Foundation of the School of Pharmacy, Fudan University, Republic of China (KC200622).

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