A new ginkgolide from *Ginkgo biloba*

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Abstract Aim: To study new chemical constituents of the leaves of *Ginkgo biloba* L. Methods: Isolation and purification were carried out by several chromatographic methods. The structures of the compounds were elucidated by detailed analysis of their UV, IR, MS, ^{1}H NMR and ^{13}C NMR spectra. Results: A new ginkgolide, ginkgolide N (1, 7, 10-trihydroxy-3, 14-dehydroginkgolide, II), along with a known compound, was isolated from the leaves of *G. biloba*. The structure of the known one was elucidated as ginkgolide L (I). Conclusion: Compound II was a new compound. The complete spectroscopic data of compound I were reported for the first time.

Key words *Ginkgo biloba* L.; ginkgolide; ginkgolide N; chemical constituents; new compound

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银杏叶中一个新的萜内酯类成分

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摘 要 **目的**:研究银杏(*Ginkgo biloba* L.)叶中新的化学成分。**方法**:运用柱色谱方法分离纯化,利用 UV、IR、MS、^{1}H NMR、^{13}C NMR等色谱技术鉴定化合物结构。**结果**:从银杏叶中分离鉴定出 1个新化合物:1, 7, 10三羟基-3, 14去氢银杏内酯(II)和 1个已知化合物:银杏内酯 L(ginkgolide L)(I)。**结论**:化合物 II为新化合物,命名为银杏内酯 N(ginkgolide N);化合物 I的光谱数据为首次报道。

关键词 银杏;银杏萜内酯;银杏内酯 N;化学成分;新化合物

Ginkgo biloba, often referred to as a "living fossil", is the oldest living tree species on earth and is the only surviving species of the ginkgoaceae family. Its use as a herbal preparation dates back to approximately 5000 years ago when ancient Chinese ingested brewed leaf extracts to treat cardiovascular and bronchial diseases[1]. In recent years, standardized extracts of ginkgo leaves are amongst the top-selling phytomedicines in the world[2]. Ginkgo leaves contain many pharmacologically active ingredients such as flavonoid glycosides and terpene trilactones. In general, flavonoid glycosides are considered to increase peripheral and cerebral blood flow, and terpene trilactones are well known for their antagonistic action to platelet-activating factor (PAF) and ginkgolide B has the strongest activity[3-4].

In order to search the biologically active and structurally unique compounds from *G. biloba*, we carried out the chemical investigation on the total lactone fraction of *G. biloba*, which led to the isolation of a new ginkgolide [1, 7, 10-trihydroxy-3, 14-dehydroginkgolide (II)] (Figure 1), together with a known compound [ginkgolide L (I)]. The new compound was named ginkgolide N. Herein, we reported the isolation and structural elucidation of the new compound. Moreover, compound I (Figure 1) had been isolated previously without NMR data. In this paper, we reported its NMR data with unprecedented full assignment for the first time.

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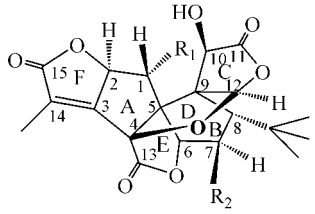

I R_1=R_2=H
II R_1=R_2=OH

Figure 1 Chemical structures of compound I and II

Compound II was obtained as white needles (MeOH). Its molecular formula was determined to be $C_{20}H_{22}O_{10}$ by HR-ESIMS (found *m/z*: 445.109 7 $[M+Na]^+$; calcd for $C_{20}H_{22}NaO_{10}$: 445.110 5). The ^{1}H NMR spectrum of compound II showed three methyl signals at δ_H 1.11 (9H, s), indicating the presence of a *tert*-butyl. The ^{13}C NMR spectrum of II displayed 20 carbon signals. The signals at δ 174.07 (C-11), 173.43 (C-15) and 169.39 (C-13) in the lowest field indicated the presence of three carbonyls. There were two carbons of double bond at δ 155.20 (C-3) and 125.19 (C-14), one carbon of hemiacetal carbon at δ 109.35 (C-12), and three methyls of *tert*-butyl at δ 28.93 (C-18～20). From the above data, it was suggested that the structure of compound II was characteristic of ginkgolide's skeleton.

Further, by comparison of the ^{1}H NMR and ^{13}C NMR spectra of compound II with those of ginkgolide K[5-6], it was observed that compound II was very similar to ginkgolide K, except for the additional signal at δ 5.68 (1H, d, J = 6.4 Hz, 7-OH) which led to the chemical shifts of H-7 (δ 4.04) moved to lower field 2 and C-7 (δ 73.93) moved to higher field 38. Based on the above evidence, compound II could be concluded to be an additional hydroxy of ginkgolide K at C-7. Moreover, comparison of the ^{1}H NMR and ^{13}C NMR spectra with those of ginkgolide C[7] revealed that compound II was similar to ginkgolide C, especially the signals of C-6, 7, 8 and H-6, 7, 8, suggesting that the spatial location of H-7 was in good agreement with those of ginkgolide C, namely, H-7 was on the α-face. In addition, the difference between compound II and ginkgolide C was that the additional double bond between C-14 and C-3 of compound II. Based on the above results, the structure of compound II might be 1, 7, 10-trihydroxy-3, 14-dehydroginkgolide.

The ^{1}H-^{1}H COSY, HMQC and HMBC spectra of compound II allowed the assignment of all protons and carbon signals (Table 1). In the ^{1}H-^{1}H COSY spectra of compound II, the correlations between δ 3.78 (1H, dd, J = 4.2, 7.9 Hz, H-1) and δ 5.52 (1H, dd, J = 2.2 Hz, 7.9 Hz, H-2), as well as those between δ 5.17 (1H, d, J = 4.0 Hz, H-6), δ 4.04 (1H, brd, J = 4.4 Hz, H-7) and δ 1.67 (1H, d, J = 12.6 Hz, H-8), indicated the presence of two spin-spin systems (C-1 to C-2, and C-6 to C-8). The HMBC correlations (Figure 2) were observed between the hydroxyl at δ 5.16 (1H, d, J = 7.9 Hz, 1-OH) and carbons bearing oxygen at δ 73.45 (C-1), 85.34 (C-2); the hydroxyl at δ 5.68 (1H, d, J = 6.4 Hz, 7-OH) and carbons bearing oxygen at δ 73.93 (C-7), 81.20 (C-6); the proton at δ 5.52 (1H, dd, J = 2.2, 7.9 Hz, H-2) and double bond carbons at δ 155.19 (C-3), 125.19 (C-14). Therefore, compound II was identified as 1, 7, 10-trihydroxy-3, 14- dehydroginkgolide, and named as ginkgolide N. It is the third example of natural product possessing double bond of ginkgolide.

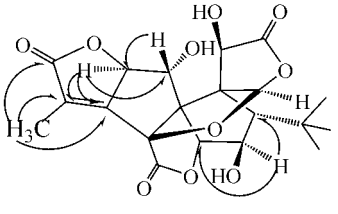

Figure 2 Key HMBC correlations of compound II

Compound I was obtained as white needle crystal (MeOH). Its molecular formula was determined to be $C_{20}H_{22}O_8$ by HR-ESIMS (Found *m/z*: 413.116 96 $[M+Na]^+$; Calcd. for $C_{20}H_{22}NaO_8$: 413.120 7). The ^{1}H NMR and ^{13}C NMR data (Table 2) of compound I were similar to those of compound II, except for two additional methylenes and two absent hydroxyls. Based on the 1D- and 2D-NMR experiments, all the proton

and carbon signals of compound I were assigned accurately (Table 2). These findings led to the assignment of compound I as 10-hydroxy-3, 14-didehydroginkgolide, namely ginkgolide L.

Table 1 NMR data of compound II (DMSO-d_6)

Position	δ_C	δ_H	HMBC
1	73.45	3.78 (dd, 4.2, 7.9)	5.16 (1-OH), 5.17 (6), 5.52 (2)
2	85.34	5.52 (dd, 2.2, 7.9)	3.78 (1), 5.16 (1-OH)
3	155.20		1.92 (16), 5.52 (2)
4	92.06		6.10 (12)
5	65.44		4.04 (7), 3.78 (1), 4.99 (10), 5.16 (1-OH), 6.10 (12)
6	81.20	5.17 (d, 4.0)	4.04 (7), 5.68 (7-OH), 3.78 (1)
7	73.93	4.04 (br. d, 4.4)	1.67 (8), 5.68 (7-OH)
8	48.61	1.67 (d, 12.6)	1.11 (18-20), 4.04 (7), 5.68 (7-OH), 5.17 (6)
9	69.18		1.67 (8), 4.04 (7), 3.78 (1), 4.99 (10), 5.17 (6), 6.10 (12)
10	71.11	4.99 (d, 5.6)	7.25 (10-OH)
11	174.07		4.99 (10), 6.10 (12)
12	109.35	6.10 (s)	1.67 (8)
13	169.39		5.17 (6)
14	125.19		1.92 (16), 5.52 (2)
15	173.44		1.92 (16)
16	8.88	1.92 (d, 2.2)	
17	31.95		1.11 (18-20), 4.04 (7), 5.68 (7-OH), 1.67 (8)
18-20	28.93	1.11 (s)	1.67 (8)
1-OH		5.16 (d, 7.9)	73.45 (C-1), 85.34 (C-2)
7-OH		5.68 (d, 6.4)	73.93 (C-7), 81.20 (C-6)
10-OH		7.25 (d, 5.6)	71.11 (C-10)

Table 2 NMR data of compound I (DMSO-d_6)

Position	δ_C	δ_H	HMBC
1α	35.08	1.84 (dd, 15.2, 7.4)	5.12 (6), 5.51 (2)
1β		2.72 (dd, 15.2, 7.4)	
2	86.99	5.51 (t, 7.4)	1.84 (1)
3	160.66		1.92 (16), 5.51 (2)
4	92.94		6.05 (12)
5	72.51		2.05 (7), 1.84 (1α), 2.72 (1β), 4.97 (10), 6.05 (12)
6	80.46	5.12 (d, 4.0)	2.05 (7), 1.84 (1α), 2.72 (1β)
7α	37.51	2.05 (m)	
7β		2.05 (m)	
8	48.67	1.71 (t, 9.6)	1.05 (18-20), 2.05 (7), 5.12 (6)
9	68.25		1.71 (8), 2.05 (7), 1.84 (1α), 2.72 (1β), 4.97 (10), 5.12 (6), 6.05 (12), 6.76 (10-OH)
10	69.06	4.97 (d, 4.2)	6.76 (10-OH)
11	174.29		4.97 (10), 6.05 (12), 6.76 (10-OH)
12	109.68	6.05 (s)	1.71 (8)
13	169.29		5.12 (6)
14	124.17		1.92 (16), 5.51 (2)
15	173.55		1.92 (16)
16	8.70	1.92 (d, 7.0)	
17	31.89		1.05 (18-20), 2.05 (7), 1.71 (8)
18-20	28.75	1.05 (s)	1.71 (8)
1-OH			
7-OH			
10-OH		6.76 (d, 4.2)	

2.1 *General experimental procedures*

Melting points were measured on an XT-4 micro melting-point apparatus (uncorrected). SCO P-1020 polarimeter. IR spectra were recorded on a Nicolet Instrument Nexus 870 FT-IR spectrometer. NMR spectra were recorded on Bruker AV-400 spectrometers, MS were determined on Agilent 6210 LC/MSD TOF spectrometer. Column chromatographic separations were performed on silica gel (200-300 mesh; Qingdao Marine Chemical Ltd., Qingdao, China). Thin-layer chromatography was performed on precoated silica gel GF_{254} plates (Qingdao Marine Chemical Ltd., Qingdao, China). All other common chemical reagents were purchased from Guangzhou Reagent Co., Ltd..

2.2 *Plant material*

The leaves of ginkgo were collected in Nanjing, Jiangsu province of China, in April 2008, and were authenticated by Prof. QIN Min-jian (China Pharmaceutical University). A voucher specimen (No. 080412) was deposited in the Herbarium of the China Pharmaceutical University, Nanjing, China.

2.3 *Extraction and isolation*

The pulverized leaves of *Ginkgo biloba* L. (8.0 kg) were extracted by refluxing with 95% ethanol (40 L ×3). The EtOH extract was concentrated under vacuum to yield the crude extract (800 g), which was dispersed in water and extracted successively with petroleum ether, EtOAc and *n*-BuOH. The EtOAc extract was subjected to silica gel column chromatography (mesh 200-300), using gradient mixture of CH_2Cl_2-MeOH (100∶1-100∶100, *v*/*v*) as eluants to yield total lactone, which can be isolated and purified by recrystallization and column chromatography. The residue (36 g) from the recrystallization mother liquor of total lactone was chromatographed on a silica gel (mesh 200-300) using gradient mixture of petroleum ether-EtOAc (1∶0-0∶1) as eluants to yield compounds I (50 mg) and II (42 mg), respectively.

Compound I: white needle crystal (MeOH), mp > 300 ℃. IR: ν_{max}^{KBr} (cm^{-1}): 3 585, 3 470, 3 350, 1 790, 1 770 cm^{-1}. HR-ESI-MS *m*/*z*: 445.109 7 $[M+Na]^+$ (calcd. for $C_{20}H_{22}NaO_{10}$: 445.110 5). The data of ^1H NMR and ^{13}C NMR (400 MHz, DMSO-d_6) can be seen from Table 2.

Compound II: white needles crystal (MeOH), mp > 300 ℃. IR: ν_{max}^{KBr} (cm^{-1}): 3 558, 3 520, 3 500, 3 130, 1 610, 1 785, 1 757 cm^{-1}. HR-ESI-MS *m*/*z*: 413.117 0 $[M+Na]^+$ (calcd. for $C_{20}H_{22}NaO_8$: 413.120 7). The data of ^1H NMR and ^{13}C NMR (400 MHz, DMSO-d_6) can be seen from Table 1.

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