Ginkgolides: Selective Acetylations, Translactonization, and Biological Evaluation

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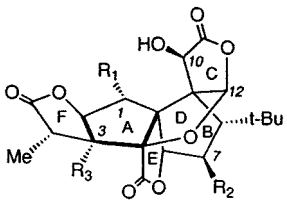
Abstract: Protocols for selective acetylation of the hydroxyl groups of ginkgolide C have been developed. These acetylations have given rise to various ginkgolide C acetates and iso-ginkgolide C acetates, the latter having a rearranged skeleton resulting from translactonization. These acetyl derivatives, as well as ginkgolides A and B acetates have been investigated for their ability to bind to a cloned platelet-activating factor (PAF) receptor.

The Ginkgo tree (*Ginkgo biloba* L.), mentioned in the Chinese Materia Medica for over 3000 years, is the last surviving member of a family of trees *Ginkgoacea* that appeared more than 250 million years ago. In 1998, Americans spent ca. 4 billion dollars on botanical medicines and *Ginkgo biloba* ranked first among herbal medications. Today over 50 million *G. biloba* trees are grown in China, France, and South Carolina producing approximately 8000 tons of dried leaves each year.[1] A number of *G. biloba* natural products have been identified,[2] including the diterpenoid ginkgolides A−C, J, and M (**1**−**5**, Figure 1),[3,4] and the sequiterpenoid bilobalide,[5] together termed terpene trilactones (TTLs). The ginkgolides have a cage skeleton consisting of six five-membered rings: a spiro[4.4]nonane carbocyclic ring, three lactones, and a tetrahydrofuran.

Ginkgolides, especially ginkgolide B (GB, **2**), are antagonists of the platelet-activating factor receptor (PAFR), an effect that might be related to the neuroprotective effects of *G. biloba*.[6] Ginkgolide C (GC, **3**) is the most abundant ginkgolide, but the 7-hydroxyl decreases binding to the PAFR by ca. 20-fold compared to GB.[7] Thus procedures have been developed to convert GC into the more potent GB,[8] and most structure−activity relationship (SAR) studies have been performed by derivatization of GB.[9] To study the interactions of ginkgolides with various targets, including PAFR, the synthesis of photoactivatable ginkgolide derivatives has recently been described.[7] We were also interested in preparing diazoacetate derivatives to be used in a photolabeling protocol that employs fluorescence rather than radioactivity.[10] However, since methods for selectively introducing diazoacetates into GC were lacking, it was first decided to study the selective introduction of acetyl groups into GC. Such acetyl derivatives of GC would also provide an insight into the potential bioactivities of GC diazoacetates. Finally, acetylation is a simple model for exploring site-specific acylations in molecules with multiple hydroxyl groups as GC.



	R_1	R_2	R_3
Ginkgolide A (GA, **1**)	H	H	OH
Ginkgolide B (GB, **2**)	OH	H	OH
Ginkgolide C (GC, **3**)	OH	OH	OH
Ginkgolide J (GJ, **4**)	H	OH	OH
Ginkgolide M (GM, **5**)	OH	OH	H

Figure 1. Ginkgolides A, B, C, J, and M.

Earlier structural studies had shown that treatment of GC with sodium acetate in acetic anhydride[3a,b,d] or a mixture of pyridine and acetic anhydride[11] gave rise to a complex mixture of products. However, acetic anhydride and a catalytic amount concentrated H_2SO_4 resulted in clean transformation of GC into 1,3,7,10-tetraAc-GC.[12] In the following we describe conditions for selective acetylation of GC and the binding affinity of ginkgolide acetates to cloned PAFR.

First acetylation of GC (**3**) was studied in DMF and CH_2Cl_2 at ambient temperature using excess acetic anhydride (10 equiv) without addition of base. When carried out in DMF, the reaction provided a mixture of products in which 10-Ac-GC (**6**) (Figure 2) was predominant (Table 1).[13] On the other hand, in CH_2Cl_2 no reaction

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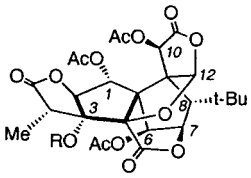
6 10-Ac-GC
7 1-Ac-GC
8 7-Ac-GC
9 7,10-diAc-GC
10 1,7-diAc-GC
11 1,7,10-triAc-GC
12 1,3,7,10-tetraAc-GC



13 1,6,10-triAc-isoGC (R=H)
14 1,3, 6,10-tetraAc-isoGC (R=Ac)

Figure 2. Acetates of ginkgolide C (**3**).

Table 1. Acetylation of GC with Acetic Anhydride (10 equiv)

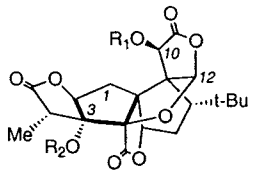
entry	base/solvent[a]	time, h	product	yield, %[b]
1	none/DMF	18	**6**	60
2	none/CH_2Cl_2	24	NR[c]	-
3	pyridine/CH_2Cl_2	24	**11**	50
4	2,6-lutidine/CH_2Cl_2	8	**6**	65
5	Et_3N/CH_2Cl_2	2	**13**	79
6	iPr$_2$EtN/CH_2Cl_2	2	**13**	90

[a] 10 equiv of base. Base/CH_2Cl_2 (1:6). [b] Determined by ^{1}H NMR. [c] NR: no reaction.

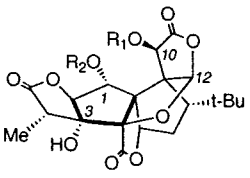
was observed. Various bases in CH_2Cl_2 were evaluated in order to determine the effect of base, the results of which are summarized in Table 1. A mild base, 2,6-lutidine, led to a mixture of products with 10-Ac-GC (**6**) as the major component (65% yield), whereas pyridine gave 1,7,10-triAc-GC (**11**, 50%) after 24 h. Stronger bases, such as tertiary amines Et_3N or iPr$_2$EtN, provided rearranged product **13** in 79% and 90% yield, respectively.[3d,e,11] Acetylation of GC with acetic anhydride and pyridine in CH_2Cl_2 (Table 2) was then evaluated in more detail to provide insight into the relative reactivity of the hydroxyl groups of GC under these conditions. Initially, acetylation yielded 10-Ac-GC (**6**) and 1-Ac-GC (**7**) in a 5:1 ratio. The next position to be acetylated in both **6** and **7** was 7-OH to form 7,10-diAc-GC (**9**) and 1,7-diAc-GC (**10**), respectively. Further reaction led to acetylation of the remaining secondary hydroxyl to form 1,7,10-triAc-GC (**11**). Addition of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) to the above acetylation mixture of acetic anhydride and pyridine in CH_2Cl_2 led to further acetylation of the tertiary 3-OH to give peracetylate **12** (Figure 2) in high yield. Heating or increasing the content of DMAP caused elimination of acetate at C-3 to form a 3,14-ene; further elimination of the 1-acetoxy can occur to give the conjugated 1,2−3,14 dianhydro derivatives.[3b,11b]

In an attempt to form GC monoacetate by decreasing the amount of acetic anhydride to 1 or 2 equiv using the above described conditions resulted in only moderate yields of 10-Ac-GC (**6**); it appears that acetic anhydride is too reactive to achieve selective monoacetylation. Therefore 2 equiv of an active ester, prepared from acetic anhydride and *N*-hydroxybenzotriazole, was reacted with GC in DMF in the presence of pyridine; this gave 10-Ac-GC (**6**, 86% yield) contaminated with only 10% of 1-Ac-GC (**7**). Using the same conditions, reaction with GB afforded 10-Ac-GB (**17**) in 91% yield and 9% of 1-Ac-GB (**18**) (Figure 3).

Acid-catalyzed acetylation was also investigated. Acetic anhydride with traces of H_2SO_4 had provided peracetate



15 10-Ac-GA (R_1=Ac, R_2=H)
16 3,10-diAc-GA (R_1=R_2=Ac)



17 10-Ac-GB (R_1=Ac, R_2=H)
18 1-Ac-GB (R_2=Ac, R_1=H)
19 1,10-diAc-GB (R_1=R_2=Ac)

Figure 3. Acetates of GA (**1**) and GB (**2**).

12 from GC.[12] Glacial acetic acid with H_2SO_4 (10 equiv) was therefore selected as a milder reagent. However, since this reaction did not proceed at ambient temperature, the mixture was heated at 50 °C for 9 h upon which 7-Ac-GC (**8**) was formed in 82% yield, with 15% residual GC; further heating at 70 °C for 12 h increased the yield of **8** to 84%, with 8% residual GC remaining and 6% of 1,7-diAc-GC (**10**). Similar results were obtained when BF_3·OEt_2 instead of H_2SO_4 was used as acid catalyst. An increase in the reaction time or temperature only led to a higher content of 1,7-diAc-GC (**10**), whereas a shorter reaction time led to lower conversion.

These results demonstrate the differences among the hydroxyl groups of GC. Acetylation in the presence of base led to higher reactivity of 10-OH which had been observed previously.[8b,c,9,10] On the other hand, the pattern for acid-catalyzed acetylation of GC is different as seen in the case of H_2SO_4-catalyzed formation of **8**. These observations can be explained by the hard−soft and acid−base (HSAB) principle: due to the hydrogen bonding between 1-OH and 10-OH, it is easy to form a delocalized alkoxy anion at 1-OH and 10-OH in the presence of a relatively mild base. This anion becomes a hard base compared to 7-OH. In the presence of acid, the alkoxy anion at 1-OH and 10-OH cannot be formed and 7-OH becomes the harder base, and thus more reactive toward acetylation.

Interestingly, use of iPr$_2$EtN (Hunigs base) causes translactonization of lactone ring E (Figure 1) from C-6 to C-7 and rapid and exclusive formation of 1,6,10-tri-Ac-isoGC (**13**).[14] This derivative readily yielded crystals, the X-ray structure (Figure 4) of which was used to support the proposed mechanism of translactonization (Scheme 1). The high yield of isoGC derivative **13** in the presence of Hunigs base is somewhat surprising. The rationale for this effect is probably higher basicity of the base that is necessary to open lactone ring E (Figure 1). The suggested mechanism of translactonization is depicted in Scheme 1. This mechanism involves stabilization of translactonized anion (**IV**) by hydrogen bonding with 3-OH that is preferred over the original structure (**II**), and capture of the stabilized anion (**IV**) by acetic anhydride to give the translactonized product (**V**, Scheme 1).

This mechanism is supported by the following observations: (i) treatment of 7-acetyl-GC (**8**) with iPr$_2$EtN in CH_2Cl_2 does not cause translactonization, (ii) treatment of 7-Ac-GC (**8**) with acetic anhydride and iPr$_2$EtN in CH_2Cl_2 furnishes 1,7,10-triAc-GC (**11**) without detectable amount of any isoGC derivative, confirming that translactonization must precede acetylation, and (iii) the

(13) Substitution of hydroxyl groups was accompanied by downfield shifts of the corresponding carbinyl protons, i.e., 1.1−1.2 ppm for 10-H, 1.3−1.5 ppm for 1-H and, 0.9 ppm for 7-H. In addition, the H−OH coupling (observed in $CDCl_3$ and DMSO-d_6) was absent.

(14) Translactonization was accompanied by further changes in the ^{1}H NMR spectra; due to conformational change of ring B, 8-H appeared as a singlet, and 12-H shifted upfield by 0.4 ppm.

Table 2. Composition of the Reaction Mixture during Acetylation of GC[a,b]

	10-Ac (**6**)	1-Ac (**7**)	7,10-diAc (**9**)	1,7-diAc (**10**)	1,7,10-triAc (**11**)	1,3,7,10-tetraAc (**12**)
3 min[c]	35	7	-	-	-	-
1 h	56	11	30	3	-	-
2 h	37	8	45	5	5	-
12 h	-	-	57	7	32	-
24 h	-	-	31	5	50	7

[a] Acetic anhydride (10 equiv) and pyridine (10 equiv) in CH_2Cl_2. [b] Relative yields determined by ^1H NMR. [c] 58% of starting material (GC).

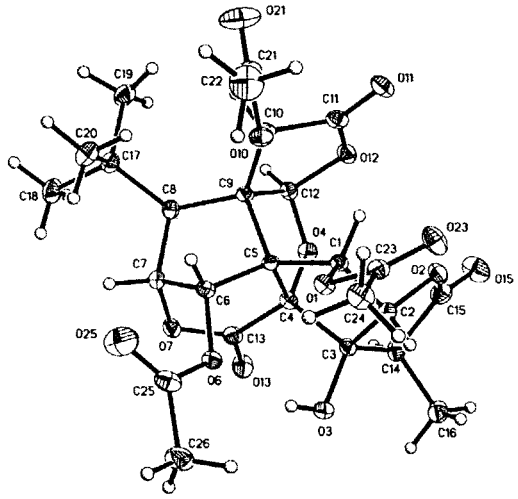
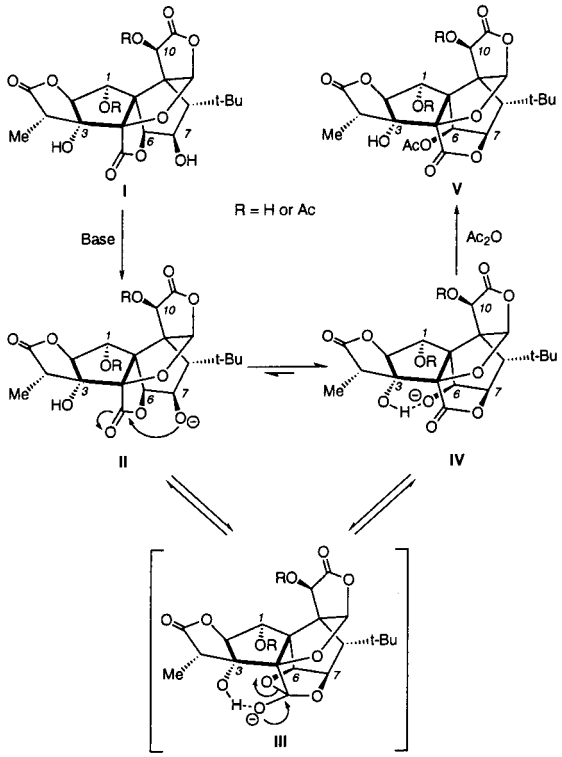


Figure 4. X-ray crystal structure of 1,6,10-triAc-isoGC (**13**). The thermal ellipsoids are drawn at 94.4% probability level.

Scheme 1. Mechanism of translactonization of GC into isoGC



parameters of stabilizing hydrogen bond in **IV** can be calculated from the crystal structure of 1,6,10-triAc-isoGC (**13**, Figure 4). Namely, the distance between 3-OH proton and 6-O is 2.12 Å with a 3O−H- -6O angle of 171.2°, typical parameters for hydrogen bond.

Table 3. Inhibition of [^{3}H]-WEB 2086 Binding to PAFR by Acetyl Derivatives of GA, GB, and GC

compound	inhib[a]	compound	inhib[a]
GA (**1**)	46	1,3,7,10-tetraAc-GC (**12**)	<10
GB (**2**)	71	1,6,10-triAc-isoGC (**13**)	<10
GC (**3**)	21	1,3, 6,10-tetraAc-isoGC (**14**)	<10
10-Ac-GC (**6**)	NT[b]	10-Ac-GA (**15**)	42
1-Ac-GC (**7**)	NT[b]	3,10-diAc-GA (**16**)	<10
7-Ac-GC (**8**)	18	10-Ac-GB (**17**)	31
7,10-diAc-GC (**9**)	NT[b]	1-Ac-GB (**18**)	NT[b]
1,7-diAc-GC (**10**)	<10	1,10-diAc-GB (**19**)	11
1,7,10-triAc-GC (**11**)	11		

[a] % Inhibition of [^{3}H]-WEB 2086 binding by a 5 μM solution of the test compound. [b] NT: not tested.

The synthesized GC acetyl derivatives, as well as GA (**1**) and GB (**2**) acetates[15] (Figure 3) were tested as competitive inhibitors of the cloned PAFR using a radioligand binding assay (Table 3).[16] Generally acetyl derivatives were less potent than the parent ginkgolides, although 10-Ac-GA (**15**) was equipotent with GA (**1**). 10-Ac-GB (**17**) is also more potent than the 1,10-diacetate (**19**), indicating that an acetyl group in the 10-position of ginkgolides is tolerated in the binding to the PAFR. Furthermore, the 7-acetyl derivative of GC (**8**) is equipotent to GC (**3**), showing that an acetyl group at C-7 is also tolerated, but does not improve the activity compared to GC. The two iso-derivatives of GC (**13** and **14**) were both devoid of activity in the concentration tested, but since the corresponding GC acetates (**11** and **12**) were essentially inactive, the significance of the rearrangement of the ginkgolide core for binding to the PAFR cannot be resolved.

In conclusion, we have provided methods for selective acetylations of GC. Treatment of GC with Hunigs base and acetic anhydride yielded the rearranged isoGC acetates according to the mechanism shown in Scheme 1. Finally, binding assays of several ginkgolide acetates showed that the binding properties of acetates to the PAFR were not diminished in several cases, thus suggesting ginkgolide diazoacetates as potential photolabeling probes for the PAFR.

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(15) Some of the acetyl derivatives originated from early structural studies, see ref 3 and 12.

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Supporting Information Available: All experimental details, and characterization of compounds. ^{1}H NMR spectra and 2D-COSY NMR of compounds **6**−**9**, **11**, **13**−**15**, **17**−**19**. Complete tables of crystallographic data, summary of structural refinement, atomic coordinates, anisotropic thermal parameters, and bond distances and angles for **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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