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Chemistry of the Ginkgolides. Part 10:[1] Access to ^{14}C-Labelled Ginkgolide A

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Abstract—In four reaction steps, ginkgolide A (**1**) was synthesized from the enol-acetate **3**, which is available in six steps from **1**. The key step was the reaction of the α-epoxy-acetate **4** with the lithium enolate of methyl propionate to give the compounds **5** and **6**. ^{14}C-Labelled ginkgolide A, which is of interest for pharmacological studies, can be synthesized using the ^{14}C-labelled methyl propionate.

Introduction

Ginkgolides are contained in a series of medicines. ^{14}C-Labelled ginkgolides are needed since they enable to follow the metabolism and to carry out pharmacological studies. Therefore we developed initially a simple path with inactive compounds for the synthesis of ginkgolide A using ginkgolide A (**1**) as starting material by partially degrading and subsequently reconstructing it. This is the way ^{14}C-labelled ginkgolide A is presently being synthesized.

Results and Discussion

The ketone **2**, which we have already reported and which can be obtained from **1** in good yield in five steps (I. H_2O elimination, II. catalytical hydrogenation, III. esterification with diazomethane, IV. methoxymethylation, V. ozonization)[2,3] was transformed into the enol-acetate **3** by reaction with acetic anhydride/pyridine.[4] Reaction of **3** with 3-chloroperbenzoic acid yields the α-epoxy-acetate **4**. Both steps proceed in more than 80% yield. As determined by X-ray analysis, the oxygen atom of the epoxy group of

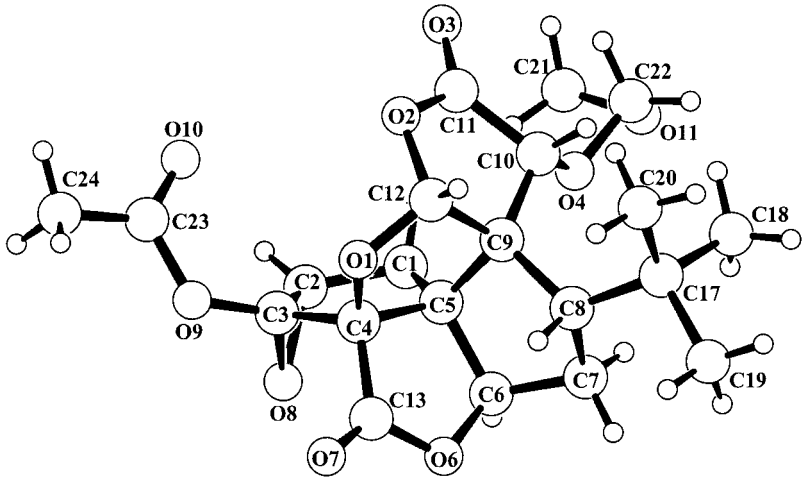

Figure 1. Molecular structure of **4**.[6]

Keywords: ginkgolide A; ^{14}C-labelling; enolacetate; epoxidation.

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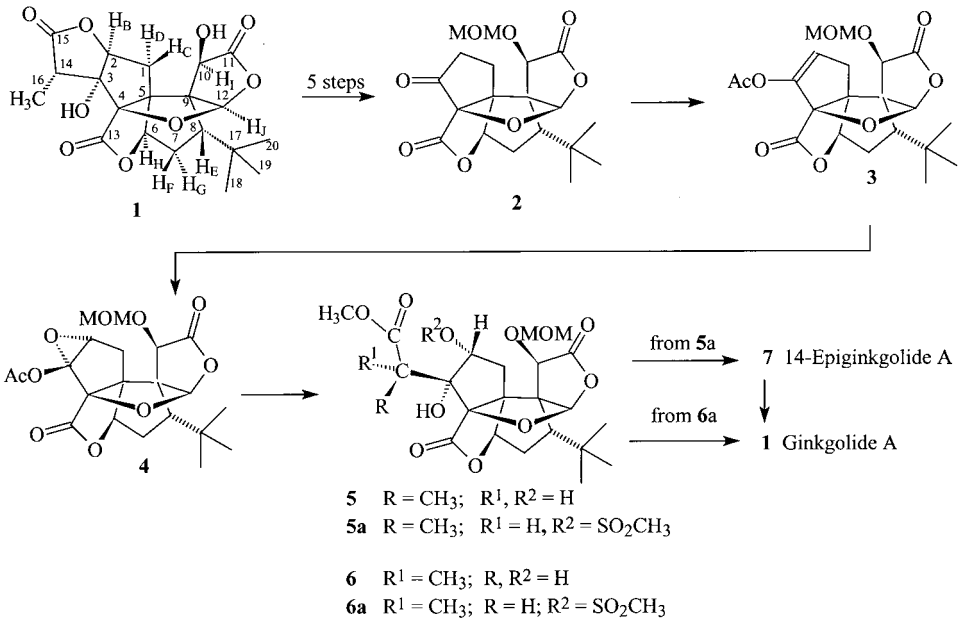

Scheme 1.

compound **4** is oriented in the direction of the neighboring lactone ring (Fig. 1).

It is well known that the ring opening of α-epoxy ethers always takes place between the oxygen of the epoxide and the C-atom where the alkoxy group is bound.[5] Accordingly, the reaction of α-epoxyacetate **4** with the lithium enolate of methyl propionate yielded **5** and **6** in a ratio of about 1:1. These compounds were separated by column chromatography and transformed into their 2-*O*-mesyl derivatives **5a** and **6a**. The yield of **5** and **6** was only ca. 41%, and could not be improved. After boiling **5a** in dioxane/HCl 14-*epi*-ginkgolide A (**7**) was obtained together with a small amount of anhydroginkgolide A, whereas **6a** produced the natural ginkgolide A (**1**) exclusively. We were able to increase the yield of **1** by epimerization of **7** to **1**, which we have already reported (Scheme 1).[2]

Concerning stereoselectivity, the following statements can be made: by the X-ray structure analysis of **4** it was proven that the 3-chloroperbenzoic acid attacks the double bond of **3** at the C-3 atom only from the re-side. In the next reaction step the attack of the nucleophile comes from the side opposite to the epoxide oxygen, generating the *cis*-diols **5** and **6**, which have the same configuration of C-3 as the natural ginkgolides. By the transformation of **5a** into 14-*epi*-ginkgolide (**7**) and of **6a** into natural ginkgolide A (**1**) the configuration of C-14 in **5** and **6** were established.

Experimental

General

Melting points were determined by a Büchi melting point apparatus and are not corrected. For TLC sheets 'Polygram Sil G/UV$_{254}$' (Macherey–Nagel) were used and for developing spray reagent hydroxylamine/iron(III)-chloride according to Stahl.[7] CC was carried out with 'Silica 32-63' (ICN Biomedicals). Optical rotations were measured with a Perkin–Elmer 241 polarimeter. The NMR spectra were recorded on Bruker instruments (AC 200 and AC 300). Mass spectra were taken on a Jeol JMS 700 instrument.

3-Acetoxy-2,3-epoxy-10-methoxymethoxy-14,15,16-trinorginkgolide (4). In a 100 ml round bottom flask 2 g (4.73 mmol) of **3** was dissolved in 25 ml of benzene and under stirring 1.5 g (6 mmol) 3-chloroperbenzoic acid (*m*-Cl-PBA) (70%) was added. After 2 h, 1 g of *m*-Cl-PBA was added again. After 24 h, a precipitate of 3-chlorobenzoic acid was formed. For work up 10 ml of an aqueous solution of $Na_2S_2O_5$ was added and stirred for 10 min. The mixture was transferred into a separatory funnel and the round bottom flask was irrigated several times with ethyl acetate. The aqueous layer was separated and the organic layer was washed twice with a solution of $NaHCO_3$ and with brine and was dried with Na_2SO_4. After removal of the solvent the residue was crystallized from a small quantity of acetone/ethanol. Colorless crystals (quantitative yield), mp 145–146°C (decomp.; R_f=0.55 (toluene/acteone, 8:2); $[\alpha]_{589}^{20}$=−2.1; $[\alpha]_{578}^{20}$=−2.4; $[\alpha]_{546}^{20}$=−3.3 (*c*=2, acetonitrile); ^{13}C NMR: Table 2; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=6.05 (s, 1H, H$_J$), 5.06 (s, 1H, H$_I$), 5.06 (d, 1H, two spin system, OC*H*$_2$O) and 4.93 (d, 1H, *J*=6 Hz, two spin system, OC*H*$_2$O), 4.70 (d, 1H, $J_{B/D}$=3.7 Hz, H$_B$), 4.60 (s$_{br}$, 1H, H$_H$), 3.35 (s, 3H, OCH$_3$), 2.15 (s, 3H, C*H*$_3$CO), 2.1–2.6 (H$_{C,D,E,F,G}$), 1.04 (s, 9H, *tert*-butyl). MS (FAB, positive-ion mode): $[M+H]^+$($C_{21}H_{27}O_{10}$) calcd. 439.16043, found 439.1612; $[M+Na]^+$ ($C_{21}H_{26}O_{10}Na$) calcd. 461.1426, found 461.1491. $C_{21}H_{26}O_{10}$ (438.4) calcd. C, 57.53, H, 5.98; found C, 57.45, H, 5.99.

X-Ray analysis of 4

Table 1 contains the crystallographic data and details of the refinement procedure. The reflections were collected with a Nonius-CAD4-diffractometer (Mo Kα-radiation, graphite monochromator, ω−2Θ-scan). Intensities were corrected

Table 1. Crystallographic data of **4**

Compound	**4**
Empirical formula	$C_{21}H_{26}O_{10}$
Molecular mass [g/mol]	438.4
Crystal size [mm]	0.5×0.45×0.45
Crystal color	Colorless
Crystal shape	Irregular
Space group	$P2_12_12_1$
a [Å]	9.205(3)
b [Å]	10.359(4)
c [Å]	22.076(7)
V [Å^3]	2105(1)
D_{calc} [Mg/m^3]	1.38
Z	4
F(000)	928
Temperature [K]	293
h_{min}/h_{max}	0/12
k_{min}/k_{max}	0/13
l_{min}/l_{max}	0/29
Θ range [°]	2.2–28.0
μ [mm^{-1}]	0.11
T_{min} [%]	94.7
T_{max} [%]	95.2
Refl. collected	2874
Refl. unique	2874
Refl. observed [$I>2\sigma(I)$]	2003
Variables	303
$(\Delta/\sigma)_{max}$	<0.01
R	0.041
R_w	0.115
S (Gof)	1.03
$(\Delta\rho)_{max}$ [e Å^{-3}]	0.20
$(\Delta\rho)_{min}$ [e Å^{-3}]	−0.15

for Lorentz and polarization effects. The structure was solved by direct methods (SHELXS-97[8]). The structural parameters of the non-hydrogen atoms were refined anisotropically according to a full-matrix least-squares technique (F^2). The parameters of the hydrogen atoms were calculated. The refinement was carried out with SHELXL-97.[9] Disorder was found at O11 and C24 (for atomic numbering see Fig. 1) with a multiplicity of 66.7 and 33.3%.[10]

Table 2. ^{13}C NMR data

C-	**3**[a]	**4**[b]	**5**[b]	**5a**[b]	**6**[a]	**6a**[b]
-1	35.60	33.87	37.09	34.53	38.14	34.91
-2	121.73	67.57	73.69	81.55	78.87	81.32
-3	141.74	85.39	80.57	81.30	81.11	80.54
-4	98.26	95.04	101.52	100.11	101.66	100.28
-5	68.03	69.69	66.80	67.04	67.21	67.34
-6	87.91	87.10	88.06	87.70	88.27	87.91
-7	35.47	35.30	35.56	35.70	35.49	35.53
-8	49.11	49.02	48.91	48.95	48.99	48.84
-9	66.71	68.08	65.72	66.30	65.85	65.44
-10	73.76	73.59	73.69	73.78	73.69	73.67
-12	109.33	109.43	108.29	108.34	108.26	108.20
-14	–	–	42.10	42.08	42.87	42.22
-16	–	–	12.55	11.78	12.61	12.87
-17	31.85	31.85	31.87	31.97	31.86	31.87
-18						
-19	28.75	28.75	28.82	28.88	28.80	28.78
-20						
-11	169.44	169.77	171.07	171.17	171.50	170.94
-13	167.74	166.81	172.25	171.82	172.21	171.80
-15	–	–	173.25	171.93	173.82	173.52
CH_3CO	172.52	172.52	–	–	–	–
OCH_2O	95.67	95.69	95.70	95.90	95.75	95.85
CH_3OCH_2	56.52	56.52	56.55	56.68	56.55	56.59
CH_3CO	20.47	20.53	–	–	–	–
$COOCH_3$	–	–	51.32	51.72	51.28	51.57
CH_3SO_3	–	–	–	37.59	–	37.38

[a] δ values, 50.32 MHz, [D$_6$]DMSO).
[b] (δ values, 75.47 MHz, [D$_6$]DMSO).

(2*R*,3*R*,10*R*,14*R*)-2,3-Dihydroxy-10-methoxymethoxy-ginkgolide-15-acid methyl ester (5) and (2*R*,3*R*,10*R*,14*S*)-2,3-dihydroxy-10-methoxymethoxy-ginkgolide-15-acid methyl ester (6). 5.65 ml (40 mmol) diisopropylamine in 30 ml THF was submitted in a three necked round bottom flask (200 ml) equipped with a 50 ml and a 100 ml dropping funnel (one with a supply for argon the other with a drying tube) and a septum. 3.85 ml (40 mmol) methyl propionate in 30 ml THF was put into one dropping funnel, into the other 3.5 g (8 mmol) of **4** dissolved in 50 ml THF. Under argon at −70 to −78°C 25 ml (40 mmol) *n*-butyllithium (1.6 M in *n*-hexane) was added slowly with a syringe through the septum under stirring. After 30 min and cooling to −100°C the solution of methyl propionate was added *slowly* drop by drop. After another 30 min the solution of **4** was also added slowly. After stirring for 1 h the cooling bath was removed and the mixture acidified with 2 M HCl. The THF was removed extensively in vacuo and the mixture was extracted with ethyl acetate. The organic layer was washed with sat. solution of $NaHCO_3$ and NaCl and dried with Na_2SO_4. The solvent was removed and the preparation separated by CC (silica gel, toluene/actetone, 9:1). Aside from the first eluate, which contained methyl-(2-methyl-3-oxo)-pentanoate, two isomeric products (**5** and **6**) were isolated.

5: R_f=0.43 (Toluene/acetone, 8:2), 0.8 g (20.7%) of colorless crystals, mp 218–219°C (decomp.); $[\alpha]_{589}^{20}=-19.8$; $[\alpha]_{578}^{20}=-20.7$; $[\alpha]_{546}^{20}=-23.8$ (c=2, acetonitrile); ^{13}C NMR: Table 2. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=5.91 (s, 1H, H$_J$), 5.22 (d, 1H, $J_{2\text{-OH/HB}}$=5.6 Hz, 2-OH), 5.12 (s, 1H, H$_I$), 5.04 (s, 1H, 3-OH), 5.04 (d, 1H, two spin system) and 4.96 (d, 1H, two spin system, J=5.9 Hz, OCH_2O), 4.69 (s$_{br}$, 1H, H$_H$), 4.4 (m$_c$, 1H, H$_B$), 3.57 (s, 3H, $COOCH_3$), 3.31 (s, 3H, OCH_3), 2.90 (q, 1H, $J_{HA/16\text{-}CH3}$=7.3 Hz, H$_A$), 2.83 (m$_c$, 1H, H$_C$), 2.2–1.5 (H$_{D,E,F,G}$), 1.23 (d, 3H, $J_{16\text{-Me/HA}}$=7.3 Hz, 16-CH_3), 1.02 (s, 9H, *tert*-butyl). $C_{23}H_{32}O_{11}$ (484.5) calcd. C, 57.02; H, 6.66; found C, 57.04, H, 6.71.

6: R_f=0.35 (Toluene/acetone, 8:2), 0.8 g (20.7%) of colorless crystals, mp 227–229°C (decomp.), $[\alpha]_{589}^{20}=+3.6$; $[\alpha]_{578}^{20}=+3.7$; $[\alpha]_{546}^{20}=+3.9$ (c=2, acetonitrile).^{13}C NMR: Table 2. ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ=5.97 (s, 1H, H$_J$), 5.38 (d, 1H, $J_{2\text{-OH/HB}}$=6.6 Hz, 2-OH), 5.16 (s,, 1H, H$_I$), 5.11 (d, 1H, two spin system) and 4.95 (d, 1H, two spin system, OCH_2O), 4.73 (s$_{br}$, 1H, H$_H$), 4.41 (s, 1H, 3-OH), 4.19 (m$_c$, 1H, H$_B$), 3.53 (s, 3H, $COOCH_3$), 3.35 (s, 3H, OCH_3), 2.94 (m$_c$, 1H, H$_C$), 2.72 (q, 1H, $J_{HA/16\text{-Me}}$=7.1 Hz, H$_A$), 2.1–1.5 (H$_{D,E,F,G}$), 1.24 (d, 3H, J=7.1 Hz, 16-CH_3), 1.02 (s, 9H, *tert*-butyl). $C_{23}H_{32}O_{11}$ (484.5) calcd. C, 57.07; H, 6.69; found C, 57.04, H, 6.71.

(2*R*,3*R*,10*R*,14*R*)-2-Mesyl-3-hydroxy-10-methoxymethoxy-ginkgolide-15-acid methyl ester (5a) and (2*R*,3*R*,10*R*,14*S*)-2-mesyl-3-hydroxy-10-methoxymethoxy-ginkgolide-15-acid methyl ester (6a). 0.97 g (2 mmol) **5** or **6**, respectively, was dissolved in 15 ml dichloromethane and at −10°C 0.42 ml (3 mmol) triethylamine and 0.21 ml (2.7 mmol) mesylchloride was added under stirring. The cooling bath

was removed and the stirring was continued for 2 h at room temperature. Since the starting material was not visible anymore on the TLC, the solution was washed sequentially with 1 N HCl, $NaHCO_3$ solution and brine and dried over Na_2SO_4. After removal of the solvent the compound was crystallized from acetone/toluene.

5a: Colorless crystals (yield: 94%), mp 162–163°C (decomp.); R_f=0.45 (toluene/acetone, 8:2), R_f=0.38 (CH_2Cl_2/acetone, 95:5); $[\alpha]_{589}^{20}=-39.2$; $[\alpha]_{578}^{20}=-40.9$; $[\alpha]_{546}^{20}=-48.4$ (c=2, acetonitrile). ^{13}C NMR: Table 2. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=6.17, (s, 1H, 3-OH), 5.92 (s, 1H, H_J), 5.28 (dd, 1H, J=7.7 Hz, J=10.3 Hz, H_B), 5.14 (s, 1H, H_I), 5.01 (d, 1H, two spin system) and 4.93 (d, 1H, two spin system OCH_2O), 4.85 (s_{br}, 1H, H_H), 3.59 (s, 3H, COOCH_3), 3.35 (s, 3H, OCH_3), 3.18 (s+m_c, 4H, SCH_3 and H_C), 2.97 (q, 1H, $J_{HA/16\text{-}Me}$=7.2 Hz, H_A), 2.49 (m_c, solvent and H_D), 2.2–1.5 ($H_{E,F,G}$), 1.28 (d, 3H, $J_{16\text{-}Me/HA}$= 7.2 Hz, 16-CH_3), 1.01 (s, 9H, *tert*-butyl). $C_{24}H_{34}O_{13}S$ (562.4) calcd. C, 51.24; H, 6.09; found C, 51.28, H, 6.11.

6a: Colorless crystals (yield: 94%), mp 184–185°C (decomp.); R_f=0.45 (toluene/acetone, 8:2), R_f=0.37 (CH_2Cl_2/acetone, 95:5); $[\alpha]_{589}^{20}=-31.5$; $[\alpha]_{578}^{20}=-32.8$; $[\alpha]_{546}^{20}=-37.6$ (c=2, acetonitrile). ^{13}C NMR: Table 2. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=6.07 (s, 1H, 3-OH), 5.97 (s, 1H, H_J), 5.18 (s+m, 2H, H_I and H_B), 5.02 (d, 1H, two spin system) and 4.95 (d, 1H, two spin system, J=5.8 Hz, OCH_2O), 4.90 (s_{br}, 1H, H_H), 3.60 (s, 3H, COOCH_3), 3.34 (HDO+OCH_3, and H_C [by H/D exchange]), 2.94 (m_c, 1H, H_A), ≅2.56 (m, H_D) with 2.49 (solvent), 2.2–1.5 ($H_{E,F,G}$), 1.27 (d, 3H, 16-CH_3), 1.02 (s, 9H, *tert*-butyl). $C_{24}H_{34}O_{13}S$ (562.4) calcd C, 51.24; H, 6.09; found C, 51.31, H, 6.11.

(3*R*,10*R*)-3,10-Dihydroxy-14-epiginkgolide (14-epiginkgolide A) (7). 0.5 g (0.9 mmol) **5a** was dissolved in 5 ml dioxane, 4 ml of 2.5 N HCl was added and refluxed for 5 days. The solution was evaporated to dryness in vacuo, ethanol was added twice and evaporated again. Since the residue showed two spots on the TLC, a separation by CC (toluene/acetone, 9:1) was necessary. Aside from **7** (10*R*)-3,14-didehydro-10-hydroxy-ginkgolide (anhydroginkgolide A; R_f=0.53, toluene/acetone, 7:3) was obtained.

7: Colorless crystals from ethanol, mp 255°C (decomp.), R_f=0.23 (toluene/acetone, 7:3), 0.13 (CH_2Cl_2/acetone 9:1). [compare lit.[2] for epimerization of **7** to **1** see lit.[2]]

(3*R*,10*R*)-3,10-Dihydroxy-ginkgolide (ginkgolide A) (1). 0.5 g (0.9 mmol) **6a** was dissolved in 5 ml of dioxane, 4 ml of 2.5 N HCl was added and refluxed for 5 days. The solution was evaporated in vacuo to dryness, ethanol was added twice and evaporated again. The residue was crystallized from a small quantity of ethanol.

1: Colorless crystals; R_f=0.35 (toluene/acetone, 7:3). All analytical data were identical with those of a sample of natural ginkgolide A.[2,11]

Note: In the meantime the synthesis has been carried out with ^{14}C-labelled methyl propionate by the firm 'BlyChem' (Billingham, UK). Thus detailed pharmacological research of ginkgolides is possible.

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