SYNTHESIS AND HIGH-RESOLUTION NMR SPECTRA OF A-*nor*-DERIVATIVES OF 11-DEOXYGLYCYRRHETIC ACID

L. R. Mikhailova,[1] L. A. Baltina,[1,2] R. M. Kondratenko,[2] O. Kunert,[3] L. V. Spirikhin,[1] F. Z. Galin,[1] and G. A. Tolstikov[4]

UDC 547.598.458.22+ 543.422.25

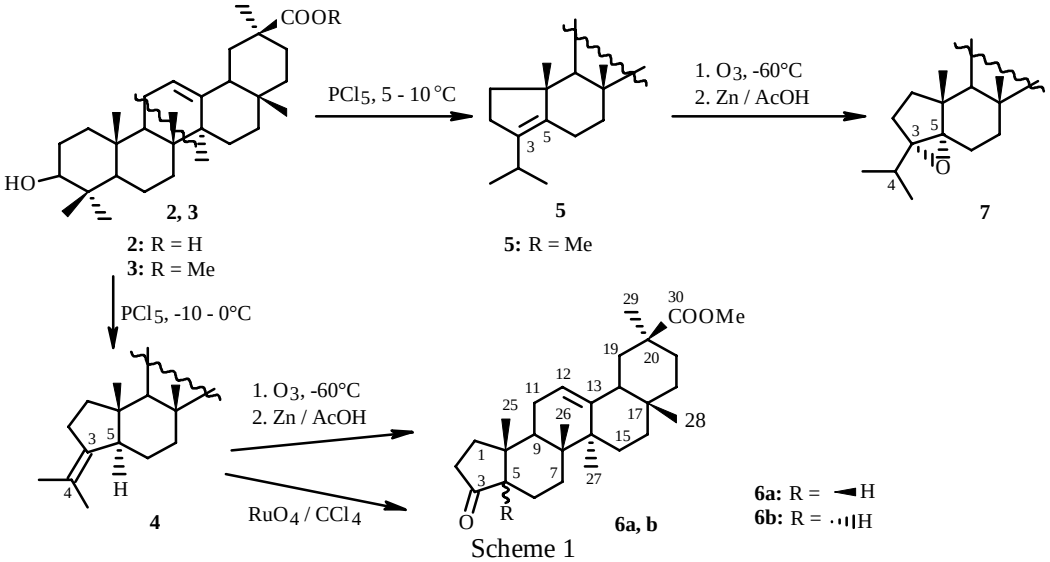
*Several skeletal and oxidative transformations of 11-deoxyglycyrrhetic acid were carried out to produce A-*nor*-derivatives. Signals for protons and C atoms in the PMR and ^{13}C NMR spectra of the A-*nor*-derivatives were assigned using high-resolution ^{1}H (400 MHz) and ^{13}C (125 MHz) NMR spectroscopy.*

Key words: 11-deoxyglycyrrhetic acid, A-*nor*-derivatives, high-resolution NMR spectra.

Transformations of biologically active natural triterpenoids that alter the pentacyclic carbon skeleton are interesting for preparing analogs with new structures and different biological activity [1-5].

Syntheses of A-*nor*-derivatives of glycyrrhetic acid (GA, **1**) [6], the principal triterpenoid of licorice root (*Glycyrrhiza glabra* L. and *G. uralensis* Fisher), oleanolic acid [1, 7], and betulin and lupeol [5, 8, 9] have been published.

The structural similarity of GA and corticosteroids (the 11-on-12-ene group) is known to lead to the appearance of side effects (hypokaliemia, increased arterial pressure, retention of sodium, etc.) with prolonged use of GA preparations, in particular, carbenoxolone [10]. Therefore, the synthesis of derivatives of 11-deoxy-GA (**2**), one of the minor triterpenoids of licorice root, is interesting. We prepared **2** by reduction of GA with Zn powder and HCl in dioxane at 0-10°C according to the literature [11] in 70% yield. Treatment of **2** in CH_3OH with an ether solution of diazomethane isolated the methyl ester of 11-deoxo-GA (**3**) [12], which was used as a substrate for preparing A-*nor*-derivatives (Scheme 1).



Scheme 1

1) Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Sciences, 450054, Ufa, pr. Oktyabrya, 71, fax (3472) 35 60 66, e-mail: baltina@anrb.ru; 2) Bashkir State Medical University, 450000, Ufa, ul. Lenina, 3; 3) Institut fur Pharmazeutische Chemnie und Pharmazeutische Technologie, Kapl-Franzens-Universitat Graz Universitatsplatz 1, A-8010 Graz, Austria, fax: +433163809846, e-mail: olaf.kunert@kfunigraz.ac.at; 4) N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Division, Russian Academy of Sciences, 630090, Novosibirsk, pr. Akad. Lavrent'eva, 9. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 445-448, September-October, 2006. Original article submitted September 7, 2006.

0009-3130/06/4205-0553

TABLE 1. Chemical Shifts in PMR and ^{13}C NMR Spectra (δ, ppm) of 11-Deoxyglycyrrhetic Acid Derivatives with a Cyclopentane Ring A (Pyridine-d_5, 310 K, TMS Internal Standard)

Atom C	**4**		**5**		**7**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	39.6	1.52; 1.02	41.9	1.72; 1.47	35.3	1.17; 1.17
2	29.0	2.10; 2.23	28.4	2.28; 2.21	23.9	1.73; 1.61
3	135.8	-	136.1	-	75.1	-
4	120.9	-	26.8	2.72	28.9	1.77
5	56.0	1.73	140.9	-	75.9	-
6	23.4	1.60; 2.19	20.0	2.37; 1.97	20.2	2.02; 1.33
7	32.9	1.31; 1.50	32.4	1.45; 1.37	29.4	2.02; 1.33
8	40.0	-	40.7	-	40.4	-
9	45.6	1.79	46.6	1.87	41.3	2.25
10	44.2	-	49.8	-	41.3	-
11	25.9	2.03; 1.86	25.6	2.03; 1.84	25.3	2.03; 1.79
12	123.1	5.42	123.5	5.45	123.0	5.44
13	145.3	-	144.8	-	145.4	-
14	42.0	-	42.2	-	42.5	-
15	26.8	1.82; 0.99	26.7	1.82; 1.02	26.7	1.83; 1.03
16	27.4	2.02; 0.88	27.4	1.99; 0.97	27.4	2.00; 0.88
17	32.4	-	32.4	-	32.5	-
18	49.1	2.14	49.3	2.14	49.2	2.14
19	43.3	2.08; 1.76	43.2	2.06; 1.74	43.1	2.07; 1.75
20	44.5	-	44.5	-	44.6	-
21	31.6	2.07; 1.39	31.6	2.06; 1.37	31.5	2.08; 1.38
22	38.9	1.49; 1.40	38.6	1.49; 1.37	38.8	1.49; 1.38
23	23.0	1.62	21.5	0.97	18.7	1.08
24	19.7	1.81	22.1	1.01	19.1	0.95
25	15.0	0.75	19.0	1.00	18.0	0.91
26	17.2	1.02	16.0	1.09	16.6	1.11
27	26.2	1.22	25.8	1.14	26.2	1.30
28	28.5	0.87	28.5	0.87	28.5	0.88
29	28.6	1.22	28.5	1.16	28.5	1.18
30	177.4	-	177.3	-	177.3	-
Me	51.5	3.71	51.5	3.68	51.1	3.70

Ring A in **3** was contracted using PCl_5 in benzene:toluene (1:1) through the classical Wagner—Meerwein rearrangement, which occurred in two directions depending on the reaction temperature. At -10 to 0°C, the methyl ester of 3-isopropylidene-A-*neo*-5α*H*-olean-12-en-30-oic acid (**4**) was formed in 79.2% yield; at 5-10°C, the endo-isomer of A-*nor*-olean-3(5)-ene (**5**) in 82.5% yield.

Oxidation of **4** in CCl_4 by an excess of RuO_4, prepared from $RuCl_3$ and HIO_4, produced a mixture of isomeric 5β*H*- and 5α*H*-A-*nor*-ketones **6a** and **6b**, which were isolated in 62% overall yield by column chromatography (CC) over Al_2O_3 as a 2:1 mixture according to NMR spectroscopy. CC of the reaction products over Al_2O_3 also afforded starting **4** (32% yield).

The ^{13}C NMR spectrum (75.5 MHz) of 4,23,24-tri-*nor*-derivatives **6a** and **6b** gave signals for C-3 and C-5 at 221.4 and 57.8 ppm (5β*H*-isomer) and 219.8 and 57.2 ppm (5α*H*-isomer), respectively. Analogous chemical shifts (CS) were previously reported for the 5β*H*-3-A-*nor*-ketone of allobetulin and the 5α*H*-3-A-*nor*-ketone of oleanolic acid [1, 5].

The PMR spectra of **6a** and **6b** showed two singlets for carbomethoxy groups at 3.64 and 3.68 ppm with a 1:2 ratio of intensities. These belonged to the 5α*H* and 5β*H* isomers, respectively. The signals for the cyclopentane protons and its closest neighbors (H-1, H-2, H-5, H-6) in the PMR spectra of **6a** and **6b** that belong to C-1, C-2, C-5, and C-6 with CS 32.0 (32.2), 38.0 (38.2), 57.2 (57.8), and 17.0 (17.1) ppm, respectively, appeared at 1.80-2.05 ppm as a multiplet.

Recrystallization of the mixture of isomers **6a** and **6b** from ethanol afforded pure single crystals. 5β*H*-A-*nor*-ketone **6a** was characterized by an x-ray structure analysis (Fig. 1).

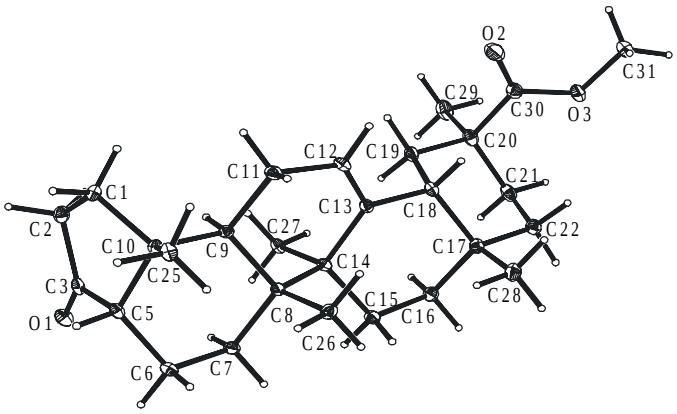


Fig. 1. X-ray structure of the methyl ester of A-*nor*-3-oxo-5βH-olean-12-en-30-oic acid.

Partial ozonolysis of **4** at -60°C in CH_2Cl_2 with subsequent reduction of the peroxide products by Zn/AcOH formed only the 5βH-A-*nor*-ketone **6a**, which was isolated by CC in 58.8% yield. The reaction mixture also contained starting triterpenoid **4** (30%). The ^{13}C NMR spectrum of pure **6a** exhibited only one signal for C-3 (C=O) at 221.4 ppm. The resonance for the 5βH-proton had CS 1.82 ppm (singlet).

Partial ozonolysis of **5** at -60°C produced the 3α,5α-epoxide of **7** in 56% yield. CC of the reaction mixture also produced starting *endo*-isomer **5**, which was identified by TLC with a marker.

The NMR spectra of 11-deoxy-GA derivatives **4**, **5**, and **7** were studied using a high-resolution NMR spectrometer (Varian Unity Inova, working frequency 400 MHz for ^{1}H and 125 MHz for ^{13}C). Signals for protons were assigned using two-dimensional (2D) homonuclear H—H COSY and heteronuclear HSQC and HMBC CH correlation spectroscopy as before [13, 14].

Table 1 gives the CS in the high-resolution PMR and ^{13}C NMR spectra for the studied compounds. It can be seen that the ^{13}C NMR spectrum of **4** contains characteristic signals for olefinic C-3 and C-4 at 135.8 and 120.9 ppm. Two singlets for the isopropylidene methyls in the PMR spectrum have CS 1.62 and 1.81 ppm. The CS for the cyclopentane C atoms of ring A confirm the 5αH-configuration and the *trans*-fusion of rings A/B in **4**. The 5αH signal is observed at 1.73 ppm and correlates with C-5, which has CS 56.0 ppm.

The PMR spectrum of isomeric **5** contains a septet for H-4 at 2.72 ppm. The ^{13}C NMR spectrum has CS 136.1 and 140.9 ppm for C-3 and C-5, respectively.

The ^{13}C NMR spectrum of epoxide **7** shows signals for C–O bonds at 75.1 and 75.9 ppm. Signals of olefinic C atoms C-3 and C-5 are absent. The double bond in ring C is not ozonated by partial ozonolysis, which is consistent with retention of signals for C-12 and C-13 in ^{13}C NMR spectra of **6a** and **7**.

The signals for cyclopentane C-1 and C-2 in the ^{13}C NMR spectrum of **7** are shifted to strong field by 4-5 ppm and correlate with protons at 1.17 and 1.73/1.61 ppm, respectively.

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded on a Varian Unity Inova spectrometer at working frequency 400 and 125 MHz and a Bruker AM 300 at working frequency 300 and 75.5 MHz in Py-d_5 or $CDCl_3$ with TMS internal standard. IR spectra were recorded on a Specord M80 spectrometer. Specific rotations were measured on a Perkin—Elmer 341 MC polarimeter in a 1-dm cuvette.

The x-ray structure analysis (XSA) was performed on a diffractometer using a Bruker SMART APEX2 CCD area detector at 100 K (Mo-Kα-radiation, 2qmax = 52.16°) with a single crystal of dimensions 0.35 × 0.25 × 0.20 mm.*

*The XSA was performed at the Center for x-ray structure analysis of the RAS INEOS, for which we thank the staff of the center.

TLC was performed on Silufol (Czech Rep.) and Sorbfil (Sorbopolimer) plates. Spots were developed using phosphotungstic acid (20%) in ethanol with subsequent heating at 110-120°C for 2-3 min. CC used Al_2O_3 (Brockmann neutral).

11-Deoxy-GA (2) was prepared by reduction of 18β-GA with Zn powder in dioxane with an excess of HCl as before [11] (70% yield), mp 322-325°C, lit. [11] mp 323-325°C. The methyl ester of 11-deoxy-GA (**3**) was isolated by treatment of a solution of **2** in CH_3OH with an ether solution of diazomethane (90% yield) and recrystallization from EtOH:$CHCl_3$, mp 232-234°C, $[\alpha]_D^{20}$ +110° (*c* 0.1, $CHCl_3$), lit. [12] mp 230-232°C, $[\alpha]_D^{20}$ +110° (*c* 0.02, $CHCl_3$).

Solvents were purified by literature methods [15] and evaporated in vacuo at <50°C.

Methyl Ester of 3-Isopropylidene-A-*neo*-5αH-18βH-olean-12-en-30-oic Acid (4). Compound **3** (2 g, 4.25 mmol) was dissolved in a mixture of dry benzene and toluene (1:1, 400 mL), cooled to -10 to 0°C, treated in one portion with PCl_5 (2.1 g), stirred for 1 h with TLC monitoring (until the spot of the starting substrate disappeared), treated with saturated aqueous Na_2CO_3 solution (120 mL), stirred for another 30 min, and warmed to room temperature. The organic layer was separated, washed with water, dried, and evaporated. The solid was recrystallized from $CHCl_3$:EtOH. The mother liquor was chromatographed over a column of Al_2O_3 with elution by benzene to afford **4** (1.52 g, 79.2%) that was homogeneous by TLC, R_f 0.62 (hexane:ethylacetate 10:1), mp 176-178°C, $[\alpha]_D^{20}$ +117.5° (*c* 0.008, $CHCl_3$). $C_{31}H_{48}O_2$, MW = 452.72. Table 1 gives the PMR and ^{13}C NMR spectra.

Methyl Ester of A-*nor*-Olean-3,12-dien-3-(2-isopropyl)-30-oic Acid (5). Compound **3** (2.1 g, 4.6 mmol) was dissolved in a mixture of dry benzene and toluene (1:1, 300 mL), cooled to +10°C, treated in one portion with PCl_5 (2.2 g), stirred for 1 h, treated with saturated aqueous Na_2CO_3 solution (150 mL), stirred for another 30 min, and warmed to room temperature. The organic layer was separated, washed with water, dried, and evaporated. The solid was recrystallized from EtOH:$CHCl_3$. The mother liquor was chromatographed over a column of Al_2O_3 with elution by benzene to afford **5** (1.63 g, 82.5%) that was homogeneous by TLC, R_f 0.57 (benzene), mp 174-176°C, $[\alpha]_D^{20}$ +153.3° (*c* 0.04, $CHCl_3$). $C_{31}H_{48}O_2$. MW = 452.72. Table 1 gives the PMR and ^{13}C NMR spectra.

Methyl Ester of A-*nor*-3-Oxo-5βH-olean-12-en-30-oic Acid (6a). 1. A mixture of CCl_4 (40 mL), CH_3CN (40 mL), and distilled water (60 mL) was vigorously stirred. The two-phase system was treated first with **4** (0.9 g, 2 mmol) and then with $NaIO_4$ (2.7 g, 2.6 mmol) and $RuCl_3 \cdot H_2O$ (30 mg, 13.2 mol% based on $RuCl_3 \cdot H_2O$) in three portions over 5 h. The mixture was vigorously stirred at 20-22°C for 3 d with TLC monitoring and diluted with CH_2Cl_2 (40 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with water, dried over $MgSO_4$, and evaporated. The solid was dissolved in ethylacetate (40 mL) and filtered through a thin layer of cellite. The filtrate was evaporated. The dry solid containing two spots on TLC was chromatographed over a column of Al_2O_3 with elution by $CHCl_3$:CH_3OH (300:1, 200:1, 100:1, v/v). Fractions that were homogeneous by TLC were combined, evaporated, and recrystallized from aqueous ethanol to afford a mixture of **6a** and **6b** (0.52 g, 62%, 2:1 ratio). IR spectrum (ν, cm^{-1}): 1700 (C=O). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm): 0.77, 1.03, 1.12, 1.20, 1.26 (15H, all s, $5CH_3$), 3.64, 3.68 (6H, both s, OCH_3), 1.80-2.05 (m, $H1_b$, H-2, H-5, H-6), 5.30 (1H, t, H-12). ^{13}C NMR spectrum (75.5 MHz, pyridine-d_5, δ, ppm): 32.0, 32.2 (C-1), 38.05, 38.21 (C-2), 219.8, 221.4 (C-3), 57.2, 57.8 (C-5), 17.1, 17.0 (C-6), 29.6 (C-7), 41.8 (C-8), 35.3 (C-9), 40.9 (C-10), 24.7 (C-11), 122.3 (C-12), 144.4 (C-13), 42.5 (C-14), 26.3 (C-15), 26.5 (C-16), 34.9 (C-17), 48.6 (C-18), 39.9 (C-19), 44.2 (C-20), 31.2 (C-21), 35.9 (C-22), 25.1, 25.15, 25.2, 25.25 (C-25, C-29), 16.05, 16.1 (C-26), 28.1, 28.15 (C-27), 28.5 (C-28), 177.6, 177.65 (C-30), 51.6, 51.65 (C-31).

The second compound (upper spot) was isolated by CC over Al_2O_3 and identified by TLC with markers as starting **4** (32%), R_f 0.62.

A second recrystallization of **6a** and **6b** from $CHCl_3$:EtOH at 20-22°C produced single crystals of **6a** (colorless platelike monoclinic crystals). $C_{28}H_{42}O_3$. MW = 426.64, characterized by XSA (Fig. 1). The structure was solved by direct methods. All nonhydrogen atoms were located in difference electron-density syntheses and refined anisotropically over F^2hkl. All calculations were performed using the SHELXTL PLUS5 programs [16].

2. Ozone (~1 mmol) was bubbled through a solution of **4** (0.45 g, 1 mmol) in dry CH_2Cl_2 (50 mL) at -60°C. The reaction mixture was warmed to room temperature. The ozonide was reduced with Zn powder (1 g) in the presence of glacial acetic acid (GAA, 20 mL) for 1 h. The mixture was filtered and neutralized with saturated Na_2CO_3 solution. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The solid was chromatographed over a column of Al_2O_3 with elution by hexane:ethylacetate (25:1, 10:1, 5:1, v/v). Fractions that were homogeneous by TLC were combined and evaporated to afford **6a** (0.30 g, 58.8%) that was recrystallized from EtOH. **6a**, mp 176-178°C, $[\alpha]_D^{20}$ +82.5° (*c* 0.04, CH_3OH). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm): 0.72, 0.98, 1.10, 1.16, 1.23, 1.25 (15H, all s, $5CH_3$), 0.88-1.75 (12H, 2H-1, $H\text{-}6_a$, 2H-7,

2H-15, H-16$_a$, H-19$_a$, H-21$_a$, 2H-22), 1.78 (1H, H-9), 1.82 (1H, s, 5βH), 1.89-2.08 (9H, m, 2H-2, H-6$_b$, 2H-11, H-16$_b$, H-18, H-19$_b$, H-21$_b$), 3.69 (3H, s, OCH_3), 5.33 (1H, t, H-12, J = 13.6). ^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 31.97 (C-1), 38.21 (C-2), 221.39 (C-3), 57.82 (C-5), 17.07 (C-6), 29.6 (C-7), 41.85 (C-8), 35.29 (C-9), 40.95 (C-10), 24.69 (C-11), 122.87 (C-12), 144.42 (C-13), 42.54 (C-14), 26.29 (C-15), 26.54 (C-16), 34.91 (C-17), 48.65 (C-18), 38.96 (C-19), 44.19 (C-20), 31.16 (C-21), 35.30 (C-22), 16.05 (C-26), 28.13, 28.45 (C-28, C-27), 25.07, 25.18 (C-25, C-29), 177.61 (C-30), 51.58 (C-31).

Methyl Ester of 3,5-Epoxy-A-*nor*-olean-12-en-3-(2-isopropyl)-30-oic Acid (7). Ozone (1 mmol) was bubbled through a solution of **5** (0.9 g, 2 mmol) in CH_2Cl_2 (90 mL) at -60°C. The reaction mixture was warmed to room temperature, treated with GAA (20 mL) and Zn powder (2 g), and stirred for 1.5 h. The mixture was filtered. The excess of GAA was neutralized with saturated Na_2CO_3 solution. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The dry solid was chromatographed over a column of Al_2O_3 with elution by benzene to afford **7** (0.51 g, 56%) that was recrystallized from aqueous ethanol, mp 177°C, $[\alpha]_D^{20}$ +35° (*c* 0.02, $CHCl_3$). $C_{29}H_{44}O_3$. MW = 440.66. Table 1 gives the PMR and ^{13}C NMR spectra.

ACKNOWLEDGMENT

The work was supported financially by Rosnauki (2005-RI-12.0/004/088, State Contract 02.434.11.7060), (2006-RI-112.0/001/409, State Contract 02.445.11.7430), the RFBR, and Austria (03-03-20004 BNTSa).

REFERENCES

1. T. Honda and G. W. Gribble, *J. Org. Chem.*, **63**, 4846 (1998).
2. T. Honda, H. J. Finlay, G. W. Gribble, N. Suh, and M. B. Sporn, *Bioorg. Med. Chem. Lett.*, **13**, 1623 (1997).
3. L. A. Tapondjon, F. N. Ngounou, D. Lontsi, B. L. Sondengam, and J. D. Connolly, *Tetrahedron*, **54**, 2099 (1998).
4. J. Sejbal, M. Homolova, I. Tislerova, and V. Krecek, *Collect. Czech. Chem. Commun.*, **65**, 1339 (2000).
5. N. I. Medvedeva, O. B. Flekhter, E. V. Tret′yakova, F. Z. Galin, L. A. Baltina, L. V. Spirikhin, and G. A. Tolstikov, *Zh. Org. Khim.*, **39**, 1140 (2004).
6. G. A. Tolstikov, Kh. A. Alibaeva, and V. M. Potapov, *Zh. Org. Khim.*, **9**, 1631 (1969).
7. T. Konoike, K. Takahashi, Y. Kitaura, and Y. Kanda, *Tetrahedron*, **55**, 14901 (1999).
8. T. Honda, G. W. Gribble, N. Suh, H. J. Finlay, B. Rounds, L. Bore, F. G. Favaloro, Y. Wang, and M. B. Sporn, *J. Med. Chem.*, **43**, 1866 (2000).
9. Y. Deng and J. K. Snyder, *J. Org. Chem.*, **67**, 2864 (2002).
10. R. Pellegata, M. Pinza, G. Pifferi, and C. Farina, *Org. Prep. Proc. Int.*, **31**, 181 (1999).
11. Jpn. Pat. No. 5970638 (1984); *Chem. Abstr.*, **101**, 1715662t.
12. S. R. Mustafina, Candidate Dissertation in Chemical Sciences, Ufa (2001), "Synthesis of new biologically active derivatives of glycyrrhizic acid."
13. W. Seebacher, N. Simic, R. Weins, and O. Kunert, *Magn. Res. Chem.*, **41**, 626 (2003).
14. M. F. Summers, L. G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, **108**, 4285 (1986).
15. A. J. Gordon and R. A. Ford, *A Chemist's Companion*, Wiley-Interscience, New York (1972).
16. G. M. Sheldrick, *SHELXTL*, 5.10, *Structure Determination Software Suite*, Bruker AXS, Madison, Wisconsin, USA (1998).