Obtaining of the Methyl Ester of 2-Cyano-3,12-dioxo-11-deoxo-18-βH-glycyrrhet-1(2),11(9)-dienoic Acid

O. V. SALOMATINA, D. V. KORCHAGINA, N. F. SALAKHUTDINOV and G. A. TOLSTIKOV

Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, Pr. Akademika Lavrentyeva 9, Novosibirsk 630090 (Russia)

E-mail: ana@nioch.nsc.ru

(Received September 24, 2008; revised September 29, 2008)

Abstract

Methyl ester of 2-cyano-3,12-dioxo-11-deoxo-18-βH-glycyrrhet-1(2),11(9)-dienoic acid has been synthesized employing a combined modification of A and C rings of 18-βH-glycyrrhetic acid

Key words: 18-βH-glycyrrhetic acid, triterpenoids, synthesis

INTRODUCTION

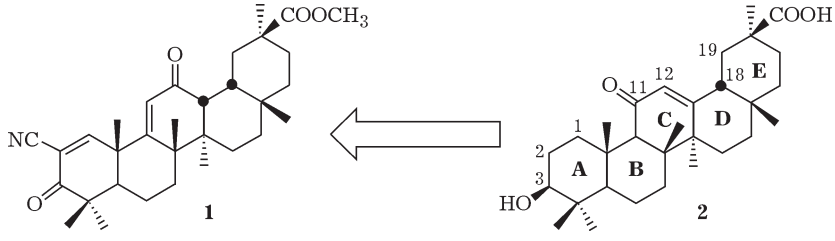
A wide spectrum of the biological activity of naturally occurring triterpenoids (anti-inflammatory, antiviral, antineoplastic (antitumor), immunopotentiating, *etc.* activities) as well as the availability of sources for obtaining them cause the compounds of the mentioned class to be promising for using in the creation of modified derivatives and pharmaceutical preparations based on these substances.

It is known that some derivatives of triterpenoids are promising as antitumor preparations [1]. So, for example, such synthetic triterpenoid as CDDO (2-cyano-3,12-dioxoolean-1,9-diene-28-oic acid) obtained from oleanolic acid exhibits antineoplastic activity with respect to a wide variety of cancer cells (IC_{50} = 10^{-6}–10^{-9} M) [2]. The CDDO also causes the apoptosis of myeloid leucaemia cells (IC_{50} ~ 10^{-6} M) [3] as well as inhibiting the growth of the cells of epithelial ovarian carcinoma (IC_{50} = 1.1–3.0 · 10^{-6} M) [4].

The aim of the present work consisted in the obtaining of methyl ester of 2-cyano-3,12-dioxo-11-deoxo-18-βH-glycyrrhet-1(2),11(9)-dienoic acid **1** (methyl ester of 2-cyano-3,12-dioxo-18-βH-olean-1,9-diene-30-oic acid), a structural analogue of the CDDO, *via* modifying 18-βH-glycyrrhetic acid **2** (Scheme 1).

EXPERIMENTAL

The elemental composition of the substances obtained was established basing on data resulted from high resolution mass spectra registered employing a DFS (Double Focusing Sector) mass spectrometer (Thermo Electron Corporation).



Scheme 1.

For the synthesis we used 94 % purity (HPLC) acetate of glycyrrhetic acid **3**, obtained *via* the deglycolization of glycyrrhizic acid in acetic acid medium [5].

^{1}H and ^{13}C NMR spectra were registered for substances dissolved in $CDCl_3$ employing Bruker NMR spectrometers such as AM-400 (operating frequency values being of 400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C) and DRX-500 (500.13 and 125.76 MHz, respectively). Signals resulted from solvent (δ_H = 7.24 and δ_C = 76.9 ppm) were used as an internal standard. The structure of compounds obtained was established basing on the analysis of ^{1}H NMR spectra attracting ^{1}H–^{1}H double resonance spectra and two-dimension spectra of homonuclear ^{1}H–^{1}H correlation, as well as the analysis of ^{13}C NMR spectra registered in the mode *J*-modulation (JMOD), with out-of-resonance suppression of signals from protons as well as two-dimension spectra of heteronuclear ^{13}C–^{1}H correlation for direct and for long-range spin-spin coupling constants (C–H COSY, $^1J_{C,H}$ = 135 Hz, and COLOC, $^{2,3}J_{C,H}$ = 10 Hz, respectively).

Obtaining of acetate of the methyl ester of 18-βH-glycyrrhetic acid (4). To a suspension of 10 g (19.0 mmol) of acetate of 18-βH-glycyrrhetic acid **3** in 200 mL of methanol under stirring was added a diazomethane solution in diethyl ether until the solution grew pale yellow in colour. After the specific colouring completely disappeared, the solvent was distilled off employing a rotary evaporator; a precipitate obtained was recrystallized from methanol-chloroform mixture. The mass of compound **4** obtained amounted to 9.1 g (the yield being of 88.6 %, or 96 % as recalculated for pure substance). M. p. 303–304 °C. Found, *m/z*: 526.3649 $[M]^+$. $C_{33}H_{50}O_5$. Calculated herefrom: *M* 526.3653. ^{1}H NMR spectrum of compound **4**, δ, ppm, (*J*, Hz): 0.76 dd (H^{5a}, $J_{5a,6a}$ 12.5, $J_{5a,6e}$ 1.5), 0.76 s ($C^{28}H_3$), 0.84 s (6H, $C^{23}H_3$, $C^{24}H_3$), 0.97 dm (H^{16e}, $J_{16e,16a}$ 13.8), 1.01 ddd (H^{1a}, $J_{1a,1e}$ 13.5, $J_{1a,2a}$ 13.5, $J_{1a,2e}$ 3.7), 1.08 s ($C^{26}H_3$), 1.10 s ($C^{29}H_3$), 1.12 s ($C^{25}H_3$), 1.14 dm (H^{15e}, $J_{15e,15a}$ 13.8), 1.23–1.39 m (H^7, H^{21a}, $2H^{22}$), 1.32 s ($C^{27}H_3$), 1.41 dddd (H^{6a}, $J_{6a,6e}$ 13.5, $J_{6a,7a}$ 13.5, $J_{6a,5a}$ 12.0, $J_{6a,7e}$ 3.2), 1.51–1.66 m (H^{6e}, H^{2e}, $H^{7'}$), 1.57 dd (H^{19a}, $J_{19a,19e}$ 13.5, $J_{19a,18a}$ 13.5), 1.66 dddd (H^{2a}, $J_{2a,2e}$ 13.5, $J_{2a,1a}$ 13.5, $J_{2a,3a}$ 11.7, $J_{2a,1e}$ 3.7), 1.78 ddd (H^{15a}, $J_{15a,15e}$ 13.8, $J_{15a,16a}$ 13.8, $J_{15a,16e}$ 4.5), 1.88 ddd (H^{19e}, $J_{19e,19a}$ 13.5, $J_{19e,18a}$ 4.2, $J_{19e,21e}$ 2.7), 1.95 dm (H^{21e}, $J_{21e,21a}$ 10), 1.98 ddd (H^{16a}, $J_{16a,16e}$ 13.8, $J_{16a,15a}$ 13.8, $J_{16a,15e}$ 4.8), 2.00 s ($C^{33}H_3$), 2.04 dd (H^{18a}, $J_{18a,19a}$ 13.5, $J_{18a,19e}$ 4.2), 2.32 s (H^{9a}), 2.76 ddd (H^{1e}, $J_{1e,1a}$ 13.5, $J_{1e,2a}$ 3.7, $J_{1e,2e}$ 3.0), 3.64 s ($OC^{31}H_3$), 4.47 dd (H^{3a}, $J_{3a,2a}$ 11.7, $J_{3a,2e}$ 4.7), 5.62 s (H^{12}). ^{13}C NMR spectrum of compound **4**, δ, ppm: 38.63 t (C^1), 23.41 t (C^2), 80.45 d (C^3), 37.88 s (C^4), 54.88 d (C^5), 17.22 t (C^6), 32.55 t (C^7), 43.03 s (C^8), 61.56 d (C^9), 36.78 s (C^{10}), 199.85 s (C^{11}), 128.34 d (C^{12}), 169.01 s (C^{13}), 45.23 s (C^{14}), 26.31 t (C^{15}), 26.26 t (C^{16}), 31.67 s (C^{17}), 48.25 d (C^{18}), 40.93 t (C^{19}), 43.87 s (C^{20}), 30.98 t (C^{21}), 37.59 t (C^{22}), 27.89 q (C^{23}), 16.52 q (C^{24}), 16.24 q (C^{25}), 18.52 q (C^{26}), 23.17 q (C^{27}), 28.36 q (C^{28}), 28.15 q (C^{29}), 176.73 s (C^{30}), 51.58 q (C^{31}), 170.77 s (C^{32}), 21.13 q (C^{33}).

Obtaining of acetate of the methyl ester of 11-deoxo-18-βH-glycyrrhetic acid (5). To a suspension of 9.1 g (17.3 mmol) of acetate of the methyl ester of glycyrrhetic acid **4** and 18.2 g of zinc dust in 300 mL of dioxane at 5 °C was added dropswise 50 mL of concentrated HCl during 2 h. Maintaining the temperature within the range of 5–10 °C, the reaction mixture was stirred during 3 h more. Then a half of dioxane volume was distilled off using a rotary evaporator and then the residue obtained was poured into 1 L of water. The precipitate obtained was filtered, dried in air, and then it was chromatographed employing a column with silica gel (the eluent represented benzene with a 0–100 % chloroform gradient). The yield amounted to 6.8 g (76.8 %). In order to obtain an analytically pure sample, we recrystallized 100 mg of compound **5** from a methanol-chloroform mixture. M. p. 265–267 °C. Found, *m/z*: 512.3649 $[M]^+$. $C_{33}H_{52}O_4$. Calculated herefrom: *M* 512.3653. ^{1}H NMR spectrum of compound **5**, δ, ppm (*J*, Hz): 0.74 s ($C^{28}H_3$), 0.81 dd (H^{5a}, $J_{5a,6a}$ 12.0, $J_{5a,6e}$ 1.6), 0.82–0.86 m (H^{16e}), 0.83 s ($C^{24}H_3$), 0.84 s ($C^{23}H_3$), 0.93 s ($C^{25}H_3$, $C^{26}H_3$), 0.94 dm (H^{15e}, $J_{15e,15a}$ 13.5), 1.02 m (H^1), 1.09 s ($C^{29}H_3$), 1.10 s ($C^{27}H_3$), 1.17–1.35 m (H^7, H^{21}, $2H^{22}$), 1.39 m (H^{6a}), 1.44–1.64 m ($H^{1'}$, $2H^2$, H^{6e}, $H^{7'}$, H^{9a}, H^{19}), 1.73 ddd (H^{15a}, $J_{15a,15e}$ 13.5, $J_{15a,16a}$ 13.5, $J_{15a,16e}$ 4.6), 1.79–1.93 m ($2H^{11}$, H^{18}, $H^{19'}$, $H^{21'}$), 1.92 m (H^{16a}), 2.01 s ($C^{33}H_3$), 3.64 s ($OC^{31}H_3$), 4.47 dd (H^{3a}, $J_{3a,2a}$ 10.0, $J_{3a,2e}$ 6.0), 5.23 t (H^{12}, $J_{12,11}$ 3.6). ^{13}C NMR spectrum of compound **5**, δ, ppm: 38.13

t (C^{1}), 23.42 t (C^{2}), 80.74 d (C^{3}), 37.56 s (C^{4}), 55.13 d (C^{5}), 18.11 t (C^{6}), 32.46 t (C^{7}), 39.65 s (C^{8}), 47.42 d (C^{9}), 36.70 s (C^{10}), 23.34 t (C^{11}), 122.34 d (C^{12}), 144.23 s (C^{13}), 41.38 s (C^{14}), 25.99 t (C^{15}), 26.82 t (C^{16}), 31.79 s (C^{17}), 48.05 d (C^{18}), 42.68 t (C^{19}), 44.12 s (C^{20}), 31.15 t (C^{21}), 38.25 t (C^{22}), 27.89 q (C^{23}), 16.54 q (C^{24}), 15.41 q (C^{25}), 16.64 q (C^{26}), 25.77 q (C^{27}), 28.03 q (C^{28}), 28.38 q (C^{29}), 177.46 s (C^{30}), 51.35 q (C^{31}), 170.77 s (C^{32}), 21.13 q (C^{33}).

Obtaining of acetate of the methyl ester of 12-oxo-11-deoxo-18-βH-glycyrrhetic acid (6). To a solution containing 3.0 g (5.7 mmol) of acetate of the methyl ester of 11-deoxo-18-βH-glycyrrhetic acid **5** in 100 mL of glacial acetic acid at 80 °C was added dropwise a mixture of 25 mL hydrogen peroxide (~30 %) with 25 mL of acetic acid during 1 h. Then the reaction mixture was stirred during 30 min more, cooled and poured into water. The precipitate formed was filtered and dried in air. The obtained mass of compound 6 amounted to 2.9 g (the yield being of 96 %). In order to obtain an analytically pure sample we recrystallized 300 mg of compound of **6** from a methanol-chloroform mixture. M. p. 296–299 °C. Found, *m/z:* 528.3649 $[M]^{+}$. $C_{33}H_{52}O_{5}$. Calculated herefrom: *M* 528.3653. ^{1}H NMR spectrum of compounds **6**, δ, ppm (*J*, Hz): 0.79–0.88 m (H^{5a}, H^{16e}), 0.82 s ($C^{28}H_3$), 0.83 s ($C^{24}H_3$), 0.84 s ($C^{23}H_3$), 0.86 s ($C^{25}H_3$), 0.90 s ($C^{27}H_3$), 0.92–1.01 m (H^{1}, H^{15e}), 1.09 s ($C^{29}H_3$), 1.10 s ($C^{26}H_3$), 1.19 dd (H^{19a}, $J_{19a,19e}$ 13.4, $J_{19a,18}$ 13.4), 1.18–1.27 m (H^{21a}, H^{22e}), 1.31–1.48 m ($2H^{7}$, H^{22a}, H^{6}), 1.49–1.64 m ($H^{1'}$, $2H^{2}$, $H^{6'}$), 1.64 dd (H^{9a}, $J_{9a,11a}$ 13.0, $J_{9a,11e}$ 5.1), 1.74 m (H^{18}), 1.76 m (H^{15a}), 1.85 ddd (H^{16a}, $J_{16a,16e}$ 13.2, $J_{16a,15a}$ 13.2, $J_{16a,15e}$ 4.2), 1.91 dm (H^{21e}, $J_{21e,21a}$ 13.2), 2.00 s ($C^{33}H_3$), 2.12 dd (H^{11a}, $J_{11a,11e}$ 17.0, $J_{11a,9a}$ 13.0), 2.23 dd (H^{11e}, $J_{11e,11a}$ 17.0, $J_{11e,9a}$ 5.1), 2.54 ddd (H^{19e}, $J_{19e,19a}$ 13.4, $J_{19e,18}$ 3.4, $J_{19e,21e}$ 2.8), 2.72 d (H^{13}, $J_{13,18}$ 4.4), 3.68 c ($OC^{31}H_3$), 4.44 dd (H^{3a}, $J_{3a,2a}$ 11.4, $J_{3a,2e}$ 4.8). ^{13}C NMR spectrum of compound **6**, δ, ppm: 37.50 t (C^{1}), 23.26 t (C^{2}), 80.28 d (C^{3}), 37.60 s (C^{4}), 55.04 d (C^{5}), 18.05 t (C^{6}), 31.63 t (C^{7}), 41.41 s (C^{8}), 49.32 d (C^{9}), 36.67 s (C^{10}), 38.28 t (C^{11}), 211.91 s (C^{12}), 50.08 d (C^{13}), 41.90 s (C^{14}), 25.84 t (C^{15}), 26.29 t (C^{16}), 32.01 s (C^{17}), 38.41 d (C^{18}), 34.05 t (C^{19}), 43.97 s (C^{20}), 31.19 t (C^{21}), 38.33 t (C^{22}), 27.75 q (C^{23}), 16.30 q (C^{24}), 15.16 q (C^{25}), 15.95 q (C^{26}), 20.78 q (C^{27}), 26.83 q (C^{28}), 28.59 q (C^{29}), 177.44 s (C^{30}), 51.36 q (C^{31}), 170.70 s (C^{32}), 21.09 q (C^{33}).

Obtaining of acetate of the methyl ester of 12-oxo-11(9)-ene-11-deoxoglycyrrhetic acid (7). To a solution of 4.0 g (7.6 mmol) of acetate of the methyl ester of 12-oxo-11-deoxo-18-βH-glycyrrhrtic acid **6** in glacial acetic acid (150 mL) at 80 °C was dropwise added 50.0 mL (9.8 mmol) of 3 % bromine solution in glacial acetic acid during 1 h. The reaction mixture was held during 1 h at the same temperature, then it was cooled and poured into 1.5 L of cold water. The precipitate obtained was filtered, washed with water and dried in air. Recrystallizing it from the mixture of methanol and chloroform we obtained 3.0 g of product **7** (the yield being of 75.3 %). M. p. 298 °C. Found, *m/z:* 526.3490 $[M]^{+}$. $C_{33}H_{50}O_{5.}$ Calculated herefrom: *M* 526.3653. ^{1}H NMR spectrum of compound **7**, δ, ppm (*J*, Hz): 0.84 m (H^{16e}), 0.85 s ($C^{24}H_3$), 0.86 s (6H: $C^{23}H_3$, $C^{28}H_3$), 0.91 s ($C^{27}H_3$), 0.94 dm (H^{5a}, $J_{5a,6a}$ 10), 1.01 dm (H^{15e}, $J_{15e,15a}$ 13.2), 1.07 s ($C^{29}H_3$), 1.16 s ($C^{25}H_3$), 1.20 dd (H^{19a}, $J_{19a,19e}$ 13.3, $J_{19a,18}$ 13.3), 1.16–1.27 m (H^{21a}, H^{22e}), 1.32 c ($C^{26}H_3$), 1.35–1.44 m (H^{1a}, H^{7e}), 1.46 ddd (H^{22a}, $J_{22a,22e}$ 14.0, $J_{22a,21a}$ 14.0, $J_{22a,21e}$ 4.2), 1.55–1.73 m ($2H^{6}$, H^{7a}, $2H^{2}$), 1.77 m (H^{15a}), 1.83 m (H^{16a}), 1.90 dm (H^{1e}, H^{21e}), 1.93 dm (H^{18}, $J_{18,19a}$ 13.3), 2.00 s ($C^{33}H_3$), 2.16 ddd (H^{19e}, $J_{19e,19a}$ 13.3, $J_{19e,18}$ 3.4, $J_{19e,21e}$ 2.8), 2.92 d (H^{13}, $J_{13,18}$ 4.7), 3.69 s ($OC^{31}H_3$), 4.43 dd (H^{3a}, $J_{3a,2a}$ 11.7, $J_{3a,2e}$ 4.5), 5.52 s (H^{11}). ^{13}C NMR spectrum of compound **7**, δ, ppm: 35.86 t (C^{1}), 23.66 t (C^{2}), 79.49 d (C^{3}), 37.95 s (C^{4}), 50.08 d (C^{5}), 17.67 t (C^{6}), 32.66 t (C^{7}), 45.29 s (C^{8}), 177.61 s (C^{9}), 39.59 s (C^{10}), 122.89 d (C^{11}), 201.14 s (C^{12}), 47.70 d (C^{13}), 41.61 s (C^{14}), 26.03 t (C^{15}), 26.03 t (C^{16}), 31.87 s (C^{17}), 37.81 d (C^{18}), 33.69 t (C^{19}), 43.88 s (C^{20}), 31.10 t (C^{21}), 38.13 t (C^{22}), 27.77 q (C^{23}), 16.46 q (C^{24}), 23.79 q (C^{25}), 23.85 q (C^{26}), 21.84 q (C^{27}), 26.90 q (C^{28}), 28.45 q (C^{29}), 177.22 s (C^{30}), 51.32 q (C^{31}), 170.59 s (C^{32}), 21.01 q (C^{33}).

Obtaining of the methyl ester of 12-oxo-11-deoxo-18-βH-glycyrrhet11 (9)-enoic acid (8). A solution of acetate of the methyl ester of 12-oxo-11(9)-ene-11-deoxo-18-βH-glycyrrhetic acid **7** 6.2 g in mass (11.8 mmol) and KOH (41 g, or 732 mmol) in metanole (400 mL) was boiled during 2 h. The reaction mixture was then cooled and the most part of methyl alcohol was distilled off employing a rotary evaporator. Then

we added ethyl acetate (100 mL) and 5 % HCl solution. After that we performed an extraction procedure using the mixture of chloroform and ethyl acetate, taken in the proportion of 1 : 4 (3 × 75 mL). Joined extract was washed with saturated solutions of $NaHCO_3$ (3 × 50 mL) and NaCl (3 × 50 mL)), then it was dried with the use of $MgSO_4$. The solvent was distilled off by means of a rotary evaporator; the mass of obtained product **8** amounted to 5.13 g (the yield being of 90.0 %). In order to obtain an analytically pure sample we recrystallized of 130 mg of the product from a methanol-chloroform mixture. For the further transformations we used the without any additional purification. M. p. 202–203 °C. Found, *m/z:* 484.3385 $[M]^+$. $C_{31}H_{48}O_4$. Calculated herefrom: *M* 484.3396. ^{1}H NMR spectrum of compound **8**, δ, ppm (*J*, Hz): 0.80 s ($C^{24}H_3$), 0.89 s ($C^{28}H_3$), 0.94 s ($C^{27}H_3$), 1.01 s ($C^{23}H_3$), 1.08 s ($C^{29}H_3$), 1.16 s ($C^{25}H_3$), 1.35 s ($C^{26}H_3$), 0.83–0.91 m (H^{5a}, H^{16e}), 1.04 dm (H^{15e}, $J_{15e,15a}$ 12.8), 1.23 dd (H^{19a}, $J_{19a,19e}$ 13.2, $J_{19a,18}$ 13.2), 1.18–1.25 m (H^{21a}), 1.27 dm (H^{22e}, $J_{22e,22a}$ 14.0), 1.31 m (H^{1a}), 1.44 dm (H^{7e}, $J_{7e,7a}$ 9.8), 1.49 ddd (H^{22a}, $J_{22a,22e}$ 14.0, $J_{22a,21a}$ 14.0, $J_{22a,21e}$ 4.2), 1.55–1.76 m ($2H^6$, H^{7a}, $2H^2$), 1.80 m (H^{15a}), 1.85 m (H^{16a}), 1.89–2.00 m (H^{1e}, H^{21e}, H^{18}), 2.19 ddd (H^{19e}, $J_{19e,19a}$ 13.2, $J_{19e,18}$ 3.2, $J_{19e,21e}$ 2.7), 2.95 d (H^{13}, $J_{13,18}$ 4.7 Hz), 3.18 dd (H^{3a}, $J_{3a,2a}$ 11.7, $J_{3a,2e}$ 4.4), 3.71 s ($OC^{31}H_3$), 5.76 s (H^{11}). ^{13}C NMR spectrum of compound **8**, δ, ppm: 36.22 t (C^1), 27.38 t (C^2), 77.83 d (C^3), 39.11 s (C^4), 50.06 d (C^5), 17.87 t (C^6), 32.79 t (C^7), 45.39 s (C^8), 178.10 s (C^9), 39.81 s (C^{10}), 122.85 d (C^{11}), 201.36 s (C^{12}), 47.76 d (C^{13}), 41.70 s (C^{14}), 26.14 t (C^{15}), 26.12 t (C^{16}), 31.95 t (C^{17}), 37.88 d (C^{18}), 33.79 t (C^{19}), 43.95 s (C^{20}), 31.17 t (C^{21}), 38.21 t (C^{22}), 27.96 q (C^{23}), 15.44 q (C^{24}), 23.81 q (C^{25}), 23.87 q (C^{26}), 21.99 q (C^{27}), 26.96 q (C^{28}), 28.52 q (C^{29}), 177.33 s (C^{30}), 51.43 q (C^{31}).

Obtaining of the methyl ester 3,12-dioxo-11-deoxo-18-βH-glycyrrhet-11(9)-enoic acid (9). To a solution of the methyl ester 12-oxo-11(9)-ene-11-deoxo-18-βH-glycyrrhetic acid **8** (5.0 g, or 10.3 mmol) in acetone (500 mL) was added dropwise 5 mL of the Jones reagent (saturated solution of $Na_2Cr_2O_7 \cdot 2H_2O$ in 33 % H_2SO_4 solution) [6]. The reaction mixture was stirred during 2.5 h, then 50 mL of ethyl alcohol was added and the mixture was stirred for 30 min more in order to decompose chromic acid. Then the reaction mixture was concentrated by means of evaporation employing a rotary evaporator to obtain the volume of ~100 mL and poured into water (1 L). The precipitate formed was filtered and dried in air. For purifying the substance was dissolved in chloroform and filtered through the layer of aluminium oxide. The filtrate obtained was concentrated via evaporation, the mass of obtained compound **9** amounted to 4.7 g (the yield being of 94.4 %). In order to obtain an analytically pure sample we recrystallized 200 mg of the product from the mixture of methanol and chloroform. For the further transformations we used the product without any additional purification. M. p. 189–192 °C. Found, *m/z:* 482.3235 $[M]^+$. $C_{31}H_{46}O_4$. Calculated herefrom: *M* 482.3240. ^{1}H NMR spectrum of compounds **9**, δ, ppm (*J*, Hz): 0.88 s ($C^{28}H_3$), 0.94 s ($C^{27}H_3$), 1.05 s ($C^{24}H_3$), 1.07 s ($C^{29}H_3$), 1.08 s ($C^{23}H_3$), 1.27 s ($C^{25}H_3$), 1.38 s ($C^{26}H_3$), 0.89 m (H^{16e}), 1.06 m (H^{15e}), 1.21 dd (H^{19a}, $J_{19a,19e}$ 13.3, $J_{19a,18}$ 13.3), 1.17–1.29 m (H^{21a}, H^{22e}), 1.43–1.50 m (H^{5a}, H^7), 1.48 ddd (H^{22a}, $J_{22a,22e}$ 14.0, $J_{22a,21a}$ 14.0, $J_{22a,21e}$ 4.3), 1.60–1.72 m ($2H^6$, $H^{7'}$), 1.75 m (H^{1a}), 1.80 m (H^{15a}), 1.85 m (H^{16a}), 1.92 m (H^{21e}), 1.96 ddd (H^{18}, $J_{18,19a}$ 13.3, $J_{18,13}$ 4.7, $J_{18,19e}$ 3.2), 2.16 m (H^{1e}), 2.17 ddd (H^{19e}, $J_{19e,19a}$ 13.3, $J_{19e,18}$ 3.2, $J_{19e,21e}$ 2.8), 2.44 ddd (H^{2e}, $J_{2e,2a}$ 15.8, $J_{2e,1a}$ 7.2, $J_{2e,1e}$ 3.8), 2.60 ddd (H^{2a}, $J_{2a,2e}$ 15.8, $J_{2a,1a}$ 11.5, $J_{2a,1e}$ 7.3), 2.98 d (H^{13}, $J_{13,18}$ 4.7), 3.70 s ($OC^{31}H_3$), 5.78 s (H^{11}). ^{13}C NMR spectrum of compound **9**, δ, ppm: 36.80 t (C^1), 33.97 t (C^2), 215.57 s (C^3), 47.35 s (C^4), 50.73 d (C^5), 18.97 t (C^6), 31.90 t (C^7), 45.46 s (C^8), 176.36 s (C^9), 39.23 s (C^{10}), 124.10 d (C^{11}), 200.78 s (C^{12}), 47.86 d (C^{13}), 41.78 s (C^{14}), 26.13 t (C^{15}), 26.11 t (C^{16}), 31.90 s (C^{17}), 37.82 d (C^{18}), 33.76 t (C^{19}), 43.90 s (C^{20}), 31.12 t (C^{21}), 38.14 t (C^{22}), 26.10 q (C^{23}), 21.25 q (C^{24}), 23.69 q (C^{25}), 23.75 q (C^{26}), 21.81 q (C^{27}), 26.94 q (C^{28}), 28.45 q (C^{29}), 177.19 s (C^{30}), 51.37 q (C^{31}).

Obtaining of the methyl ester of 2-hydroxymethylene-3,12-dioxo-11-deoxo-18-βH-glycyrrhet-11(9)-enoic acid (10). To a solution of the methyl ester of 3-oxo-12-oxo-11(9)-ene-11-deoxo-18-βH-glycyrrhetic acid **9** (4.5 g, or 9.3 mmol), ethyl formate (3.25 mL, or 39.5 mmol) in benzene (48 mL) was added sodium methylate (2.1 g, or 38.9 mmol) a little at a time, under stirring. The reaction mixture was stirred during 2 h at a room temperature. Then the

reaction mixture was added with diethyl ether (100 mL) and 5 % HCl solution for pH < 7 to be reached. Then it was extracted by means of a 1 : 3 chloroform-diethyl ether mixture (3 × 50 mL). Joined extract was washed by saturated solutions of $NaHCO_3$ (3 × 50 mL) and NaCl (3 × 50 mL)), then it was dried with the use of magnesium sulphate. The solvent was distilled off employing a rotary evaporator; the mass of product **10** obtained amounted to 4.5 g (the yield being of 95.0 %). In order to obtain an analytically pure sample we purified 100 mg of the reaction mixture by means of column cromatography on silica gel (the eluent represented hexane with a 10–25 % ethyl acetate gradient). For the further transformations the product was used without any additional purification. Found, *m/z*: 510.3101 $[M]^+$. $C_{32}H_{46}O_5$. Calculated herefrom: *M* 510.3177. ^{1}H NMR spectrum of compound **10**, δ, ppm (*J*, Hz): 0.90 s ($C^{28}H_3$), 0.96 s ($C^{27}H_3$), 1.09 s ($C^{29}H_3$), 1.13 s ($C^{24}H_3$), 1.15 s ($C^{25}H_3$), 1.20 s ($C^{23}H_3$), 1.38 s ($C^{26}H_3$), 0.91 m (H^{16e}), 1.07 m (H^{15e}), 1.23 dd (H^{19a}, $J_{19a,19e}$ 13.3, $J_{19a,18}$ 13.3), 1.18–1.31 m (H^{21a}, H^{22e}, H^{5a}), 1.49 ddd (H^{22a}, $J_{22a,22e}$ 14.0, $J_{22a,21a}$ 14.0, $J_{22a,21e}$ 4.1), 1.47–1.53 m (H^7), 1.59–1.67 m ($2H^6$, $H^{7'}$), 1.82 m (H^{15a}), 1.86 m (H^{16a}), 1.94 dddd (H^{21e}, $J_{21e,21a}$ 13.3, $J_{21e,22a}$ 4.1, $J_{21e,22e}$ 3.4, $J_{21e,19e}$ 2.7), 1.99 ddd (H^{18}, $J_{18,19a}$ 13.3, $J_{18,13}$ 4.6, $J_{18,19e}$ 3.3), 2.21 ddd (H^{19e}, $J_{19e,19a}$ 13.3, $J_{19e,18}$ 3.3, $J_{19e,21e}$ 2.7), 2.26 d (H^1, $J_{1,1'}$ 14.5 Hz) и 2.58 d ($H^{1'}$, $J_{1',1}$ 14.5) – system AB, 3.02 d (H^{13}, $J_{13,18}$ 4.6), 3.71 s ($OC^{31}H_3$), 5.90 s (H^{11}), 8.70 d (H^{32}, $J_{32,OH}$ 2.4), 14.81 d (OH, $J_{OH,32}$ 2.4). ^{13}C NMR spectrum of compound **10**, δ, ppm: 36.85 t (C^1), 104.80 s (C^2), 188.08 s (C^3), 40.32 s (C^4), 48.07 d (C^5), 18.80 t (C^6), 31.25 t (C^7), 45.55 s (C^8), 175.36 s (C^9), 38.89 s (C^{10}), 124.37 d (C^{11}), 200.73 s (C^{12}), 47.86 d (C^{13}), 41.80 s (C^{14}), 26.22 t (C^{15}), 26.14 t (C^{16}), 31.93 s (C^{17}), 37.82 d (C^{18}), 33.74 t (C^{19}), 43.93 s (C^{20}), 31.14 t (C^{21}), 38.18 t (C^{22}), 28.14 q (C^{23}), 20.65 q (C^{24}), 23.36 q (C^{25}), 23.23 q (C^{26}), 21.82 q (C^{27}), 26.96 q (C^{28}), 28.48 q (C^{29}), 177.22 s (C^{30}), 51.42 q (C^{31}), 189.65 d (C^{32}).

Obtaining of the methyl ester of isoxazolo[4,5-*b*]-12-oxo-11-deoxo-18-βH-glycyrrhet-11(9)-enoic acid (11). A mixture of the methyl ester of 2-hydroxymethylene-3-oxo-11-deoxo-18-βH-glycyrrhetic acid **10** (4.4 g, or 8.6 mmol), ethyl alcohol (120 mL) and hydroxylamine hydrochloride (6.0 g, or 86.0 mmol) and water (12 mL) was boiled using a backflow condenser during 2 h. The reaction mixture was cooled, ethyl alcohol was distilled off by means of a rotary evaporator; and then we added water and extracted the mixture by ethyl acetate (2 × 50 mL). Joined extract was washed by saturated solutions of $NaHCO_3$ (3 × 50 mL) and NaCl (3 × 50 mL), then it was dried with the use of magnesium sulphate. The solvent was distilled off employing a rotary evaporator; the residue obtained was chromatographed using a column packed with silica gel (the eluent represented hexane with a 10–20 % ethyl acetate gradient). The mass of obtained product **11** amounted to 3.2 g (the yield being of 73.3 %). Found, *m/z*: 507.3164 $[M]^+$. $C_{32}H_{45}NO_4$. Calculated herefrom: *M* 507.3181. ^{1}H NMR spectrum of compound **11**, δ, ppm (*J*, Hz): 0.91 s ($C^{28}H_3$), 0.98 s ($C^{27}H_3$), 1.10 s ($C^{29}H_3$), 1.15 s ($C^{25}H_3$), 1.24 s ($C^{24}H_3$), 1.32 s ($C^{23}H_3$), 1.40 s ($C^{26}H_3$), 0.91 m (H^{16e}), 1.06–1.11 m (H^{15e}), 1.24 dd (H^{19a}, $J_{19a,19e}$ 13.2, $J_{19a,18}$ 13.2), 1.19–1.34 m (H^{21a}, H^{22e}), 1.44–1.57 m (H^{5a}, H^{22a}, H^7), 1.64–1.79 m ($2H^6$, $H^{7'}$), 1.84 m (H^{15a}), 1.88 m (H^{16a}), 1.95 dddd (H^{21e}, $J_{21e,21a}$ 13.3, $J_{21e,22a}$ 4.2, $J_{21e,22e}$ 3.2, $J_{21e,19e}$ 2.7), 2.00 ddd (H^{18}, $J_{18,19a}$ 13.2, $J_{18,13}$ 4.6, $J_{18,19e}$ 3.2), 2.21 ddd (H^{19e}, $J_{19e,19a}$ 13.2, $J_{19e,18}$ 3.2, $J_{19e,21e}$ 2.7), 2.38 d (H^1, $J_{1,1'}$ 15.0) и 2.75 d ($H^{1'}$, $J_{1',1}$ 15.0) – system AB, 3.04 d (H^{13}, $J_{13,18}$ 4.6), 3.73 s ($OC^{31}H_3$), 5.89 s (H^{11}), 8.04 s (H^{32}). ^{13}C NMR spectrum of compound **11**, δ, ppm: 33.42 t (C^1), 108.38 s (C^2), 171.94 s (C^3), 35.01 s (C^4), 49.53 d (C^5), 18.16 t (C^6), 31.24 t (C^7), 45.75 s (C^8), 175.87 s (C^9), 41.09 s (C^{10}), 124.65 d (C^{11}), 200.77 s (C^{12}), 47.90 d (C^{13}), 41.79 s (C^{14}), 26.27 t (C^{15}), 26.13 t (C^{16}), 31.95 s (C^{17}), 37.85 d (C^{18}), 33.79 t (C^{19}), 43.95 s (C^{20}), 31.16 t (C^{21}), 38.18 t (C^{22}), 28.65 q (C^{23}), 21.26 q (C^{24}), 24.52 q (C^{25}), 23.28 q (C^{26}), 21.87 q (C^{27}), 26.99 q (C^{28}), 28.51 q (C^{29}), 177.26 s (C^{30}), 51.47 q (C^{31}), 150.06 d (C^{32}).

Isoxazole ring cleavage in compound (11). To a solution of compound **11** (3.0 g, or 5.9 mmol) in the mixture diethyl ester (170 mL) and methanol (85 mL) under stirring was added sodium methylate (11 g) a little at a time, on ice bath cooling. The mixture was stirred during 1 h at a room temperature. Then we added ethyl acetate (~50 mL) and 5 % HCl solution until obtaining pH < 7 was reached. The reaction mixture was then extracted by ethyl

acetate (2 × 50 mL). Joined extract was washed by saturated solutions of $NaHCO_3$ (3 × 50 mL) and NaCl (3 × 50 mL), then it was dried with the help of magnesium sulphate. The solvent was distilled off employing a rotary evaporator; the mass of the reaction mixture (the mixture of tautomeric compounds) **12** amounted to 3.0 g (quantitative yield). ^1H and ^{13}C NMR spectra of the compounds obtained indicated the presence of several (more than one) compounds in the reaction mixture; however the disappearance of such a signal as 8.04 s (H^{32}) indicated the cleavage of the isoxazole ring.

Obtaining of the methyl ester of 2-cyano-3,12-dioxo-11(9)-diene-11-deoxo-18-βH-glycyrrhet-1(2),11(9)-dienoic acid (1). A solution of tautomeric mixture **12** (2.8 g, or 5.5 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (1.5 g, or 6.5 mmol) in benzene (160 mL) was boiled with a backflow condenser during 4 h. The reaction mixture was filtered; the filtrate obtained was concentrated by means of evaporation. After performing column chromatography on silica gel (the eluent represented benzene with a 0–100 % chloroform gradient) we obtained 2.3 g of a crystalline precipitate. Recrystallizing the latter from a methanol-chloroform mixture we obtained 1.7 g of target product **1** (with the yield amounting to 61 %). M. p. 247–249 °C. Found, *m/z*: 505.3025 $[M]^+$. $C_{32}H_{43}NO_4$. Calculated herefrom: *M* 505.3181. ^1H NMR spectrum of compound **1**, δ, ppm (*J*, Hz): 0.90 s ($C^{28}H_3$), 0.96 s ($C^{27}H_3$), 1.09 s ($C^{29}H_3$), 1.14 s ($C^{24}H_3$), 1.22 s ($C^{23}H_3$), 1.44 s ($C^{26}H_3$), 1.47 s ($C^{25}H_3$), 0.93 dm (H^{16e}, $J_{16e,16a}$ 13.3), 1.08 m (H^{15e}), 1.20 dd (H^{19a}, $J_{19a,19e}$ 13.2, $J_{19a,18}$ 13.2), 1.18–1.32 m (H^{21a}, H^{22e}), 1.48 ddd (H^{22a}, $J_{22a,22e}$ 14.0, $J_{22a,21a}$ 14.0, $J_{22a,21e}$ 4.2), 1.55 dm (H^{7e}, $J_{7e,7a}$ 13.5), 1.67–1.79 m (H^{5a}, $2H^6$, H^{7a}), 1.82 m (H^{15a}), 1.87 m (H^{16a}), 1.94 dddd (H^{21e}, $J_{21e,21a}$ 13.3, $J_{21e,22a}$ 4.2, $J_{21e,22e}$ 3.2, $J_{21e,19e}$ 2.8), 2.02 ddd (H^{18}, $J_{18,19a}$ 13.2, $J_{18,13}$ 4.7, $J_{18,19e}$ 3.2), 2.17 ddd (H^{19e}, $J_{19e,19a}$ 13.2, $J_{19e,18}$ 3.2, $J_{19e,21e}$ 2.8), 3.02 d (H^{13}, $J_{13,18}$ 4.7), 3.72 s ($OC^{31}H_3$), 5.97 s (H^{11}), 8.01 s (H^1). ^{13}C NMR spectrum of compound **1**, δ, ppm: 165.65 d (C^1), 114.46 s (C^2), 196.42 s (C^3), 44.86 s (C^4), 47.57 d (C^5), 18.12 t (C^6), 31.58 t (C^7), 45.82 s (C^8), 168.18 s (C^9), 42.36 s (C^{10}), 124.17 d (C^{11}), 199.52 s (C^{12}), 48.04 d (C^{13}), 42.10 s (C^{14}), 26.03 t (C^{15}), 26.00 t (C^{16}), 31.88 s (C^{17}), 37.75 d (C^{18}), 33.61 t (C^{19}), 43.91 s (C^{20}), 31.11 t (C^{21}), 38.14 t (C^{22}), 26.88 q (C^{23}), 21.40 q (C^{24}), 26.61 q (C^{25}), 24.77 q (C^{26}), 21.81 q (C^{27}), 26.96 q (C^{28}), 28.46 q (C^{29}), 177.13 s (C^{30}), 51.48 q (C^{31}), 114.22 s (C^{32}).

RESULTS AND DISCUSSION

One of accessible compounds with strongly pronounced physiological properties is presented by 18-βH-glycyrrhetic acid **2** (see Scheme 1) which represents the aglycon of glycyrrhizic acid, the glycoside prevailing in licorice roots (~90 % with respect to total triterpene glycosides) [7].

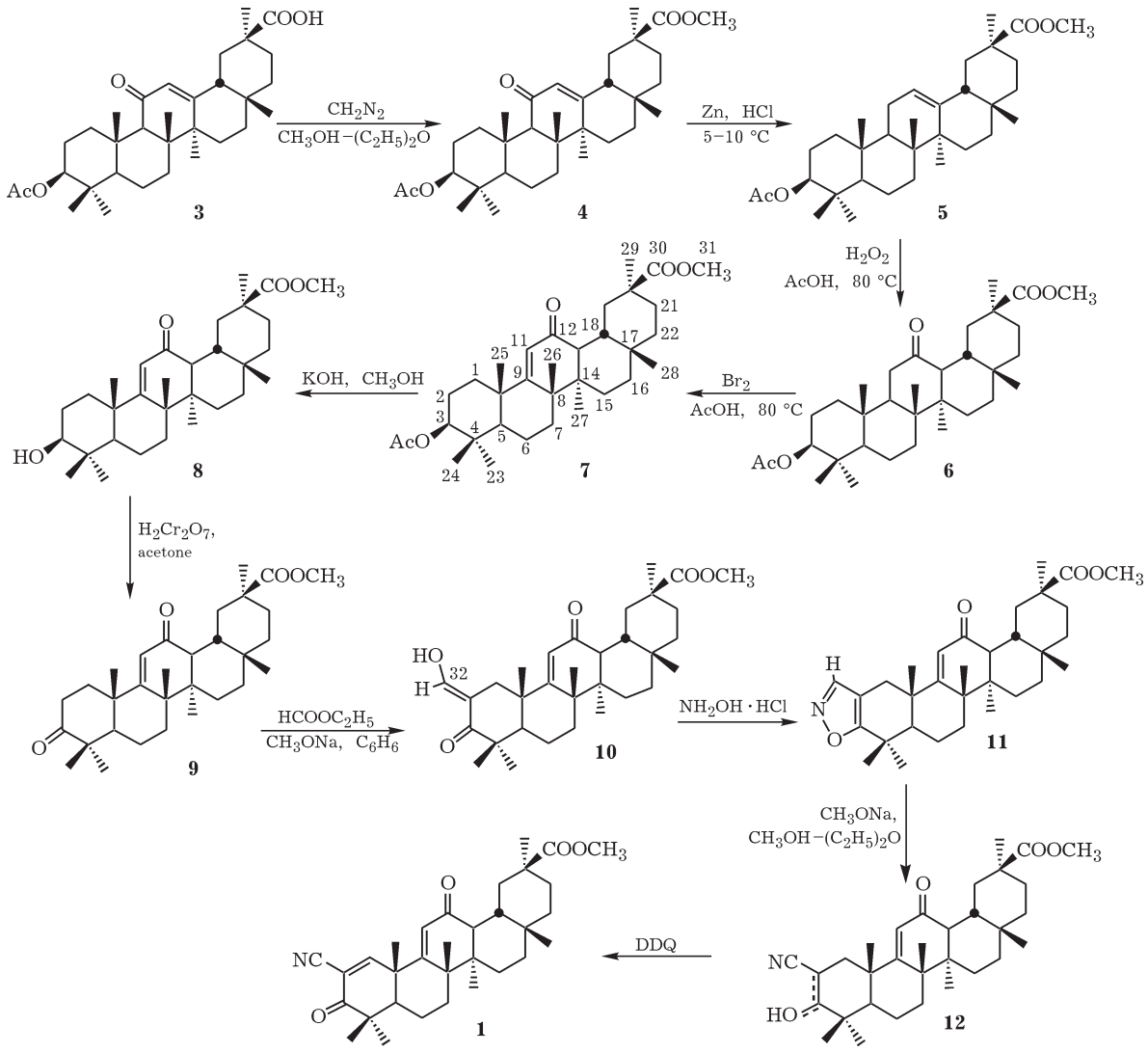
We have obtained the methyl ester of 2-cyano-3,12-dioxo-11(9)-diene-11-deoxo-18-βH-glycyrrhet-1(2),11(9)-dienoic acid **1** as the result of a combined modification of A and C rings in 18-βH-glycyrrhetic acids **2** according to Scheme 2. As the initial compound we employed 18-βH-glycyrrhetic acid acetate **3** obtained *via* glycyrrhizic acid deglycolization in acetic acid medium [5].

The methyl ester of 18-βH-glycyrrhetic acid acetate **4** was obtained through the interaction of compound **3** with diazomethane.

We have first of all carried out the modification of ring C. The reduction of compound **4** by zinc in HCl solution results in the formation of the methylene group at C-11 position. The oxidation of compound **5** by means of hydrogen peroxide in the acetic acid medium results in the formation of the carbonyl group at C-12 position, whereas the subsequent interaction of the obtained ketone with bromine in the acetic acid media (as the result of the bromination-dehydrobromination reaction) results in the formation of 9,11-double bond, *i.e.* in the formation of compound **7**.

The further 6-staged modification of ring A (the oxidation of the hydroxyl group into the ketone group at C-3 position, formylation, condensation with hydroxylamine, isoxazole ring cleavage and the oxidation by means of dichlorodicyanoquinone results in the formation of 2-cyano-3-oxo-1-ene fragment and, correspondingly, in the formation of compound **1**.

Compound **1** was obtained earlier by the authors of [8] *via* the interaction between methyl ester of 2-iodo-3,12-dioxo-11(9)-diene-11-deoxo-18-βH-glycyrrhet-1(2),11(9)-dienoic acid and copper (I) cyanide. The method of synthe-



Scheme 2.

sis we have proposed differs from the known ones in using less expensive and more accessible reagents as well as in the simplicity of reaction mixture treatment.

CONCLUSION

Thus, basing on 18-βH-glycyrrhetic acid we have obtained methyl ester of 2-cyano-3,12-dioxo-11(9)-diene-11-deoxo-18-βH-glycyrrhet-1(2),11(9)-dienoic acid **1**. All the intermediate products have been isolated in the individual form and characterized employing ^{1}H and ^{13}C NMR as well as mass spectrometry technique.

REFERENCES

T. Honda, T. Janosik, Y. Honda *et al.*, *J. Med. Chem.*, 47 (2004) 4923.
2 N. Suh, Y. Wang, T. Honda *et al.*, *Cancer Res.*, 59 (1999) 336.
3 M. Konopleva, T. Tsao, Z. Estrov *et al.*, *Ibid.*, 64 (2004) 7927.
4 B. Melichar, M. Konopleva, Wei Hu *et al.*, *Gynecologic Oncology,* 93 (2004) 149.
5 G. A. Tolstikov, Sinteticheskiye Issledovaniya v Oblasti Fiziologicheski Aktivnykh Vysshikh Terpenoidov i Steroidov (Doctoral Dissertation in Chemistry), Ufa, 1969.
6 Sweden Pat. 307574, 1970.
7 G. A. Tolstikov, L. A. Baltina, V. P. Grankina *et al.*, Solodka: Bioraznoobraziye, Khimiya, Primeneniye v Meditsine, GEO, Novosibirsk, 2007.
8 G. Chadalapaka, I. Jutooru, A. McAlees *et al.*, *Bioorg.&Med. Chem. Lett.,* 18 (2008) 2633.