SYNTHESIS AND ANTIULCER ACTIVITY OF 3-O-ACYLATED GLYCYRRHETIC ACID METHYLATES

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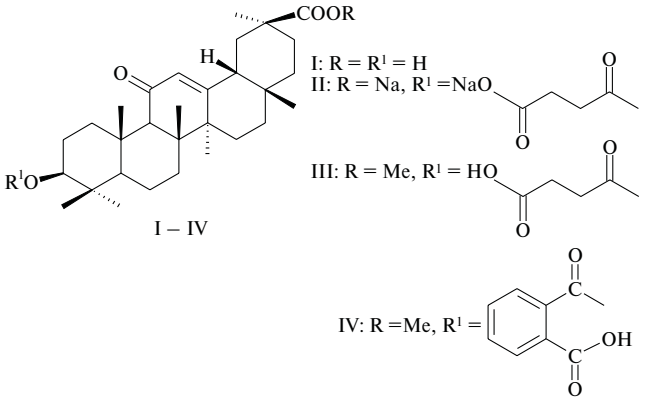
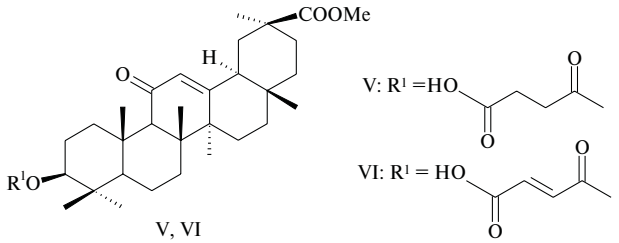
Typically possessing low toxicity and a broad spectrum of biological activity, plant triterpenoids are valuable raw materials for the creation of new drugs [1 – 4]. Of special interest in this respect are the major triterpenoids contained in the root extract obtained from plants of the common licorice (*Glycyrrhiza glabra L.*) and Ural licorice (*Glycyrrhiza uralensis Fisher*) species. These substances are represented by 18β-glycyrrhetic acid (I) and its modified analogs, which serve as the base for effective antiinflammatory, antiallergic, and antiulcer preparations [2, 5 – 9]. The antiulcer drugs include the sodium salt of acid I (glycyrrhenate sodium) [2] and the disodium salt of acid I succinate (carbenoxolone, II) [6, 7].

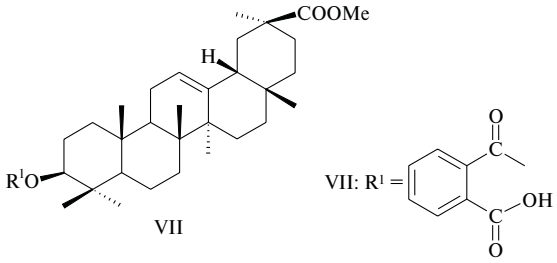
In continuation of our previous study devoted to the synthetic transformation of triterpenoids extracted from licorice root [10], we have synthesized a series of 3-O-acylates of methyl esters of 18α- and 18β-glycyrrhetic acids and their 11-deoxo- and 18,19-dehydro analogs (III – IX).



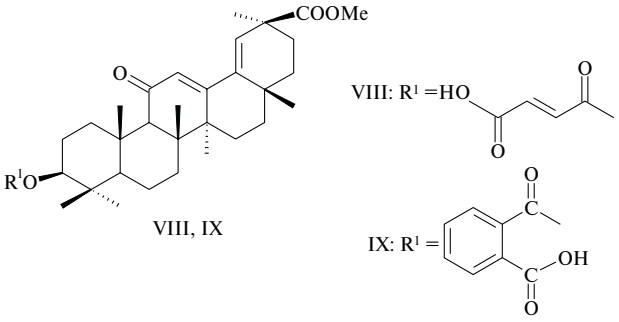
I – IV



V, VI



VII



VIII, IX

Methyl esters of 18β-glycyrrhetic acid and its analogs were acylated by succinic, maleic, or phthalic anhydrides in dry pyridine (Py), pyridine – triethylamine (Py – TEA), or pyridine – tributylamine (Py – TBA) mixtures. Maximum yield (85 – 90%) of the target 3-O-acylates was achieved by etherification of the corresponding triterpenic alcohols in a Py – TBA (15 : 1, v/v) mixture in the presence of 4-Å molecular sieves. For the same methyl esters of glycyrrhetic acid and its analogs acylated in a Py – TEA (5 : 1, v/v) mixture, the target product yields did not exceed 60 – 65%, while

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the yields from Py were as low as 40 – 50%. The main side compounds isolated from the products were the initial methyl esters of triterpenic acids (20 – 40%).

The proposed structures were confirmed by spectroscopic data. The ^{13}C NMR spectrum of acid succinate (III) of glycyrrhetic acid methylate exhibits signals from 31 carbon atoms (which are analogous to signals observed in the ^{13}C NMR spectrum of the initial methyl ester [11]) and additional signals from C32 – C35 carbons of the succinic acid residue (Table 1). The formation of the ester bond is also confirmed by a 2 – 3 ppm weak-field shift of the carbinol carbon (C3) in the spectra of compounds III – IX. The ^{13}C NMR spectrum of ester V exhibits a shift of the C18 carbon signal by 6 ppm toward stronger fields as compared to an analogous signal in the spectrum of 18β-stereomer III; this effect was previously observed in the spectra of 18α-glycyrrhetic acid derivatives [11].

The ^{13}C NMR spectra of 3-O-acylates VI and VIII are characterized by the appearance of additional signals from olefin carbons at 134.5 – 134.8 and 164.7 – 166.0 ppm (C33 and C32, respectively). In the ^{1}H NMR spectrum, protons involved in the double bonds at C32 and C33 are manifested at ~ 6 ppm by doublets with a spin – spin coupling constant of J = 15.8 Hz (compound VI), which is evidence that the protons are in the *trans* configuration. The ^{13}C NMR spectra of acid phthalates IV, VII, and IX exhibit additional signals due to aromatic carbons in the region of 128.4 – 133.6 ppm.

The antiulcer activity of 3-O-acylates IV, V, and IX was studied using an experimental model of gastric ulcers induced by acetylsalicylic acid in rats (Table 2). It was found that esters IV and V possess an antiulcer activity comparable to that of carbenoxolone.

EXPERIMENTAL CHEMICAL PART

The IR spectra were measured on a Specord M-80 spectrophotometer using samples prepared as nujol mulls. The electronic (UV) absorption spectra were recorded on a Specord UV-400 spectrophotometer using methanol solutions. The NMR spectra were measured on a Bruker AM-300 spectrometer (Germany) operating at a working frequency of 75.5 and 300 MHz in the ^{1}H and ^{13}C modes, respectively. The spectra were recorded with a broadband off-resonance proton signal suppression, using deuterated chloroform as the solvent and TMS as the internal standard. The optical activity was determined on a Perkin-Elmer Model 241MC polarimeter in a 10-dm tube cell. The melting points were determined using a Boetius heating stage.

TLC analyses were performed on Silufol UV-254 plates (Czech Republic) eluted in chloroform – methanol, 25 : 1 (A) or 10 : 1 (B), and benzene – methanol 25 : 1 (C) solvent systems. The spots were visualized by treating the plates in a 20% phosphotungstic acid solution in ethanol, followed by heating for 2 – 3 min at a temperature of 110 – 120°C. The column chromatography was performed on silica gel L (100/160 μm) (Chemapol, Czech Republic) or alumina (Al_2O_3).

The initial methyl esters of 18α-, 18β-, 11-deoxo-, and 18,19-dehydroglycyrrhetic acids were synthesized as described previously [12, 13].

General method for the synthesis of esters III – IX.

M e t h o d A . A mixture of 5 mmole triterpenic alcohol and 15 – 20 mmole acid anhydride in 50 ml of pyridine was boiled for 15 – 16 h without access to water. Then the reaction mixture was diluted with 300 ml of cold water and the solution was acidified with hydrochloric acid to pH ~ 3 – 4. The residue was filtered, washed with hot water, dried, and chromatographed in a column filled with silica gel L (eluted in a methylene chloride – methanol system, step gradient mode, 300 :1 – 100 : 1, v/v) or with Al_2O_3 (eluted in a benzene – methanol system, 200 : 1 – 50 : 1, v/v). The products were crystallized from a chloroform – methanol mixture or from an aqueous methanol or ethanol solution.

M e t h o d B . A mixture of 5 mmole triterpenic alcohol, 15 – 20 mmole acid anhydride, 50 ml of pyridine, and 10 ml TEA was boiled for 14 – 15 h without access to water and then treated as described above.

M e t h o d C . A mixture of 5 mmole triterpenic alcohol, 10 – 15 mmole acid anhydride, 3 – 5 g 4-Å molecular sieve, 50 ml of pyridine, and 10 ml TBA was heated for 9 – 10 h at 90 – 100°C. Then the reaction mixture was diluted with 20 ml of acetone and the target product was isolated as described above.

3-O-β-Carboxypropionyl-18β-olean-12-en-3-yl-30-ic acid methyl ester (III).

(1) A mixture of 2.4 g (5 mmole) of 18β-glycyrrhetic acid methyl ester and 2.4 g (20 mmole) of succinic anhydride treated by method A yielded 1.3 g (45%) of ester III in the form of a cream-colored powder; R_f, 0.33 (solvent system A), 0.30 (system B); m.p., 262 – 264°C (reported m.p., 267 – 268°C [5]); $[\alpha]_D^{20}$, + 156 ± 2° (c, 0.05; chloroform); IR spectrum (ν_{max}, cm^{-1}): 1740 (COOR), 1665 (C=O); ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 0.83, 0.92, 0.92, 1.10, 1.13, 1.13, 1.45 (7s, 21H, 7 CH_3), 1.25 – 1.40 (m, CH_2, CH), 2.33 (s, 1H, H-9), 2.65 (m, 4H, 2H-33, 2H-34, –O(C=O)CH_2–), 2.77 (d, 1H, J 13.7 Hz, H-18), 3.68 (s, 3H, OCH_3), 4.52 (dd, 1H, J_1 4.9 Hz, J_2 4.6 Hz, H-3), 5.65 (s, 1H, H-12); ^{13}C NMR spectrum, see Table 1.

The reaction products contained 0.9 g (40%) of the initial 18β-glycyrrhetic acid methyl ester: R_f, 0.45 (solvent system A); $[\alpha]_D^{20}$, + 163 ± 2° (c, 0.05; chloroform), reported $[\alpha]_D^{20}$, + 160 ± 2° (c, 0.01; methanol).

(2) A mixture of 2.4 g of 18β-glycyrrhetic acid methyl ester and 2.4 g of succinic anhydride treated by method B yielded 1.8 g (62%) of ester III and 0.5 g (23%) of the initial 18β-glycyrrhetic acid methyl ester.

(3) A mixture of 2.4 g of 18β-glycyrrhetic acid methyl ester and 2.4 g of succinic anhydride treated by method C yielded 2.5 g (85.5%) of ester III.

3-O-β-Carboxyphthaloyl-18β-olean-12-en-3-yl-30-ic acid methyl ester (IV). A mixture of 2.4 g of 18β-glycyrrhetic acid methyl ester and 2.2 g (15 mmole) of phthalic anhydride treated by method C yielded 2.84 g (90%) of ester IV in the form of a yellowish powder; R_f, 0.44 (solvent system C); m.p., 154 – 157°C (aqueous methanol); $[\alpha]_D^{20}$, + 141 ± 2° (c, 0.03; methanol); IR spectrum (ν_{max}, cm^{-1}): 1730 (COOR), 1660 (C=O), 1630, 1600, 1580 (Ph); UV spectrum (λ_{max}, nm): 242 (log ε, 4.2); ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 0.75, 0.80, 0.90, 0.94, 1.05, 1.37, 1.57 (7s, 21H, 7 CH_3), 1.26 – 2.10 (m, CH_2, CH), 2.33 (s, 1H, H-9), 3.62 (s, 3H, OCH_3), 4.65 (dd, 1H, J_1 4.7 Hz, J_2 11 Hz, H-3), 5.61 (s, 1H, H-12); 7.20 – 7.65 (m, 4H, H arom); ^{13}C NMR spectrum, see Table 1; $C_{39}H_{52}O_7$.

3-O-β-Carboxypropionyl-18α-olean-12-en-3-yl-30-ic acid methyl ester (V). A mixture of 2.4 g (5 mmole) 18α-glycyrrhetic acid methyl ester and 2.4 g (20 mmole) of succinic anhydride treated by method B yielded 1.75 g (60%) of ester V in the form of a white powder; R_f, 0.27 (solvent system A); m.p., 195 – 196°C (aqueous ethanol); $[\alpha]_D^{20}$, + 104 ± 2° (c, 0.12; methanol); IR spectrum (ν_{max}, cm^{-1}): 1740 (COOR), 1660 (C=O); UV spectrum (λ_{max}, nm): 244 (log ε, 4.05); ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 0.67, 0.78, 0.81, 1.08, 1.12, 1.20, 1.30 (7s, 21H, 7 CH_3), 1.30 – 1.60 (m, CH_2, CH), 2.24 (s, 1H, H-9), 2.56 (m, 4H, H-33, H-34, –C(=O)–CH_2–), 3.64 (s, 3H, OCH_3), 4.44 (d, 1H, J 8.2 Hz, H-3), 5.52 (s, 1H, H-12); ^{13}C NMR spectrum, see Table 1; $C_{35}H_{52}O_7$.

3-O-β-Carboxy-*trans*-propenoyl-18α-olean-12-en-3-yl-30-ic acid methyl ester (VI). A mixture of 2.4 g 18α-glycyrrhetic acid methyl ester and 2.1 g (15 mmole) of maleic anhydride treated by method A yielded 1.3 g (46%) of ester VI in the form of a white powder; R_f, 0.29 (solvent system A); $[\alpha]_D^{20}$, + 82 ± 2° (c, 0.04; chloroform); IR spectrum (ν_{max}, cm^{-1}): 1730 (COOR), 1700 (COOH), 1610 (–CH=CH–); UV spectrum (λ_{max}, nm): 243 (log ε, 4.2); ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 0.64, 0.74, 0.83, 0.88, 1.08, 1.24, 1.32 (7s, 21H, 7 CH_3), 1.40 – 1.60 (m, CH_2, CH), 2.20 (s, 1H, H-9), 3.60 (s, 3H, OCH_3), 4.54 (d, 1H, J 10.3 Hz, H-3), 5.55 (s, 1H, H-12); 6.74 (d, 1H, J 15.8 Hz, H-33), 6.78 (d, 1H, J 15.8 Hz, H-34); ^{13}C NMR spectrum, see Table 1; $C_{35}H_{50}O_7$.

3-O-β-Carboxyphthaloyl-11-deoxo-olean-12-en-3-yl-30-ic acid methyl ester (VII). A mixture of 2.3 g (5 mmole) of 11-deoxo-glycyrrhetic acid methyl ester and 2.0 g (15 mmole) of phthalic anhydride treated by method C yielded 2.2 g (86%) of ester VII in the form of a yellowish powder; R_f, 0.25 (solvent system C); $[\alpha]_D^{20}$, + 130 ± 2° (c, 0.05; chloroform); IR spectrum (ν_{max}, cm^{-1}): 1740 (COOR), 1610, 1600, 1580 (Ph); ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 0.82, 0.93, 1.10, 1.15, 1.36, 1.40 (7s, 21H, 7 CH_3), 1.65, 1.86 (m, CH_2, CH), 2.36 (s, 1H, H-9), 3.66 (s, 3H, OCH_3), 4.70 (m, 1H, H-3), 5.28 (bs, 1H, H-12); 7,26, 7.48, 7,76 (s, 4H, H-34, H-35, H-36, H-37). ^{13}C NMR spectrum, see Table 1; $C_{39}H_{54}O_6$.

3-O-β-Carboxy-*trans*-propenoyl-12(13),18(19)-dien-3-yl-30-ic acid methyl ester (VIII). A mixture of 4.6 g (10 mmole) of 18,19-dihydroglycyrrhetic acid methyl ester and 4.6 g (51 mmole) of maleic anhydride treated by method A yielded 2.2 g (40%) of ester VIII in the form of an amorphous yellowish substance; R_f, 0.38 (solvent system A); $[\alpha]_D^{20}$, +236 ± 2° (c, 0.04; methanol); IR spectrum (ν_{max}, cm^{-1}): 1730 (COOR), 1670 (C_{11}=O); UV spectrum (λ_{max}, nm): 278 (log ε, 3.95); ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 0.87, 0.87, 0.93, 1.13, 1.21, 1.23, 1.28 (7s, 21H, 7 CH_3), 1.20 – 2.00 (m, CH_2, CH), 2.26 (s, 1H, H-9), 3.67 (s, 3H, OCH_3), 4.62 (dd, 1H, J_3,2c 5 Hz, J_3,2a 11.4 Hz, H-3), 5.76 (s, 1H, H-12); 5.78 (s, 1H, H-19), 6.32 (d, 1H, J 12.7 Hz, H-33), 6.36 (d, 1H, J 12.7 Hz, H-34); ^{13}C NMR spectrum, see Table 1; $C_{35}H_{48}O_7$.

3-O-β-Carboxyphthaloyl-olean-12(13),18(19)-dien-3-yl-30-ic acid methyl ester (IX). A mixture of 4.6 g

TABLE 1. Parameters of the ^{13}C NMR Spectra of 3-O-Acylates ($CDCl_3$; 25°C; 75.5 MHz; δ, ppm)

C	III	IV	V	VI	VII	VIII	IX
C1	38.65	38.56	38.56	38.69	38.23	38.42	38.75
C3	81.00	80.62	80.99	81.69	81.45	81.63	81.84
C5	54.94	54.97	54.91	54.66	54.73	54.91	55.26
C7	32.58	32.38	33.53	33.31	32.29	33.70	33.82
C9	61.62	61.44	60.58	60.29	47.04	60.45	60.72
C11	200.17	199.68	199.70	199.65	23.10	199.85	199.93
C12	128.36	128.06	123.91	123.60	122.47	129.49	129.64
C13	169.39	169.03	165.80	164.39	143.97	162.95	162.78
C14	45.32	45.07	44.77	44.92	41.28	45.29	45.16
C16	26.37	26.05	37.87	37.79	26.78	24.86	25.91
C17	31.74	31.48	36.52	36.34	31.72	31.57	31.60
C18	48.31	48.05	40.23	40.06	47.95	142.60	142.74
C19	40.97	40.77	35.75	35.54	42.35	123.81	124.04
C20	43.97	43.65	43.90	43.53	44.05	44.20	44.37
C22	37.64	37.44	26.28	26.10	38.33	37.90	36.82
C24	15.93	16.06	16.48	16.42	16.54	16.63	16.73
C26	18.57	18.37	20.61	20.40	16.96	18.18	18.37
C28	28.25	27.92	15.89	15.45	28.40	27.85	27.88
C30	176.93	176.48	177.44	176.72	176.66	176.72	176.66
C31	51.75	51.33	51.85	51.93	51.38	52.09	52.06
C32	171.95	168.05	171.92	167.76	167.79	167.68	165.28
C33 –	29.40	C33 –	28.91	134.81	C33 –	134.50	C33 –
C34	29.18	C39:	28.30	165.98	C39:	164.70	C39:
C35	177.55	126.15	178.74	178.56	126.88	171.50	126.99
		127.44			127.69		127.83
		127.88			128.10		128.52
		129.88			128.57		130.23
		131.24			131.38		132.62
		139.89			142.32		138.83
		173.23			171.85		170.96

TABLE 2. Antiulcer Activity of Glycyrrhetic Acid Derivatives with Respect to Acetylsalicylic Acid Model Ulcers in Rats *

Compound	Average number of ulcers
IV	4.6 ± 0.5 ($p < 0.02$)
V	3.6 ± 0.3 ($p < 0.002$)
IX	8.0 ± 0.7
Carbenoxolone	4.8 ± 0.3 ($p < 0.02$)
Control	6.2 ± 0.3

* Each compound tested in a group of 6 animals in a dose of 50 mg/kg.

(10 mmole) of 18,19-dehydroglycyrrhetic acid methyl ester and 4.0 g (30 mmole) of phthalic anhydride treated by method B yielded 4.0 g (65%) of ester IX in the form of a yellowish substance; R_f, 0.4 (solvent system B); $[\alpha]_D^{20}$, +135 ± 2° (c, 0.04; methanol); IR spectrum (ν_{max}, cm^{-1}): 1740 (COOR), 1680 (C=O), 1620 (Ph); UV spectrum (λ_{max}, nm): 219 (log ε, 4.24), 278 (log ε, 4.04); ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 0.82, 0.85, 1.10, 1.12, 1.15, 1.18, 1.30 (7s, 21H, 7 CH_3), 2.28 (s, 1H, H-9), 3.66 (s, 3H, OCH_3), 4.70 (d, 1H, J 7 Hz, H-3), 5.76, 5.80 (2s, 2H, H-12, H-19), 7.20 – 7.90 (m, 4H, H arom); ^{13}C NMR spectrum, see Table 1; $C_{39}H_{50}O_7$.

EXPERIMENTAL PHARMACOLOGICAL PART

The antiulcer activity of some glycyrrhetic acid derivatives was studied on white mongrel rats (weighing 150 – 200 g) with an experimental damage of the mucous membrane of the stomach caused by acetylsalicylic acid. Prior to the gastric damage induction, the animals were deprived of meals and water and kept at a reduced ambient temperature (14 – 16°C) for 24 h. The test preparations were introduced into the stomach via gastric tubes in a dose of 50 mg/kg (as aqueous suspensions) 1 h before inducing model ulcers (animals in the test group received an equivalent amount of distilled water). Acetylsalicylic acid was suspended in distilled water and introduced in a dose of 150 mg/kg via the gastric tube 1 and 6 h after the compounds studied.

The test animals were kept in a cold room (14 – 16°C) for 24 h and then killed under ether narcosis. The stomachs were extracted, cut along the lesser curvature, and examined using a magnifying glass with respect to the state of the mucous membrane. The antiulcer effect was evaluated according to [14, 15] by the decrease in the number of damaged sites in the mucous membrane of the stomach of test rats relative to control. The reference drug was carbenoxolone.

ACKNOWLEDGMENTS

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