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ANTIULCER ACTIVITY OF 3-HYDROXYIMINO DERIVATIVES OF MINOR TRITERPENOIDS OF LICORICE ROOT

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3-Hydroxyimino derivatives of minor licorice triterpenoids possessed pronounced antiulcer activity and protected rat gastric mucosa from ulceration in indomethacin and orthophen ulcer models at a dose of 50 mg/kg. 3-Hydroxyimino-11-deoxoglycyrrhetic acid was the most active antiulcerogenic substance and was superior in activity to carbenoxolone.

Keywords: triterpenoids, licorice, oximes, antiulcer activity.

Pentacyclic triterpenoids are widely distributed in the plant world, possess a variety of biological activity, and are extensively studied as a platform for producing new biologically active compounds with medical significance [1 – 5]. Glycyrrhetic acid (GLA, **1**), the aglycon of the triterpene glycoside glycyrrhizic acid (GA), the major constituent of licorice roots (*Glycyrrhiza glabra* L. and *G. uralensis* Fisher), is an available representative oleanane-type pentacyclic triterpenoid. GLA possesses a broad spectrum of pharmacological activity (anti-inflammatory, antiulcer, hepatoprotective, antiallergic, antimicrobial, antidiabetic, etc.) [6 – 11], induces apoptosis of cancer cells, and exhibits cytotoxic activity against various types of tumor cells [10, 11]. The high anti-inflammatory activity of GLA is combined with pronounced antiulcer (AU) action. This makes it an attractive natural scaffold for producing new biologically active compounds with a complex of medically valuable properties [6, 7, 11]. The succinic acid ester derivative of GLA (**II**) is well known and was previously studied for treating gastric and duodenal ulcers (carbenoxolone) [12]. Methyl esters of 18β-GLA hemiphthalate and hemisuccinate at a dose of 50 mg/kg possessed AU activity analogous to that of carbenoxolone [13]. Besides GA and GLA, several minor triterpenoids, the main ones of which were 18α-GLA, 24-hydroxy-GLA, 11-deoxo-GLA, liquiritic acid, etc., were isolated from roots of *G. glabra* and *G. uralensis* [6] and were also interesting as platforms for producing new biologically active compounds.

The present work focused on a search for new AU compounds among C3-modified minor licorice triterpenoids, i.e., 3α-hydroxy-18β*H*-olean-12-en-30-oic acid (11-deoxo-GLA, **III**), 3β-hydroxy-18β*H*-olean-9(11),12(13)-dien-30-oic acid (9,12-dien-30-oic acid, **IV**), and 3β-hydroxy-18β*H*-olean-11-oxo-12(13),18(19)-dien-30-oic acid (18,19-dehydro-GLA, **V**).

Triterpenoids **III**-**V** were used to synthesize target compounds as methyl esters of 3-oxo derivatives **VI**-**VIII**, which were obtained via oxidation of the corresponding methyl esters by pyridinium dichromate [6, 14 – 16].

Refluxing 3-oxo derivatives **VI**-**VIII** with hydroxylamine hydrochloride in Py for 2 h produced the target 3-hydroxyimino derivatives **IX**-**XI** in 78 – 82% yields after recrystallization from EtOH.

EXPERIMENTAL CHEMICAL PART

PMR and ^{13}C NMR spectra were recorded with TMS internal standard on a Bruker Avance III 500 MHz pulsed spectrometer at operating frequency 500.13 MHz (^{1}H) and 125.47 MHz (^{13}C). IR spectra were recorded in Vaseline-oil mulls on a Prestige-21 IR spectrometer (Shimadzu).

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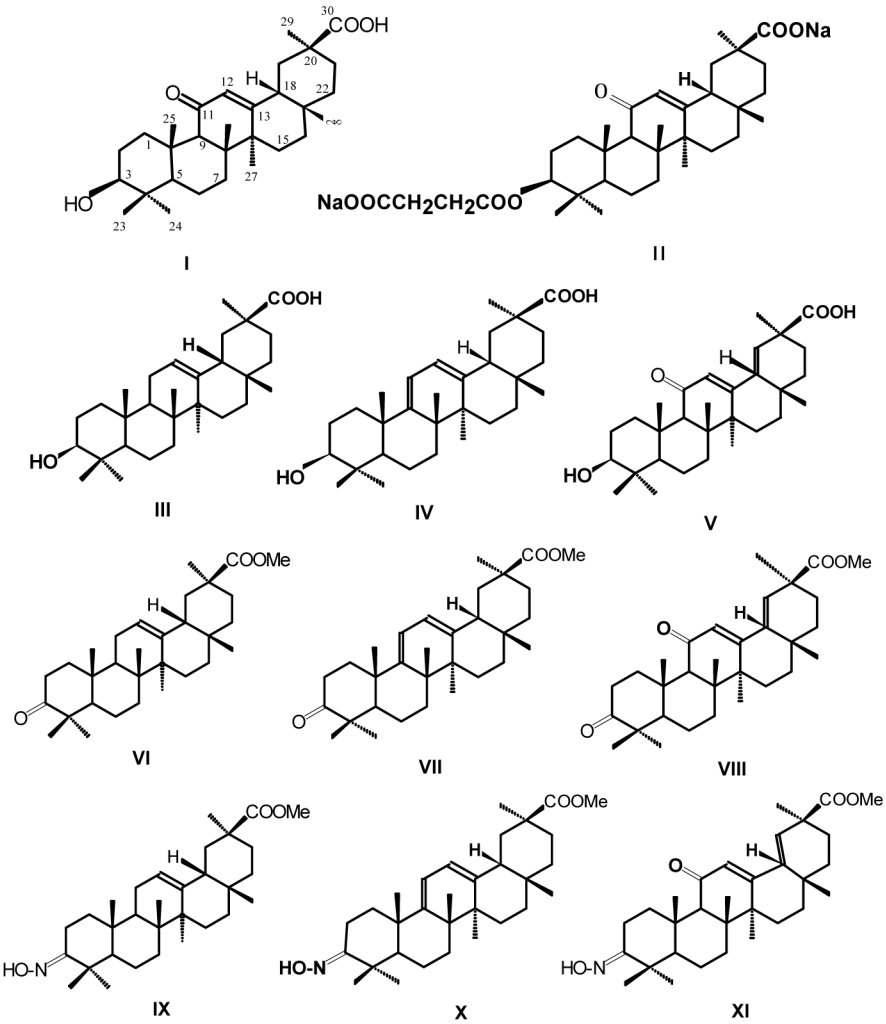

Molecular ions were determined by liquid-chromatography–mass-spectrometry (LC-MS) on an LCMS-2010 instrument (Shimadzu) using chemical ionization at atmospheric pressure of MeCN solutions.

Optical activity was measured on a PerkinElmer 341 polarimeter in a 1-dm tube at 20 – 22°C (λ_{Na} 546 nm). Melting points were determined on a Boetius apparatus. Column chromatography (CC) used silica gel (SG, L grade, 50/150 μm, Sorbpolimer, Russia) and C_6H_6 and C_6H_6–EtOH eluents (200:1, 100:1; v/v). TLC was performed on Sorbfil plates (Sorbpolimer). Spots of compounds were detected by H_2SO_4 solution (5%) in EtOH followed by heating at 110 – 120°C for 2 – 3 min.

Glycyrrhetic acid (I) was prepared by acid hydrolysis of glycyrrhizic acid [5]. Mp 293 – 295 °C; $[\alpha]_D^{20}$ +168° (*s* 0.05; $CHCl_3$). Lit. [6]: mp 290 – 292 °C, $[\alpha]_D^{20}$ +165° (*s* 0.03; $CHCl_3$).

11-Deoxo-GLA (III) was prepared via reduction of GLA by Zn and HCl in dioxane [6]. Mp 320 – 322°C, $[\alpha]_D^{20}$ +140° (c 0.04, CH_2Cl_2). Lit. [6]: mp 323 – 325°C.

3β-Hydroxy-18β*H*-olean-9(11),12(13)-dien-30-oic acid (IV) was prepared via reduction of GLA by $NaBH_4$ in THF and aqueous NaOH [15]. Mp 310 – 312°C; $[\alpha]_D^{20}$ +345° (s 0.06; $CHCl_3$). Lit. [15]: mp > 300°C; $[\alpha]_D^{20}$ +343° (s 0.06; $CHCl_3$).

18,19-Dehydro-GLA (**V**), the substance of glyderinin preparation [6], was used in the work.

3-Oxo triterpenoid derivatives **VI-VIII** were prepared via oxidation of the corresponding methyl esters by pyridinium dichromate as before [14 – 16].

3-Oxo-11-deoxoglycyrrhetic acid methyl ester (VI). Mp 190 – 191°C. Lit. [14]: mp 186 – 188°C; [17]: mp 190°C.

3-Oxo-18β*H*-olean-9(10),11(12)-dien-30-oic acid methyl ester (VII). Mp 233 – 235°C. Lit. [15]: mp 232 – 235°C.

3,11-Dioxo-18β*H*-olean-12(13),18(19)-dien-30-oic acid (VIII). Mp 193 – 195°C. Lit. [16]: mp 192 – 194°C; [18]: mp 193 – 195°C.

General method for preparing 3-(hydroxyimino)-triterpenoic acids

A solution of **VI-VIII** (1 mmol) in anhydrous Py (10 mL) was treated with $NH_2OH{\cdot}HCl$ (13 – 14 mmol), refluxed for 2 h, and diluted with cold H_2O. The precipitate was filtered off, rinsed with H_2O, dried, and crystallized from aqueous EtOH.

3-Hydroxyimino-11-deoxo-18β*H*-olean-12-en-30-oic acid methyl ester (IX). Yield 82%. mp 262 – 264°C. Lit. [14]: mp 265 – 267°C. LC-MS, *m*/*z*: 484.5 $[M + H]^+$. Calc., %: N 2.74. $C_{31}H_{49}O_3N$. Calc., %: N 2.89. M.w. 483.7.

3-Hydroxyimino-18β*H*-olean-9(11),12(13)-dien-30-oic acid methyl ester (X). Yield 80%. mp 233 – 235°C. Lit. [15]: mp 234 – 236°C; LC-MS, *m*/*z*: 482.5 $[M + H]^+$. Calc., %: N 2.80. $C_{31}H_{47}O_3N$. Calc., %: N 2.91. M.w. 481.7.

3-Hydroxyimino-18β*H*-olean-12(13),18(19)-dien-30-oic acid methyl ester (XI). Yield 78%. mp 226 – 228°C; $[\alpha]_D^{20}$ +172° (c 0.02, $CHCl_3$). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm): 5.80, 5.78 (2H, dd, J_1 7.5, J_2 15.9 Hz, H-12, H-19), 3.67 (3H, s, OCH_3), 3.01 (1H, m, CH), 2.76 (1H, m, CH), 2.28 (1H, s, H-9), 2.04 (2H, br.s, CH_2), 1.60 – 1.52 (10H, m, CH, CH_2), 1.30 (3H, s, CH_3), 1.28 (3H, s, CH_3), 1.23 (2H, s, CH_2), 1.18 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.14 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.04 – 1.02 (2H, m, CH_2), 0.93 (3H, s, CH_3). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 199.84 (C-11), 176.81 (C-30), 166.99 (C-3), 162.97 (C-13), 142.77 (C-18), 129.74 (C-19), 124.13 (C-12), 60.40 (C-9), 55.71 (C-5), 52.22 (C-31), 45.29 (C-14), 44.45 (C-8), 43.48 (C-20), 40.44 (C-4), 38.96 (C-1), 36.96 (C-22), 36.14 (C-10), 34.92 (C-16), 34.79 (C-17), 33.62 (C-7), 27.92 (C-21), 27.27 (C-23), 25.93 (C-15), 25.07 (C-29), 24.39 (C-28), 23.34 (C-24), 19.66 (C-27), 18.43 (C-6), 17.37 (C-26), 16.05 (C-25). LC-MS, *m*/*z*: 496.5 $[M + H]^+$. Calc., %: C 74.92, H 8.95, N 2.72. $C_{31}H_{45}NO_4$. Calc., %: C 75.11, H 9.15, N 2.82. M.w. 495.7.

EXPERIMENTAL PHARMACOLOGICAL PART

The experiments used 60 inbred white rats of both sexes (150 – 200 g) in models of experimental gastric ulcers induced by indomethacin and orthophen [19, 20]. Animals were kept under standard vivarium conditions with free access to feed and water. Animal experiments were conducted in compliance with international rules [*European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*, No. 123, Strasbourg, 1986; *Protocol of Amendment to the European Convention for the Protection of Vertebrate Animals Use for Experimental and Other Scientific Purposes*, Strasbourg, Jun. 22, 1998] and were approved by the Biomedical Ethics Commission of Ufa Institute of Chemistry, Subdivision of UFRC, RAS.

Animals were deprived of feed and water for one day before the modeling. Tested compounds and reference drug **II** were administered through a stomach catheter at a dose of 50 mg/kg. Indomethacin (tablets, 25 mg, Ozon Farmatsevtika, Russia) at a dose of 20 mg/kg was injected intraperitoneally (i.p.) 1 h before administering the tested compounds in the first series of tests; orthophen (tablets, 25 mg, Tatkhimfarmpreparaty, Russia) at a dose of 50 mg/kg through a stomach catheter, likewise in the second series of tests. The animals were euthanized by inhalation of CO_2 after 1 d. The stomach was excised, opened along the small flexure, and rinsed with cold normal saline. The condition of the gastric mucosa was inspected with a binocular microscope. The number of destructions was counted visually. The calculated average number of destructions in each group was compared with those of the control groups. Table 1 presents the experimental results.

Statistical analysis used the Statistica 10 program. Data were presented as averages *M* and their standard errors (±*m*). The statistical significance of intergroup differences was evaluated using the Student *t*-criterion. Differences were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

The physicochemical and spectral characteristics of **IX** and **X** agreed with those in the literature [14, 15]. Compound **XI** was prepared for the first time. Its structure was confirmed by PMR and ^{13}C NMR spectra. The ^{13}C NMR spectrum of **XI** showed the C3 resonance at δ 167.0 ppm (C=N–), which was close to the C3 resonances of oximes **IX** (166.5 ppm) [14] and **X** (166.6 ppm) [15].

The AU activity of 3-hydroxyimino derivatives **IX**-**XI** was studied after peroral administration to inbred white rats of both sexes (150 – 200 g) at a dose of 50 mg/kg in models of experimental gastric ulcers induced by indomethacin (20 mg/kg, i.p.) and orthophen (50 mg/kg, *per os*). The refer-

TABLE 1. Effect of Triterpenoid Derivatives on Experimental Gastric Mucosa Ulcers in Rats ($n = 6$)

Compound	Dose, mg/kg	Average number of destructions,* induced by	
		indomethacin	orthophen
Oxime **IX**	50	8.9 ± 0.5 $p < 0.001$	11.9 ± 0.7 $p < 0.01$
Oxime **X**	50	9.1 ± 0.4 $p < 0.001$	13.2 ± 0.6 $p < 0.01$
Oxime **XI**	50	9.0 ± 0.5 $p < 0.001$	12.4 ± 0.9 $p < 0.01$
Carbenoxolone (**II**)	50	10.3 ± 1.1 $p < 0.01$	13.6 ± 0.8 $p < 0.01$
Control	–	17.3 ± 1.3	19.8 ± 1.3

* Data given as $M \pm m$, differences statistically significant vs. the control group for $p < 0.05$.

ence drug was **II** at a dose of 50 mg/kg. Table 1 presents the experimental results.

Control animals exhibited much hemorrhagic damage to the gastric mucosa (GM) and clotted blood in the stomach cavity 1 d after administration of indomethacin at a dose of 20 mg/kg. Animals that received oximes **IX-XI** had pale pink GM without hemorrhages. The tested derivatives of licorice triterpenoic acids exhibited pronounced AU activity, reducing the number of GM destructions by 1.9 times ($p < 0.001$) as compared to the control. Compound **II** decreased the number of destructions by 1.7 times ($p < 0.01$) as compared to the control.

The second series of tests studied the AU activity of **IX-XI** in the experimental ulcer model induced by orthophen. Oxime **IX** reduced the number of GM destructions by 1.7 times ($p < 0.01$) as compared to the control. Compounds **X** and **XI**, analogously to **II**, reduced the amount of damage by 1.5 times ($p < 0.01$) as compared to the control. Thus, the derivatives of minor licorice triterpenoids modified at the C3-position by a hydroxyimino group possessed pronounced AU activity and protected the GM of rats from ulceration analogously or slightly more effectively than **II**. The 11-deoxo-GLA derivative (**IX**) turned out to be the most active AU compound in both ulcer models.

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REFERENCES

1. R. A. Hill and J. D. Connolly, *Nat. Prod. Rep.*, **35**, 1294 – 1329 (2018).
2. R. Paduch and M. Kandefer-Szerszen, *Mini-Rev. Org. Chem.*, **11**, 262 – 268 (2014).
3. H. Sheng and H. Sun, *Nat. Prod. Rep.*, **28**(3), 543 – 593 (2011).
4. S. Xiao, Z. Tian, Y. Wang, et al., *Med. Res. Rev.*, **38**, 951 – 976 (2018).
5. G. Peron, G. Marzaro, and S. Dall'Acqua, *Curr. Med. Chem.*, **25**, 1259 – 1269 (2018).
6. G. A. Tolstikov, L. A. Baltina, V. P. Grankina, et al., *Licorice: Biodiversity, Chemistry, Use in Medicine* [in Russian], Akad. Izd. Geo, Novosibirsk (2007).
7. B. Xu, G.-R. Wu, X.-Y. Zhang, et al., *Molecules*, **22**, 924 – 948 (2017).
8. I. Beseda, L. Czollner, P. S. Shah, et al., *Bioorg. Med. Chem.*, **18**(1), 433 – 454 (2010).
9. A. Alqahtani, K. Hamid, A. Kam, et al., *Curr. Med. Chem.*, **20**, 908 – 931 (2013).
10. H. Li, J.-Zh. Qiu, Zh.-Q. Yang, et al., *Fitoterapia*, **83**, 241 – 248 (2012).
11. A. Roohbakhsh, M. Iranshahy and M. Iranshahi, *Curr. Med. Chem.*, **23**, 498 – 517 (2016).
12. D. V. Parke, *Acta Gastroenterol. Belg.*, **46**, 437 – 447 (1983).
13. R. M. Kondratenko, S. R. Mustafina, L. A. Baltina, et al., *Pharm. Chem. J.*, **35**(5), 243 – 246 (2001).
14. L. R. Mikhailova, M. V. Khudobko and L. A. Baltina, *Chem. Nat. Compd.*, **45**(4), 519 – 521 (2009).
15. L. R. Mikhailova, M. V. Khudobko, L. A. Baltina Jr., et al., *Chem. Nat. Compd.*, **45**(3), 393 – 397 (2009).
16. M. V. Khudobko, L. R. Mikhailova, L. A. Baltina, and L. V. Spirikhin, *Chem. Nat. Compd.*, **47**(1), 76 – 78 (2011).
17. V. O. Jeger and W. Hofer, *Helv. Chim. Acta*, **31**, 157 – 160 (1948).
18. L. A. Baltina, H.-Ch. Lai, Ya-Chi Liu, et al., *Bioorg. Med. Chem.*, **41**, 116204 (2021).
19. A. N. Mironov (ed.), *Handbook for Preclinical Drug Trials* [in Russian], Grif i K, Moscow (2012), pp. 764 – 765.
20. L. A. Baltina, T. A. Sapozhnikova, S. F. Gabdrakhmanova, et al., *Khim.-farm. Zh.*, **54**(3), 16 – 19 (2020); *Pharm. Chem. J.*, **54**(3), 225 – 228 (2020).