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The Synthesis and Hepatoprotective Activity of Esters of the Lupane Group Triterpenoids

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Abstract—Hemisuccinates, hemiphthalates, acetylsalicylates, cinnamates, and *p*-methoxycinnamates of lupeol, betulin, and 3-*O*-acetylbetulin were synthesized via interaction with corresponding acid anhydrides or acid chlorides. A number of betulin esters in position 3 and 28 were shown to exhibit a pronounced hepatoprotective effect similar to that of betulin and silibor. These experimental data were in a good agreement with the computer prediction of their biological activity. Betulin 3,28-bishemiphthalate was more effective than carsil in models of experimental hepatitis caused by carbon tetrachloride, tetracycline, and ethanol.

Key words: lupane group triterpenoid esters, computer prediction of biological activity, hepatoprotective activity

INTRODUCTION

In some cases, chemical modification of natural bioactive substances leads to interesting results: new compounds turn out to be more effective and exhibit a wider spectrum of activities. Triterpenoids of the lupane group attracted our attention due to their availability and wide spectrum of biological activities. White birch (*Betula pendula*) bark is a rich source of lupeol (**I**) and betulin (**II**): the content of these substances reaches up to 35–40 wt %. Recent data about hepatoprotective, antibacterial, anti-inflammatory, antitumor, antiviral and other activities of these triterpenoids [1–7] prompted our studies in this area. In particular, betulin acetates have been shown to decrease the level of lipids in the blood [1], hemidimethylsuccinates and hemidimethylglutarates of betulinic acid and dihydrobetulinic acid are promising anti-HIV agents [8], and lupeol esters with palmitic and linoleic acids exhibit antiarthritic activity [9].

RESULTS AND DISCUSSION

We started the search for new biologically active compounds among lupane triterpenoids from the synthesis of esters. The boiling of lupeol (**I**), betulin (**II**), and 3-*O*-acetylbetulin (**III**) with succinic and phthalic anhydrides in pyridine yielded hemisuccinates (**IV**)–(**VI**) and hemiphthalates (**VII**)–(**IX**), respectively. The structures of these esters were determined by NMR spectroscopy and by comparison of their NMR spectra with those described for similar compounds [10–15]. The NMR spectra of the aglycone parts of the synthesized substances were analogous to those of the starting triterpenoids. The low-field shifts (by 2–4 ppm) of resonances from the C3 atoms in (**IV**) and (**VII**) and from the C28 atoms in (**V**), (**VI**), (**VIII**), and (**IX**), and the appearance of resonances at 166.7–172.5 ppm in ^{13}C NMR spectra confirmed the formation of ester bonds. Moreover, resonances from the CH_2CH_2-groups of succinates (**IV**)–(**VI**) at 29.0–29.5 ppm were observed, and, in ^1H NMR spectra, the $OCOCH_2CH_2COOH$ group of these compounds resonated as a characteristic multiplet at 2.60–2.70 ppm. The characteristic resonances of aromatic carbon atoms in (**VII**)–(**IX**) were observed at 128–140 ppm in their ^{13}C NMR spectra. The corresponding aromatic protons resonated at 7.30–7.97 ppm in the ^1H NMR spectra. Resonances from OAc groups (at ~2.0 ppm) were also observed in the ^1H NMR spectra of (**VI**) and (**IX**).

Esters (**X**)–(**XVII**) were prepared by the interaction of betulin with chlorides of acetylsalicylic, cinnamic, and methoxycinnamic acids in a mixture of pyridine and tributylamine. The 28-monosubstituted compounds (**X**)–(**XII**) were selectively formed at room temperature when the molar ratio of betulin and the acid chloride was 1 : 1.5 in yields of 58–64%. The 3,28-disubstituted esters (**XIII**)–(**XV**) were obtained in yields 81–86% by treatment with the excess acid chlorides on heating. The formation of 28-monosubstitued

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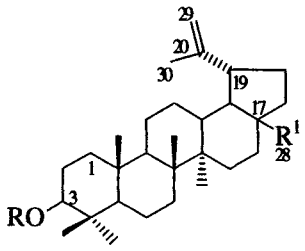

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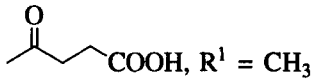
(II) R = H, R^1 = CH_2OH

(III) R = Ac, R^1 = CH_2OH

(IV) R =  , R^1 = CH_3

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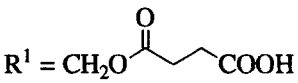
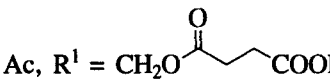
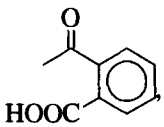
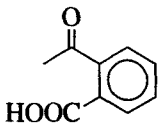
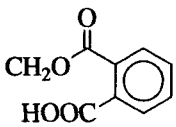
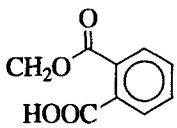
R^1 = 

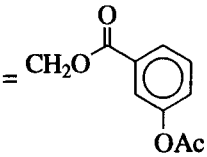
(VI) R = Ac, R^1 = 

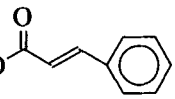
(VII) R =  , R^1 = CH_3

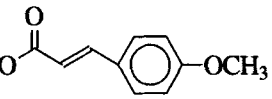
(VIII) R =  ,

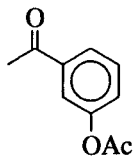
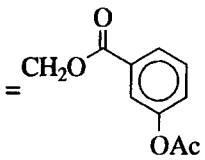
R^1 = 

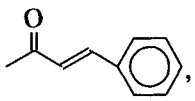
(IX) R = Ac, R^1 = 

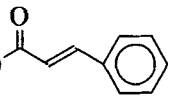
(X) R = H, R^1 = 

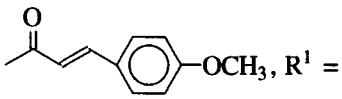
(XI) R = H, R^1 = 

(XII) R = H, R^1 = 

(XIII) R =  , R^1 = 

(XIV) R =  ,

R^1 = 

(XV) R =  , R^1 =

(XVI) R = H, R^1 = CH_2OAc

(XVII) R = Ac, R^1 = CH_2OAc

esters was confirmed by low-field shifts of the C28 resonances by 2–3 ppm, while C2 and C3 atoms resonated at 27.4 and 78.9 ppm, as in the case of the starting betulin. The appearance of carbon resonances of C=O groups at 165–167 ppm, aromatic carbon atoms (at 114–161 ppm), and also proton resonances (at 6.80–7.60 ppm) in the NMR spectra unambiguously confirmed the structure of these compounds. The NMR spectra of 3,28-bissubstituted esters (XIII)–(XV) exhibited resonance shifts from C2, C3, H3, C28, and

Table 1. Prediction of the spectrum of biological activities for (II), (III), (V), (VIII), and (XIII)–(XVII)*

Compound	Predicted activities	P_a
(II)	Inhibitor of 14-α-lanosteroldemethylase**	1.00
	Antagonist of the B-receptors of endothelin	1.00
	Antagonist of A-receptors of endothelin	0.92
	Dermatological	0.87
	Hepatoprotector	0.80
	Inhibitor of reverse transcriptase (EC 2.7.7.49)	0.79
	Agonist of the nerve growth factor	0.78
	Antagonist of gestagens	0.71
(III)	Hepatoprotector	0.80
	Inhibitor of 14-α-lanosteroldemethylase**	0.78
	Agonist of the nerve growth factor	0.78
	Inhibitor of reverse transcriptase (EC 2.7.7.49)	0.75
	Antagonist of A-receptors of endothelin	0.75
	Antagonist of gestagens	0.71
(V)	Dermatological	0.87
	Hepatoprotector	0.78
	Inhibitor of 14-α-lanosteroldemethylase**	0.78
	Agonist of the nerve growth factor	0.78
	Antagonist of gestagens	0.75
	Inhibitor of reverse transcriptase (EC 2.7.7.49)	0.74
	Antagonist of gestagens	0.71
(VIII)	Hepatoprotector	0.78
	Antagonist of gestagens	0.71
	Antagonist of A-receptors of endothelin	0.71
	Inhibitor of reverse transcriptase (EC 2.7.7.49)	0.69
(XIII)	Hepatoprotector	0.78
	Agonist of the nerve growth factor	0.78
	Antagonist of gestagens	0.71
(XIV)	Hepatoprotector	0.79
	Inhibitor of 14-α-lanosteroldemethylase**	0.78
	Inhibitor of reverse transcriptase (EC 2.7.7.49)	0.75
	Antagonist of gestagens	0.71
	Antagonist of A-receptors of endothelin	0.71
(XV)	Dermatological	0.97
	Hepatoprotector	0.75
	Inhibitor of 14-α-lanosteroldemethylase**	0.71
	Antagonist of gestagens	0.71
(XVI)	Antagonist of the B-receptors of endothelin	1.0
	Inhibitor of 14-α-lanosteroldemethylase**	0.89
	Dermatological	0.88
	Hepatoprotector	0.80
	Inhibitor of reverse transcriptase (EC 2.7.7.49)	0.79
	Agonist of the nerve growth factor	0.78
	Antagonist of A-receptors of endothelin	0.75
	Antagonist of gestagens	0.71
(XVII)	Hepatoprotector	0.80
	Inhibitor of 14-α-lanosteroldemethylase**	0.78
	Inhibitor of reverse transcriptase (EC 2.7.7.49)	0.78
	Agonist of the nerve growth factor	0.78
	Antagonist of A-receptors of endothelin	0.75
	Antagonist of gestagens	0.71

*The prediction was performed by the PASS-C computer program [16–18]. Activities with $P_a > 0.7$ were given.

** 14-α-lanosterol demethylase (CYP51, EC 1. Oxidoreductases; EC 1. Lanosterol-14-α-demethylase).

Table 2. The effect of betulin esters on the functional state of rat liver*

Compound	Rate of bile secretion, mg/min after				Total weight of bile for 4 h, mg/100 g
	1 h	2 h	3 h	4 h	
(III)	**8.2 ± 0.9**	**9.7 ± 0.8**	8.5 ± 0.9#	8.1 ± 0.9#	**2075 ± 210**
(V)	**8.4 ± 0.9**	8.4 ± 1#	8.2 ± 1.0#	**10.8 ± 0.9**	**2148 ± 228**
(VIII)	**10.8 ± 0.53**	**11.2 ± 0.8**	**11.5 ± 0.56**	**9.9 ± 0.72**	**2590 ± 156**
(XIII)	**6.1 ± 0.24**	3.76 ± 0.78	4.6 ± 0.51	4.9 ± 0.53	1160 ± 123
(XIV)	4.5 ± 0.9	4.8 ± 0.77	4.3 ± 0.8	4.0 ± 0.83	1060 ± 198
(XV)	4.8 ± 0.9	5.0 ± 1.1	4.7 ± 0.9	4.3 ± 0.91	1130 ± 228
(XVI)	**7.1 ± 0.23**	6.4 ± 0.65	5.7 ± 0.53	7.0 ± 0.6#	1570 ± 120#
(XVII)	3.1 ± 0.51	4.4 ± 1.0	4.85 ± 1.2	9.0 ± 0.72#	1280 ± 200
Extract of birch bark	4.5 ± 0.52	7.1 ± 0.85#	7.3 ± 0.66#	5.8 ± 0.88	1480 ± 174#
Betulin **(II)**	5.3 ± 1.0	4.7 ± 1.0	4.6 ± 0.9	4.1 ± 0.85	1120 ± 225
Silibor	4.6 ± 0.5	6.7 ± 1.0	6.9 ± 0.63#	7.1 ± 0.6#	1520 ± 162#
Control (intact animals)	3.2 ± 0.51	4.8 ± 0.6	4.3 ± 0.63	4.2 ± 0.4	985 ± 125

*The group of experimental animals consisted of 6 rats. The dose of the compound was 50 mg/kg of body weight.
Statistically reliable results ($P < 0.05$) in comparison with the control (intact animals) are marked.
The statistically reliable results in comparison both with the control ($P < 0.05$) and with silibor ($P < 0.002$) are given in bold.

Table 3. Effect of **(III)** on the functional state of liver of rats suffering from CCl_4 hepatitis*

Compound	Dose, mg/kg	Rate of the bile secretion, mg/min after				Total weight of bile for 4 h, mg/100 g
		1 h	2 h	3 h	4 h	
(VIII)	20	4.95 ± 0.3#	4.4 ± 0.1#	4.7 ± 0.2#	4.4 ± 0.2#	1109.5 ± 53#
Carsil	50	2.1 ± 0.2	3.7 ± 0.15**	3.8 ± 0.1**	3.2 ± 0.2**	771 ± 36*
Control (hepatitis)		1.8 ± 0.1	1.9 ± 0.1	1.6 ± 0.1	1.8 ± 0.15	430 ± 42
Control (intact animals)		3.8 ± 0.3	3.6 ± 0.2	3.2 ± 0.2	3.65 ± 0.2	855 ± 38

*The experimental group of animals consisted of 6 rats.
**The statistically reliable results ($P < 0.05$) in comparison with the control (hepatitis) were marked.
The statistically reliable results ($P < 0.002$) in comparison with the control (intact animals) were marked.

H28 and the appearance of additional doubled signals from ester groups and aromatic rings in comparison with those of 28-monosubstituted esters **(X)**–**(XII)**.

We performed a computer prognosis of the spectrum of biological activities for a number of known {**(II)**, **(III)** [16], **(VIII)** [17], **(XVI)** [18], **(XVII)** [19]} and new compounds [**(V)**, **(XIII)**, **(XIV)**, and **(XV)**] by using the PASS-C computer program [20–22]. Hepatoprotective activity and antagonistic activity toward gestagens were predicted for all the compounds (Table 1). The effect of esters **(III)**, **(V)**, **(VIII)**, and **(XIII)**–**(XVII)** on the functional state of rat liver was studied in order to find effective hepatoprotectors among betulin derivatives. The bile production and secretion in intact and experimental animals were used to compare the liver function. The extract of *Betula pendula* birch bark, betulin **(II)**, and silibor (the known hepatoprotector) [23] were used for comparison. We found that the hepatoprotective effect of **(XIII)**–**(XVII)** was similar to that of the extract, betulin **(II)**, and silibor. Esters **(II)**, **(V)**, and **(VIII)** were 1.5–2 times more effective than the comparison preparations at all stages of the experiment (Table 2). Betulin bishemiphthalate **(VIII)** was most active and was chosen for more detailed tests.

In the further series of experiments, the effect of **(VIII)** at a dose of 20 mg/kg [ED_{50} of **(VIII)** was 19.5 mg/kg] on the hepatoprotective (bile production and secretion) function of rat liver was studied in models of experimental hepatitis induced by carbon tetrachloride, tetracycline, and ethanol. Ester **(VIII)** exhibited a pronounced hepatoprotective effect that exceeded the effect of carsil (the known hepatoprotector) in the case of CCl_4-induced hepatitis [23] with respect to the increase in bile secretion at all stages of the experiment (Table 3). The administration of **(VIII)** resulted in decreased intoxication and in the restoration of hepato-

Table 4. Effect of (**VIII**) on the functional state of the liver of rats suffering from tetracycline hepatitis*

Compound	Dose, mg/kg	Rate of the bile secretion, mg/min after				Total weight of bile for 4 h, mg/100 g
		1 h	2 h	3 h	4 h	
(**VIII**)	20	6.2 ± 0.3#	5.5 ± 0.5#	5.1 ± 0.4#	5.2 ± 0.5**	1330 ± 74#
Carsil	50	4.5 ± 0.5	4.0 ± 0.5	4.15 ± 0.65	4.0 ± 0.7	1005 ± 95
Control (hepatitis)		4.5 ± 0.2	4.1 ± 0.3	3.4 ± 0.04	3.7 ± 0.2	958 ± 32
Control (intact animals)		5.5 ± 0.1	5.0 ± 0.2	4.2 ± 0.2	4.5 ± 0.25	1160 ± 26

* The group of experimental animals consisting of 6 rats.

** The statistically reliable results ($P < 0.05$) in comparison with the control (hepatitis) were marked.

The statistically reliable results ($P < 0.002$) in comparison with carsil were marked.

Table 5. Effect of (**VIII**) on the functional state of the liver of animals suffering from hepatitis caused by ethanol*

Compound	Dose, mg/kg	Rate of bile secretion, mg/min after				Total weight of bile for 4 h, mg/100 g
		1 h	2 h	3 h	4 ~	
(**VIII**)	20	5.5 ± 0.3**	4.9 ± 0.4**	3.9 ± 0.6	4.2 ± 0.5#	1115 ± 40**
Carsil	50	4.5 ± 0.5	3.5 ± 0.6	3.2 ± 0.3	2.8 ± 0.3	845 ± 93
Control (hepatitis)		4.4 ± 0.4	3.0 ± 0.4	3.2 ± 0.4	3.17 ± 0.4	834 ± 90
Control (intact animals)		3.5 ± 0.6	3.4 ± 0.5	4.0 ± 0.3	3.7 ± 0.5	892 ± 56

* The group of experimental animals consisted of 6 rats.

** The statistically reliable results ($P < 0.05$) in comparison with the control (hepatitis) were marked.

The statistically reliable results ($P < 0.002$) in comparison with carsil were marked.

cyte function. In addition, (**VIII**) caused a statistically reliable enhancement of bile secretion in the cases of medicinal (tetracycline) and ethanol damage to the liver in comparison with carsil (Tables 4 and 5).

The prediction of the biological activity for (**VIII**) is given in Table 6. For this substance, 56 activities might be probable among the 408 activities predicted by the program, since the $P_a > P_i$ condition was valid only for them. Moreover, the greater the P_a value, the greater is the probability of finding this activity experimentally. At the same time, activities with lower P_a values could be newer and more unexpected. The antiviral and antitumor activities of the lupane group triterpenoids confirm this assumption [4–7].

Thus, betulin esters (**III**), (**V**), (**VIII**), and (**XIII**)–(**XVII**) exhibit a hepatoprotective effect similar to that of the birch bark extract, betulin (**II**), and silibor. Betulin bishemiphthalate (**VIII**) is a more effective hepatoprotector than the known medicine, carsil, in the model of experimental hepatitis induced by carbon tetrachloride, tetracycline, and ethanol and even at a lower dose.

EXPERIMENTAL

Silufol plates (Chemapol, Czech Republic) were used for TLC, with the chromatographic system being 20 : 1 chloroform–methanol. Spots of substances were detected after spraying with a 20% solution of phosphotungstic acid in ethanol and heating at 100–120°C for 2–3 min. Neutral alumina (Russia) was used for column chromatography.

^{13}C- and ^1H NMR spectra were obtained on a Bruker AM-300 spectrometer (75.5 and 300 MHz, respectively) in deuterochloroform with $SiMe_4$ used as an internal standard. Chemical shifts and coupling constants, J, were expressed in ppm and in Hz, respectively. Melting points were determined on a Boetius apparatus. Acetylsalicylic chloride, cinnamic chloride, and *p*-methoxycinnamic chloride were prepared according to the known procedure in [10]. The crushed bark of the *Betula pendula* birch was extracted with 90% isopropyl alcohol [18], and the extract was subjected to column chromatography on alumina. Elution with benzene afforded lupeol (mp 169–171°C) and betulin (mp 256–258°C). Betulin 3-acetate, 28-acetate, and 3,28-diacetate were prepared according to the procedures in [16, 18, 19].

General procedure for preparation of esters (IV)–(IX). A mixture of triterpenoid alcohols (**I**)–(**III**) (1 mmol) and excess acid anhydride were refluxed in anhydrous pyridine (10–15 ml) for 15 h. The reaction mixture was diluted with cool water (200 ml), acidified with 10% HCl, and extracted with benzene. The extract was washed with water, 5% solution of HCl, and again with water, dried over Na_2SO_4, and evaporated in a vacuum.

Table 6. Prediction of biological activity of (**VIII**)

Activity	P_a	Activity	P_a
Hepatoprotector	0.78	Antiviral	0.32
Dermatological	0.73	Inhibitor of cAMP-phosphodiestrase (EC 3.1.4.17)	0.32
Antagonist of gestagens	0.71	Inhibitor of 5α-reductase (EC 1.3.1.22)	0.32
Antagonist of the endothelin A-receptors	0.71	Antiacne	0.31
Inhibitor of the reverse transcriptase (EC 2.7.7.49)	0.70	Antihelminthic	0.30
Treatment of male reproductive function	0.63	Antiinflammatory	0.29
Antagonist of the endothelin B-receptors	0.57	Agonist of mineralocorticosteroids	0.29
Regulator of the lipid metabolism	0.56	Treatment of the female sterility	0.29
Inhibitor of 14-α-lanosterol demethylase**	0.56	Abortive	0.29
Agonist of the nerve growth factor	0.55	Antitumor:	0.28
Treatment of the kidney diseases	0.53	Treatment of osteoporosis	0.28
Immunomodulator	0.46	Contraceptive	0.27
No mutagenic activity	0.45	Cholagogue	0.25
Teratogenic and/or embryotoxic agent	0.44	Inhibitor of the microtubules formation	0.25
Cardiodepressant	0.44	Cardiotonic	0.25
Antiarthritic	0.42	Treatment of prostate diseases	0.23
Immunosuppressive agent	0.41	Anabolic	0.22
Chemoprotector	0.40	Nootropic	0.22
No carcinogenic activity	0.39	Inhibitor of cholesterol esterase	0.20
General anesthetic	0.38	Increase in the level of lipids in blood	0.20
Cholesterol decreasing	0.37	Anesthetic (intravenous)	0.18
Stimulator of hair growth	0.33	Endothelin antagonist	0.17
Antagonist of estrogens	0.33	Androgen antagonist	0.16
Keratolytic	0.33	Contraceptive (postcoital)	0.13
Nonsteroidal antiinflammatory	0.33	Androgen agonist	0.11
Analeptic	0.33	Inhibitor of neuromuscular transmission	0.10
Treatment of psoriasis	0.33	Progesteron antagonist	0.10
Antiviral (AIDS)	0.33	Gestagen	0.03

*Activities with $P_a > 0.03$ were given in the table.

**See notes to Table 1.

Lupeol hemisuccinate (IV). The treatment of lupeol (0.43 g, 1 mmol) with succinic anhydride (0.2 g, 2 mmol) gave (**IV**), yield 0.50 g (95%); R_f 0.54; mp 206–208°C; ^{1}H NMR: 0.77, 0.82, 0.84, 0.93, 1.01 (18 H, 5 s, 6 CH_3), 1.05–1.90 (m, CH_2 and CH of aglycone), 1.70 (3 H, s, CH_3), 2.28–2.32 (1 H, m, H19), 2.60–2.70 (4 H, m, $OOCCH_2CH_2COOH$), 4.45–4.53 (1 H, m, H3), and 4.55 and 4.67 (1 H, d each, J 1.9, H29); ^{13}C NMR: 38.4 (C1), 23.6 (C2), 81.6 (C3), 37.9 (C4), 43.0 (C17), 48.3 (C19), 151.0 (C20), 18.1 (C28), 109.4 (C29), 19.3 (C30), 29.1 and 29.4 (CH_2CH_2), 171.9 (OC=O), and 178.1 (COOH).

Found, %: C 77.93, H 9.91. Calcd. for $C_{34}H_{54}O_4$, %: C 77.52, H 10.33.

Betulin bishemisuccinate (V). The treatment of betulin (**II**) (0.44 g, 1 mmol) with succinic anhydride (0.5 g, 5 mmol) led to (**V**), yield 0.59 g (92%); R_f 0.33; mp 110–112°C; ^{1}H NMR: 0.76, 0.81, 0.95, 1.00 (15 H, 4 s, 5 CH_3), 1.00–2.00 (m, CH_2 and CH of aglycone), 1.66 (3 H, s, CH_3), 2.40–2.45 (1 H, m, H19), 2.53–2.68 (8 H, m, $OOCCH_2CH_2COOH$), 3.88 and 4.30 (1 H, d each, J 11, H28), 4.48–4.50 (1 H, m, H3), and 4.59 and 4.69 (1 H, broad s each, H29); ^{13}C NMR: 38.3 (C1), 23.5 (C2), 81.5 (C3), 37.7 (C4), 46.3 (C17), 48.7 (C19), 150.0 (C20), 63.7 (C28), 109.8 (C29), 19.2 (C30), 29.0, 29.3, and 29.5 (CH_2CH_2), 171.8 and 172.4 (OC=O), and 178.1 and 178.2 (COOH).

Found, %: C 70.78, H 9.31. Calcd. for $C_{38}H_{58}O_8$, %: C 70.99, H 9.09.

Betulin 3-acetate 28-hemisuccinate (VI). The treatment of 3-*O*-acetylbetulin (**III**) (0.48 g, 1 mmol) with succinic anhydride (0.2 g, 2 mmol) resulted in

(**VI**); yield 0.56 g (94%); R_f 0.57; mp 189–192°C; ^{1} NMR: 0.78, 0.81, 0.93, 0.99 (15 H, 4 s, 5 CH_3), 1.00–2.00 (m, CH_2 and CH of aglycone), 1.60 (3 H, s, CH_3), 2.04 (3 H, s, OAc), 2.39–2.44 (1 H, m, H19), 2.64 (4H, broad s, $OOCCH_2CH_2COOH$), 3.84 and 4.43 (1H, d each, *J* 10.9, H28), 4.43 (1 H, dd, *J* 5.5 and 9.1, H3), and 4.55 and 4.65 (1 H, broad s each, H29); ^{13}C NMR: 38.2 (C1), 23.5 (C2), 80.8 (C3), 37.6 (C4), 47.5 (C17), 48.6 (C19), 149,9 (C20), 62.9 (C28), 109.8 (C29), 19.0 (C30), 29.0 and 29.4 (CH_2CH_2), 171.0 and 172.5 (OC=O), 21.2 ($OCO\underline{C}H_3$), and 176.8 (COOH).

Found, %: C 72.28, H 9.11. Calcd. for $C_{36}H_{56}O_7$, %: C 71.96, H 9.39.

Lupeol hemiphthalate (VII). The treatment of lupeol (**I**) (0.43 g, 1 mmol) with phthalic anhydride (0.3 g, 2 mmol) yielded (**VII**) (0.53 g, 93%); R_f 0.58; mp 167–169°C; ^{1} NMR: 0.79, 0.85, 0.88, 0.95, 1.03 (18 H, 5 s, 6 CH_3), 1.05–1.90 (m, CH_2 and CH), 1.70 (3 H, s, CH_3), 2.40–2.45 (1 H, m, H19), 4.58 and 4.70 (1H, d each, *J* 1.5, H29), 4.76 (1 H, dd, *J* 5 and 11.6, H3), 7.53–7.62, 7.68–7.73, and 7.86–7.90 (4 H, all m, aromatic H); ^{13}C NMR: 38.5 (C1), 23.1 (C2), 83.1 (C3), 38.0 (C4), 43.0 (C17), 48.3 (C19), 150.9 (C20), 18.0 (C28), 109.5 (C29), 19.3 (C30), 167.8 (OC=O), 128.5, 129.7, 130.3, 130.7, 132.0, and 133.8 (aromatic C), and 172.9 (COOH).

Found, %: C 78.95, H 9.78. Calcd. for $C_{38}H_{54}O_4$, %: C 79.40, H 9.46.

Betulin bishemiphthalate (VIII). The treatment of betulin (**II**) (0.44 g, 1 mmol) with phthalic anhydride (0.74 g, 5 mmol) yielded (**VIII**) (0.71 g, 96%); R_f 0.30; mp 173–175°C (lit. [17] mp 175°C); ^{1}H NMR: 0.85, 0.88, 0.94, 1.00 (15 H, 4 s, 5 CH_3), 1.00–2.00 (m, CH_2 and CH), 1.71 (3 H, s, CH_3), 2.49–2.53 (1 H, m, H19), 4.05 and 4.25 (1H, d each, *J* 11, H28), 4.49–4.54 (1 H, m, H3), 4.70 and 4.80 (1 H, broad s each, H29), 7.30–7.40, 7.50–7.65, 7.75–7.80, and 7.83–7.97 (8 H, all m, aromatic H); ^{13}C NMR: 38.3 (C1), 22.9 (C2), 82.8 (C3), 37.8 (C4), 46.3 (C17), 48.1 (C19), 149.8 (C20), 64.4 (C28), 109.8 (C29), 19.0 (C30), 167.5 and 168.3 (OC=O), 128.1, 128.6, 129.4, 129.5, 129.9, 130.1, 130.4, 130.5, 131.7, 131.9, 133.4, and 133.6 (aromatic C), and 171.3 and 172.4 (COOH).

Found, %: C 74.34, H 8.33. Calcd. for $C_{46}H_{58}O_8$, %: C 74.76, H 7.91.

Betulin 3-acetate 28-hemiphthalate (IX). The treatment of 3-*O*-acetylbetulin (**III**) (0.48 g, 1 mmol) with phthalic anhydride (0.3 g, 2 mmol) yielded (**IX**) (0.59 g, 93%); R_f 0.52; mp 174–176°C; ^{1}H NMR: 0.75, 0.81, 0.96, 0.97, and 1.03 (15 H, 5 s, 5 CH_3), 1.05–2.00 (m, CH_2 and CH), 1.68 (3 H, s, CH_3), 2.00 (3 H, s, OAc), 2.45–2.50 (1 H, m, H19), 4.38–4.43 (1 H, m, H3), 3.98 and 4.41 (1 H, d each, *J* 11, H28), 4.60 and 4.71 (1 H, broad s both, H29), 7.35–7.40, 7.45–7.55, and 7.65–7.75 (4 H, all m, aromatic H); ^{13}C NMR: 38.3 (C1), 23.6 (C2), 81.0 (C3), 37.7 (C4), 46.4 (C17), 48.9 (C19), 150.0 (C20), 64.4 (C28), 109.9 (C29), 19.1 (C30), 168.5 and 171.2 (OC=O) 20.7 ($OCO\underline{C}H_3$), 128.7, 129.7, 130.1, 130.6, 132.0, and 133.7 (aromatic C), and 172.0 (COOH).

Found, %: C 75.64, H 8.58. Calcd. for $C_{40}H_{56}O_6$, %: C 75.91, H 8.92.

The general procedure of preparation of esters (X)–(XV). A freshly prepared acid chloride (1.5 mmol and 3 mmol for (**X**)–(**XII**) and (**XIII**)–(**XV**), respectively) was added to a solution of betulin (**II**) (1 mmol) in a mixture of anhydrous pyridine and tributylamine upon cooling to 0–5°C and stirring. The mixture was warmed to room temperature, and (**X**)–(**XII**) were isolated after 4 h, whereas (**XIII**)–(**XV**) were isolated after stirring and heating to 60–70°C for 4 h. The reaction mixtures were treated as described for (**IV**)–(**IX**). The monosubstituted esters were purified by column chromatography under elution with benzene.

Betulin 28-acetylsalicylate (X). The treatment of betulin (**II**) (0.44 g) with acetylsalicyl chloride (0.3 g) yielded (**X**) (0.39 g, 64%); R_f 0.46; mp 92–94°C; ^{1}H NMR: 0.80, 0.82, 0.92, 0.99, and 1.03 (15 H, 5 s, 5 CH_3), 1.00–2.00 (m, CH_2 and CH), 1.68 (3 H, s, CH_3), 2.11 (3 H, s, OAc), 2.40–2.46 (1 H, m, H19), 3.22 (1 H, dd, *J* 5.2 and 10.5, H3), 3.92 and 4.33 (1 H, d each, *J* 11, H28), 4.63 and 4.73 (2 H, broad s each, H29), 7.10–7.15, 7.60–7.65, and 8.03–8.08 (4 H, all m, aromatic H); ^{13}C NMR: 38.7 (C1), 27.4 (C2), 78.9 (C3), 38.8 (C4), 47.7 (C17), 48.8 (C19), 150.1 (C20), 63.4 (C28), 109.9 (C29), 19.1 (C30), 164.7 and 169.7 (OC=O), 21.1 ($OCO\underline{C}H_3$),123.4, 126.0, 128.3, 131.5, 133.8, and 150.8 (aromatic C).

Found, %: C 77.83, H 9.18. Calcd. for $C_{39}H_{56}O_5$, %: C 77.44, H 9.33.

Betulin 28-cinnamate (XI). The treatment of betulin (**II**) (0.44 g) with cinnamyl chloride (0.26 g) yielded (**XI**) (0.35 g, 61%); R_f 0.48; mp 127–128°C; ^{1}H NMR: 0.76, 0.83, 0.97, 0.99, and 1.03 (15 H, 5 s, 5 CH_3), 1.00–2.00 (m, CH_2 and CH), 1.70 (3 H, s, CH_3), 2.45–2.53 (1 H, m, H19), 3.19 (1 H, dd, *J* 5.6 and 10.4, H3), 4.00 and 4.40 (1 H, d each, *J* 11, H28), 4.60 and 4.71 (1 H, broad s each, H29), 6.48 (1 H, d, *J* 16, ArCH=C$\underline{H}$), 7.38–7.43 and 7.49–7.53 (5 H, both m, aromatic H), and 7.70 (1 H, d, *J* 16, ArC$\underline{H}$=CH); ^{13}C NMR: 38.8 (C1), 27.5 (C2), 78.9 (C3), 38.9 (C4), 46.7 (C17), 48.9 (C19), 150.1 (C20), 62.9 (C28), 110.0 (C29), 19.3 (C30), 166.5 (OC=O), 128.1, 128.1, 128.9, 128.9, 130.3, and 134.5 (aromatic C), 118.3 (ArCH=$\underline{C}$H), and 144.7 (Ar$\underline{C}$H=CH).

Found, %: C 82.03, H 9.43. Calcd. for $C_{39}H_{56}O_3$, %: C 81.76, H 9.85.

Betulin 28-*p*-methoxycinnamate (XII). The treatment of betulin (**II**) (0.44 g) with *p*-methoxycinnamyl chloride (0.3 g) yielded (**XII**) (0.35 g, 58%); R_f 0.50; mp 182–184°C; ^{1}H NMR: 0.74, 0.81, 0.95, 0.97, and 1.03 (15 H, 5 s, 5 CH_3), 1.00–2.00 (m, CH_2 and CH),

1.68 (3 H, s, CH_3), 2.40–2.47 (1H, m, H19), 3.81 (3 H, s, OCH_3), 3.96 and 4.37 (1 H, d each, *J* 11, H28), 4.58 and 4.69 (1 H, broad s each, H29), 6.31 (1 H, d, ArCH=C<u>H</u>, *J* 16), 6.88 and 7.46 (4 H, both d, *J* 8.4, aromatic H), and 7.63 (1 H, d, *J* 16, ArC<u>H</u>=CH); ^{13}C NMR: 38.7 (C1), 27.4 (C2), 78.8 (C3), 38.8 (C4), 47.7 (C17), 48.8 (C19), 150.1 (C20), 62.6 (C28), 109.8 (C29), 19.1 (C30), 55.3 (OCH_3), 167.8 (OC=O), 114.3, 114.3, 127.1, 128.3, 129.7, and 161.3 (aromatic C), 115.7 (ArCH=<u>C</u>H), and 144.3 (Ar<u>C</u>H=CH).

Found, %: C 79.28, H 9.84. Calcd. for $C_{40}H_{58}O_4$, %: C 79.69, H 9.70.

Betulin bisacetylsalicylate (XIII). The treatment of betulin (**II**) (0.44 g) with acetylsalicyl chloride (0.60 g) yielded amorphous (**XIII**) (0.66 g, 86%); R_f 0.86; ^1H NMR: 0.85, 0.86, 0.90, 0.93, and 1.03 (15 H, 5 s, 5 CH_3), 1.00–2.00 (m, CH_2 and CH), 1.68 (3 H, s, CH_3), 2.03 and 2.05 (6 H, s, 2 OAc), 2.37–2.43 (1 H, m, H19), 3.90 and 4.35 (1 H, d each, *J* 11, H28), 4.46–4.53 (1 H, m, H3), 4.60 and 4.73 (1 H, broad s each, H29), 6.80–7.00, 7.40–7.50, and 8.80–8.90 (8 H, all m, aromatic H); ^{13}C NMR: 38.3 (C1), 23.7 (C2), 80.7 (C3), 37.9 (C4), 47.6 (C17), 48.7 (C19), 149.9 (C20), 62.7 (C28), 109.9 (C29), 19.1 (C30), 164.6, 164.7, 170.4, and 170.8 (OC=O), 20.9 and 21.0 ($OCO\underline{C}H_3$), 119.0, 119.2, 123.5, 123.6, 125.1, 125.8, 128.1, 128.2, 135.6, 136.4, 150.0, and 150.0 (aromatic C).

Found, %: C 75.35, H 7.98. Calcd. for $C_{48}H_{62}O_8$, %: C 75.16, H 8.15.

Betulin biscinnamate (XIV). The treatment of betulin (**II**) (0.44 g) with cinnamyl chloride (0.50 g) yielded (**XIV**) (0.57 g, 81%); R_f 0.88; mp 107–109; ^1H NMR: 0.80, 0.83, 0.86, 0.92, and 0.99 (15 H, 5 s, 5 CH_3), 1.00–2.00 (m, CH_2 and CH), 1.63 (3 H, s, CH_3), 2.40–2.48 (1 H, m, H19), 3.92 and 4.35 (1 H, d each, *J* 11, H28), 4.50–4.58 (2 H, m, H3 and H29), 4.68 (1 H, broad s, H29), 6.36 and 6.39 (1 H, d both, *J* 16, ArCH=C<u>H</u>), 7.25–7.37 and 7.40–7.50 (10 H, both m, aromatic H), 7.59 and 7.62 (1 H, d each, *J* 16, ArC<u>H</u>=CH); ^{13}C NMR: 38.5 (C1), 23.8 (C2), 81.0 (C3), 38.8 (C4), 46.6 (C17), 48.9 (C19), 150.0 (C20), 62.8 (C28), 109.9 (C29), 19.2 (C30), 166.7 and 167.3 (OC=O), 128.0, 128.0, 128.3, 128.3, 128.5, 128.8, 128.8, 129.0, 130.0, 130.2, 134.5, and 134.6 (aromatic C), 118.3 and 118.9 (ArCH=<u>C</u>H), and 144.2 and 144.6 (Ar<u>C</u>H=CH).

Found, %: C 81.75, H 8.58. Calcd. for $C_{48}H_{62}O_4$, %: C 82.00, H 8.89.

Betulin bis-*p*-methoxycinnamate (XV). The treatment of betulin (**II**) (0.44 g) with *p*-methoxycinnamyl chloride (0.59 g) yielded (**XV**) (0.65 g, 85%); R_f 0.85; mp 106–108°C; ^1H NMR: 0.80, 0.82, 0.85, 0.92, and 0.98 (15 H, 5 s, 5 CH_3), 1.00–2.00 (m, CH_2 and CH), 1.63 (3 H, s, CH_3), 2.38–2.45 (1 H, m, H19), 3.73 (6 H, s, 2 OCH_3), 3.90 and 4.34 (1 H, d each, *J* 11, H28), 4.50–4.57 (2 H, m, H3 and H29), 4.68 (1 H, broad s, H29), 6.23 and 6.26 (1 H, d each, *J* 16, ArCH=C<u>H</u>), 6.77–6.88 and 7.38–7.45 (8 H, both m, aromatic H) 7.55–7.57 (1 H, d each, *J* 16, ArC<u>H</u>=CH); ^{13}C NMR: 38.4 (C1), 23.8 (C2), 80.6 (C3), 38.0 (C4), 47.7 (C17), 48.8 (C19), 150.0 (C20), 62.5 (C28), 109.8 (C29), 19.1 (C30), 55.2 and 55.2 (OCH_3), 167.0 and 167.6 (OC=O), 114.2, 114.2, 127.1, 127.2, 128.2, 128.2, 129.6, 129.6, 129.7, 129.7, 161.2, and 161.3 (aromatic C), 115.6 and 116.2 (ArCH=<u>C</u>H), 143.8 and 144.2 (Ar<u>C</u>H=CH).

Found, %: C 78.45, H 8.34. Calcd. for $C_{50}H_{66}O_6$, %: C 78.70, H 8.72.

Spectrum of the biological activity was predicted using a PASS-C 4.00 computer program [20–22] forecasting 408 pharmacological effects, mechanisms of action, mutagenicity, carcinogenicity, teratogenicity, and embryotoxicity. A detailed description of the PASS computer program can be found at the internet web site <http://www.ibmh.msk.su/PASS>. Biological activity is qualitatively described (yes or no) in this system. In addition, the results of this prediction include the probabilities (within the range from 0 to 1) of the presence (P_a) or the absence (P_i) for each activity. The sum of these probabilities is not equal to 1 because they are calculated independently. The results of the prediction for (**II**), (**III**), (**V**), and (**XIII**)–(**XVII**) with $P_a > 0.7$ are presented in Table 1. The prediction of 56 possible activities with $P_a > 0.03$ for (**VIII**) is given in Table 6.

The effect of (II), (III), (V), and (VIII)–(XVII) on the functional state of liver were determined according to bile production and secretion [24] in intact rats. For (**VIII**), animals with experimental (CCl_4), medicinal (tetracycline), and alcohol (ethanol) hepatitis were also tested. The experiments were carried out on white mongrel rats of 180–200 g body weight. The effect of the compounds on the functional state of the liver of intact animals was studied at the first stage. The extract of birch bark, betulin (**II**), and silibor were used for comparison. The bile production by hepatocytes was expressed in mg/min per 100 g of rat weight. The bile secretion was determined according to the amount of bile produced for 4 h and expressed in mg per 100 g of rat weight [25]. The results of experiments after statistical processing were given in Table 2. The effect of (**VIII**) on the functional activity of the liver during hepatitis caused by CCl_4, tetracycline, and ethanol was studied in the next series of experiments according to the techniques in [26]. The effective dose for (**VIII**) (EF_{50} = 19.5 mg/kg) and the toxicity of all the compounds (LD_{50} = 6500 mg/kg) were determined as described in [27]. The results of the experiments after statistical processing were presented in Tables 3–5.

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