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SYNTHESIS OF 3β-HYDROXY-20-OXO-30-NORLUPAN-28-OIC (PLATANIC) ACID AND ITS GLYCOSIDES†

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All available literature data on the chemical synthesis and isolation from natural sources of 3β-hydroxy-20-oxo-30-norlupan-28-oic (platanic) acid and its derivatives and their biological activity were reviewed. A one-step synthesis of platanic acid from betulin was developed. Its glycosylation by α-acetobromoglucose (ABG) catalyzed by Ag_2O in various solvents such as pyridine, CH_2Cl_2, and their mixture was studied. The optimal synthetic schemes for platanic acid monoglucosides and diglucoside were found. NMR spectra of platanic acid and its glycosides were studied.

Keywords: betulin, platanic acid, glucosides, PMR, ^{13}C NMR.

The first synthesis of 3β-hydroxy-20-oxo-30-norlupan-28-oic acid (**1**) was probably carried out in 1943 by Ruzicka and Rey [1] via oxidation of 3β-acetoxy-28-hydroxy-20-oxo-30-norlupane by CrO_3 in AcOH. In 1956, Djerassi and Hodges [2] prepared **1** by successive oxidation of betulinic acid by OsO_4 and HIO_4. In 1963, Aplin et al. prepared methyl-3β-hydroxy-20-oxo-30-norlupan-28-oate (methyl ester of **1**) by ozonolysis of methylbetulinoate in order to confirm by a convergent synthesis the structure of a hydroxyketo acid isolated by them from the bark extract of platanic trees *Platanus × hybrida* Brot. It was identical to a hydroxyketo acid that was isolated in 1961 from the bark of *P. occidentalis* by Thomas and Muller, who called it platanic acid [3]. In 1969, Vystrcil and Budesinsky passed a stream of O_2 containing about 2% ozone through a solution of 3β-acetoxybetulinic acid in $CHCl_3$ cooled in dry ice. Then, the reaction mixture was evaporated, reduced by Zn powder in AcOH, dissolved again in $CHCl_3$, and separated by column chromatography over silica gel to afford 3β-acetoxy-20-oxo-30-norlupan-28-oic and 3β-acetoxy-20-oxo-29-hydroxy-30-norlupan-28-oic acids in 15% yield each [4]. In 1991, we studied the oxidation of betulin, dihydrobetulin, and 3β,28-dihydroxy-18-lupene by RuO_4 ($RuO_2 \cdot xH_2O$–$NaIO_4$) in the biphasic solvent system EtOAc–H_2O. Betulin produced **1** in 60% yield and the aldehyde corresponding to it in 5% yield [5]. Also in 1991, Pradhan et al. prepared the corresponding derivative of **1** in about 50% yield by refluxing in $CHCl_3$ the methyl ester of 3β-acetoxybetulinic acid and *m*-chloroperbenzoic acid [6]. In 2001, Kim et al. prepared **1** in 75% yield by oxidation of betulinic acid by OsO_4/$NaIO_4$ in a dioxane–H_2O solvent mixture [7].

Thus, **1** was synthesized almost 20 years earlier than it was found in a natural source, i.e., the bark of platanic trees, and was called platanic acid [3]. Later **1** was observed most frequently in plants of the Myrtaceae family [8–11]. In 1994, it was isolated from leaves of the tree *Syzigium claviflorum* Roxb. Wall (Myrtaceae) [8]. In 1996, **1** was isolated from the minor components of the ether extract of the aerial part of the New Zealand medicinal bush *Leptospermum scoparium* Forst. (Myrtaceae) [9]. Acid **1** was isolated in Taiwan from the acetone extract of leaves of the tree *Melaleuca leucadendron* L. (Myrtaceae) [10] and in Brazil from the alcohol extract of ground leaves and twigs of the tree *Eugenia moraviana* Berg (Myrtaceae) [11]. The extract of fresh plants *Melilotus messanensis* (Leguminosae) afforded several triterpenoids of the lupane and oleanane series and three norlupane triterpene acids, i.e., the previously known 3,20-dioxo-30-norlupan-28-oic acid and the previously undescribed 29-hydroxy-3,20-dioxo-30-norlupan-28-oic and 3β,29-dihydroxy-20-oxo-30-norlupan-28-oic acids [12]. Column chromatography over silica gel of the evaporated MeOH extract of air-dried fresh leaves and twigs of *Viburnum awabuki* isolated several triterpenoids and 6β-hydroxy-3,20-dioxo-30-norlupan-28-oic acid [13].

† Dedicated to the memory of Professor Nina Ivanovna Uvarova (July 5, 1928-July 6, 2008), the initiator of this investigation.

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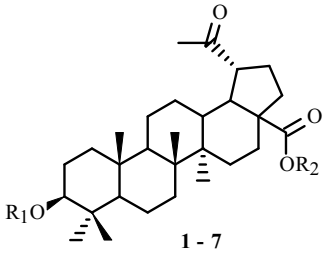
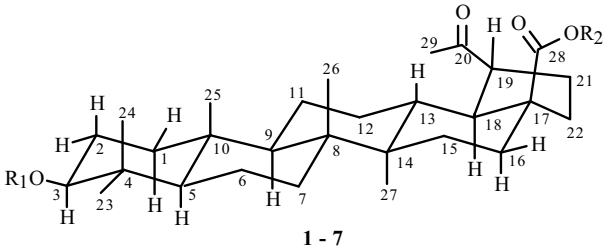
TABLE 1. Ag_2O-Catalyzed Synthesis of Acetylated Glucosides of Platanic Acid

Starting compound (SC)	SC:ABG ratio (mmol)	Solvent	Yield of **2**, %	Yield of **3**, %	Yield of **4**, %	SC recovery, %
1	1:3	Py	49.2	–	–	–
1	1:3	Py–CH_2Cl_2 1:1	84.0	–	–	–
1	1:4	CH_2Cl_2	5.2	67.5	7.0	12.4
1	1:6	CH_2Cl_2	11.5	34.5	17.8	10.2
2	1:3	CH_2Cl_2		–	57.2	27.8

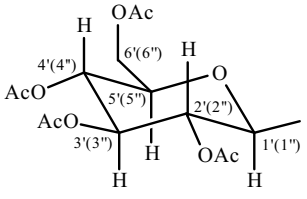


1 - 7

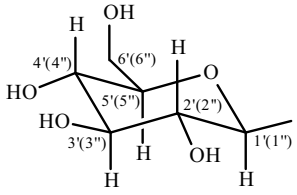


1 - 7

1: R_1= R_2= H; **2:** R_1 = H, R_2 = Glc(Ac)$_4$; **3:** R_1 = Glc(Ac)$_4$, R_2 = H; **4:** R_1 = R_2 = Glc(Ac)$_4$
5: R_1= H, R_2= Glc; **6:** R_1 = R_2 = Glc; **7:** R_1 = Glc, R_2 = H



= Glc(Ac)$_4$



= Glc

The first glycoside of platanic acid that was obtained from a natural source was probably 29-hydroxyplatanic acid 28-*O*-β-D-glucopyranosyl ester, which was isolated from the MeOH extract of leaves of *Eugenia florida* DC. (Myrtaceae) [14]. Three new glycosides of triterpenoids of the lupane series including 3α-hydroxy-20-oxo-30-norlupan-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester were recently isolated from the MeOH extract of dried leaves of the bush *Acanthopanax koreanum* (Araliaceae). The compound exhibited moderate cytotoxicity *in vitro* against the human cancer cell lines (IC_{50}, μg/mL) lung adenocarcinoma A549 (23.4 ± 2.0), leukemia HL-60 (36.5 ± 3.2) and U937 (22.6 ± 1.2), and breast cancer MCF-7 (18.5 ± 1.7) [15].

In 1994, anti-HIV activity was observed for triterpenoids of the lupane series, i.e., betulinic acid, which inhibited replication of HIV in H9 lymphocytes with EC_{50} = 1.4 μM and inhibited a culture of un-infected H9 cells with IC_{50} = 13 μM, and platanic acid (**1**), which showed the corresponding values EC_{50} = 6.5 μM and IC_{50} = 90 μM [8]. Acid **1** and its 29-oxime, 29-methoxime, and 29-hydroxy derivatives showed slight cytotoxic activity against the cell lines HST-116 carcinoma and M14-MEL, SK-MEL-2, and UACC-257 melanoma [7]. 6β-Hydroxy-3,20-dioxo-30-norlupan-28-oic acid exhibited moderate cytotoxicity *in vitro* against the cell lines P388 lymphatic leukemia (IC_{50} = 19.8 μg/mL) and HT-29 human colon carcinoma (IC_{50} = 35.6 μg/mL) [13].

We synthesized **1** together with 3,20-dioxo-30-norlupan-28-oic acid in yields of 15% each via oxidation of betulin by RuO_4 (RuO_2·xH_2O–$NaIO_4$) in the biphasic solvent system EtOAc–H_2O. Although we were unable to reproduce its synthesis with the same efficiency as in our first work [5], our synthesis based on the most available triterpenoid of the lupane series (betulin) also made **1** more available for further investigations. We studied Ag_2O-catalyzed glycosylation of **1** by α-acetobromoglucose (ABG) in various solvents such as pyridine (Py), CH_2Cl_2, and their mixture. Depending on the ratio of reagents and solvents, 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-3β-hydroxy-20-oxo-30-norlupan-28-oate (**2**), 3β-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-20-oxo-30-norlupan-28-oic acid (**3**), and 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-3β-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-20-oxo-30-norlupan-28-oate (**4**) were obtained in various yields (Table 1).

TABLE 2. ^{13}C NMR Chemical Shifts of Platanic Acid (**1**) and the Triterpene Skeleton of Its Acetylated (**2**–**4**) (in $CDCl_3$) and Free (**5**–**7**) (in C_5D_5N) Glucosides (δ, ppm vs. TMS)

C atom	Solutions in $CDCl_3$				Solutions in C_5D_5N			
	1	**2**	**3**	**4**	**1**	**5**	**6**	**7**
1	38.65	38.69	38.68	38.66	39.24	39.18	39.01	38.94
2	27.28	27.38	25.91	25.88	28.28	28.27	26.76	26.73
3	78.94	78.84	90.56	90.54	78.05	77.99	88.83	88.73
4	38.83	38.86	38.99	38.97	39.50	39.47	39.62	39.59
5	55.26	55.28	55.63	55.60	55.86	55.82	55.90	55.81
6	18.24	18.29	18.13	18.16	18.75	18.69	18.43	18.38
7	34.20	34.34	34.26	34.34	34.72	34.51	34.53	34.59
8	40.57	40.61	40.64	40.62	41.01	41.06	41.10	40.93
9	50.33	50.41	50.41	50.43	50.87	50.81	50.76	50.68
10	37.19	37.21	36.94	36.92	37.51	37.40	37.12	37.71
11	20.84	20.88	20.88	20.90	21.21	21.07	21.13	21.14
12	27.19	27.21	27.27	27.25	27.74	27.66	27.67	27.67
13	37.51	37.32	37.50	37.29	37.79	37.45	37.54	37.34
14	42.23	42.28	42.26	42.20	42.64	42.57	42.63	42.59
15	29.69	29.70	29.71	29.68	30.27	30.11	30.15	30.22
16	31.45	31.14	31.47	31.13	32.35	31.72	31.77	32.30
17	56.21	56.67	56.18	56.64	56.49	56.85	56.91	56.46
18	49.21	48.92	49.14	48.83	49.80	49.70	49.75	49.71
19	51.21	50.99	51.12	50.43	52.06	51.58	51.66	52.01
20	212.25	211.66	212.07	211.74	211.57	211.20	211.18	211.59
21	28.27	28.03	28.26	28.02	28.78	28.38	28.44	28.75
22	36.70	36.14	36.70	36.14	37.38	36.71	36.75	37.06
23α	27.96	27.97	27.61	27.59	28.64	28.62	28.16	28.08
24β	15.34	15.35	16.13	16.14	16.32	16.33	16.86	16.81
25β	16.08	16.08	16.01	16.01	16.39	16.36	16.28	16.24
26β	15.94	15.94	15.93	15.93	16.32	16.25	16.32	16.27
27α	14.71	14.74	14.69	14.70	14.90	14.87	14.89	14.83
28	181.49	174.14	180.74	174.11	178.68	174.88	174.91	178.69
29	30.07	30.06	30.17	30.16	29.61	29.69	29.67	29.60

Table 1 shows that the 28-monoglucoside **2** was more convenient to obtain from a Py:CH_2Cl_2 mixture (up to 50% resinous products formed in pure Py) whereas the diglucoside **4** was obtained better from **2** by repeated glycosylation in CH_2Cl_2.

The free glycosides were prepared from tetraacetylated glucoside **2** and octaacetylated diglucoside **4** using a general method of mild deacetylation by treatment with MeONa in MeOH for 3–4 h at room temperature with TLC monitoring. The solutions were neutralized by KU-2-8 ion-exchange resin (H^+-form) and evaporated to produce in about 95% yield the corresponding free glucosides *β*-D-glucopyranosyl-3*β*-hydroxy-20-oxo-30-norlupan-28-oate (**5**) and *β*-D-glucopyranosyl-3*β*-(*β*-D-glucopyranosylxoy)-20-oxo-30-norlupan-28-oate (**6**).

Deacetylated 3-monoglucoside 3*β*-(*β*-D-glucopyranosyloxy)-20-oxo-30-norlupan-28-oic acid (**7**) was prepared in 75.9% yield via saponification of the same diglucoside **4** by KOH solution (10%) in MeOH. This was a more rational route because octaacetylated diglucoside **4** was easier to synthesize than tetraacetylated 3-monoglucoside **3**, which must be isolated from a mixture of three products and the starting compound if synthesized directly.

We observed similar glycosylation trends in preparing glycosides of betulinic and dihydrobetulinic acids [16].

The empirical formulas of the synthesized compounds were confirmed using high-resolution mass spectrometry with electrospray ionization (ESI) and detection of cations. Mass spectra of all compounds **1–7** showed peaks for the cationic molecules $[M + Na]^+$ (monoisotopic masses).

Because of contradictory literature data [9, 11, 14] regarding resonances in ^{13}C NMR spectra of **1**, we performed our own analysis of its PMR and ^{13}C NMR spectra taken at 700 and 175 MHz, respectively, in $CDCl_3$ and C_5D_5N solutions. Our assignment of resonances in the ^{13}C NMR spectrum of **1** in $CDCl_3$ (Table 2) agreed with one study [9] with the exception of the assignments of resonances at 28.3 ppm to C-15 and 29.7 ppm to C-21.

TABLE 3. ^{13}C NMR Chemical Shifts of the Carbohydrate Part of Acetylated (**2**–**4**) and Free (**5**–**7**) Glucosides of Platanic Acid (δ, ppm vs. TMS)

C atom	**2**	**3**	**4**	**5**	**6**	**7**
1′	91.24	102.98	102.97	95.50	106.89	106.89
2′	69.92	71.72	71.72	74.25	75.86	75.81
3′	72.83	72.93	72.93	78.87	78.82	78.76
4′	68.01	68.87	68.85	71.04	71.96	71.66
5′	72.60	71.58	71.59	79.51	78.34	78.33
6′	61.49	62.33	62.31	62.19	63.12	63.03
1″			91.25		95.55	
2″			69.93		74.29	
3″			72.82		78.92	
4″			68.00		71.16	
5″			72.61		79.52	
6″			61.48		62.30	

2, δ_C, ppm $CH_3\underline{C}OO$: 170.47, 170.06, 169.34, 168.94; $\underline{C}H_3COO$: 20.65, 20.53 (3C).
3, δ_C, ppm $CH_3\underline{C}OO$: 170.60, 170.33, 169.41, 169.13; $\underline{C}H_3COO$: 20.62 (4C).
4, δ_C, ppm $CH_3\underline{C}OO$: 170.57, 170.46, 170.31, 170.04, 169.39, 169.34, 169.14, 168.94; $\underline{C}H_3COO$: 20.72, 20.67, 20.65, 20.60, 20.59, 20.53 (3C).

We assigned the resonance at 29.69 ppm to C-15 because of an observed correlation with the H_3-27α methyl protons at 1.00 ppm in the HMBC spectrum and the resonance at 28.78 ppm to C-21 because of an analogous correlation with the H-19 proton at 3.24 ppm. Our assignment of resonances of **1** in C_5D_5N (Table 2) agreed with one study [11] with the exception of the assignment of resonances at 28.8 ppm to C-15 and at 30.3 ppm to C-21 and with another study [14] with the exception of the assignments of the resonances at 21.1 ppm to C-6 and at 18.8 ppm to C-11. We assigned the resonance at 30.27 ppm to C-15 because of an observed correlation with the H_3-27α methyl protons at 1.10 ppm in the HMBC spectrum and the resonance at 28.78 ppm to C-21 because of an analogous correlation with the H-19 proton at 3.69 ppm. The resonance at 21.21 ppm was assigned to C-11 because of an observed HMBC correlation with the H-13 proton at 2.54 ppm. The resonance at 18.75 was assigned to C-6 because of an analogous correlation with the H-5 proton at 0.81 ppm, which correlated with C-5 at 55.86 ppm in the HSQC spectrum.

PMR spectra of all glucosides **2–7** (Table 4), like **1**, exhibited singlets for all six methyls. Their assignments were made based on correlations with resonances of C atoms that were found in HMBC spectra (Table 2). Thus, resonances of CH_3-23α and CH_3-24β methyls correlated with C-3, C-4, C-5 and also with C-24β and C-23α, respectively. Correlations with resonances of C-1, C-5, C-9, and C-10 were observed for the resonance of the CH_3-25β methyl. The resonance of CH_3-26 correlated with resonances of C-7, C-9, and C-14; of CH_3-27α, with C-8, C-13, C-14, and C-15; of CH_3-29, with C-19 and C-20. Correlations of the doublets for the H-1′ anomeric protons of the 28-glucosides (Table 4) for **2** and **5** and H-1″ for **4** and **6** were observed with resonances of the corresponding C-28; of the doublets for the H-1′ anomeric protons of 3β-glucosides **3**, **4**, **6**, and **7**, with resonances of the corresponding C-3 (Table 2). Spin–spin coupling constants for all H-1′ and H-1″ anomeric protons of **2–7** (Table 4) had values of about 8 Hz. This indicated that the glycosidic bond in all these glucosides had the *trans*-configuration.

TABLE 4. PMR Spectral Data (δ, ppm, J/Hz) of Acetylated (**2**–**4**) ($CDCl_3$) and Free (**5**–**7**) (C_5D_5N) Glucosides of Platanic Acid

H atom	**2**	**3**	**4**	**5**	**6**	**7**
3α	3.18 m	3.06 (dd, J = 11.8, 4.6)	3.06 (dd, J = 11.8, 4.6)	3.44 m	3.39 (dd, J = 11.8, 4.5)	3.41 (dd, J = 11.8, 4.5)
5α	0.68 (dd, J = 11.6, 2.1)	0.68 (dd, J = 11.1, 2.2)	0.67 (dd, J = 11.3, 2.2)	0.79 (dd, J = 11.2, 1.8)	0.71 (dd, J = 11.8, 2.3)	0.72 m
19	3.19 m	3.24 (td, J = 11.3, 11.2, 4.9)	3.19 (td, J = 11.2, 11.0, 4.7)	3.59 m	3.57 (td, J = 11.1, 10.5, 4.9)	3.70 (td, J = 11.3, 11.1, 4.6)
αCH_3-23	0.96 s	0.89 s	0.88 s	1.22 s	1.30 s	1.31 s
βCH_3-24	0.75 s	0.71 s	0.71 s	1.01 s	0.97 s	0.98 s
βCH_3-25	0.81 s	0.81 s	0.80 s	0.82 s	0.74 s	0.74 s
βCH_3-26	0.87 s	0.90 s	0.85 s	1.13 s	1.09 s	0.99 s
αCH_3-27	0.98 s	0.99 s	0.97 s	1.06 s	1.09 s	1.13 s
CH_3-29	2.17 s	2.18 s	2.18 s	2.18 s	2.16 s	2.22 s
1′	5.69 (d, J = 8.3)	4.53 (d, J = 8.0)	4.53 (d, J = 8.0)	6.45 (d, J = 8.2)	4.96 (d, J = 7.8)	4.98 (d, J = 7.8)
2′	5.21 (dd, J = 8.3, 9.5)	5.02 (dd, J = 8.0, 9.7)	5.02 (dd, J = 8.0, 9.7)	4.21 (dd*, J = 8.2, 8.9)	4.05 (dd*, J = 7.8, 8.8)	4.07 (dd, J =7.8, 8.6)
3′	5.28 (dd*, J = 9.5, 9.3)	5.20 (dd*, J = 9.7, 9.4)	5.20 (dd*, J = 9.7, 9.4)	4.32 (dd*, J = 8.9, 8.9)	4.27 (dd*, J = 8.8, 8.6)	4.29 (dd*, J = 8.6, 8.8)
4′	5.16 (dd*, J= 9.3, 10.0)	5.04 (dd*, J = 9.4, 10.0)	5.04 (dd*, J = 9.4, 10.0)	4.34 (dd*, J = 8.9, 9.5)	4.24 (dd*, J = 8.8, 8.5)	4.25 (dd*, J = 8.8, 8.8)
5′	3.83 (ddd, J = 10.0, 4.3, 2.4)	3.68 (ddd, J = 10.0, 5.5, 2.6)	3.68 (ddd, J = 10.0, 5.5, 2.6)	4.09 (dd, J = 9.5, 4.4, 2.5)	4.03 (ddd, J = 8.0, 2.5, 2.6)	4.04 (ddd, J = 8.8, 5.4, 2.5)
6′a	4.30 (dd, J = 12.4, 4.3)	4.25 (dd, J = 12.1, 5.5)	4.25 (dd, J = 12.1, 5.5)	4.43 (dd, J = 12.0, 4.4)	4.50 (dd, J = 11.9, 2.5)	4.44 (dd, J = 11.7, 5.4)
6′b	4.09 (dd, J = 12.4, 2.4)	4.10 (dd, J = 12.1, 2.6)	4.11 (dd, J = 12.1, 2.6)	4.50 (dd, J = 12.0, 2.5)	4.60 (dd, J = 11.7, 2.6)	4.62 (dd, J = 11.7, 2.5)
1″			5.68 (d, J = 8.2)		6.43 (d, J = 8.2)	
2″			5.22 (dd, J = 8.2, 9.5)		4.21 (dd, J = 8.2, 9.0)	
3″			5.28 (dd, J = 9.5, 9.2)		4.31 (dd, J = 8.9, 9.0)	
4″			5.16 (dd, J =9.2, 10.0)		4.38 (dd, J = 8.9, 9.6)	
5″			3.83 (ddd, J = 10.1, 4.2, 2.3)		4.08 (ddd, J = 9.6, 2.0, 2.5)	
6″a			4.30 (dd, J = 12.5, 4.2)		4.40 (dd, J = 11.8, 3.0)	
6″b			4.08 (dd, J = 12.5, 2.3)		4.46 (dd, J = 11.8, 2.6)	
OCOC<u>H</u>$_3$	2.08, 2.04, 2.03, 2.03	2.07, 2.02, 2.02, 2.00	2.08, 2.07, 2.04, 2.03, 2.02 (9H), 2.00			

*Doublet of doublets appeared as an asymmetric triplet in the spectrum.

EXPERIMENTAL

Melting points were determined on a Boetius heating stage and were uncorrected. Mass spectra were recorded in an Agilent 6510 Q-TOF LC/MS quadrupole time-of-flight mass spectrometer in cation-mode with electrospray ionization at capillary potential 3500 V, drying gas temperature 325°C, and fragmentor potential 215 V. Samples were dissolved in MeOH (0.001 mg/mL) and introduced into the mass spectrometer using a KD Scientific spray pump (flow rate 5 μL/min). The accuracy of the masses was better than $3 \cdot 10^{-6}$. IR spectra were recorded in $CDCl_3$ or KBr pellets on a Bruker Vector 22 spectrophotometer. PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ for acetylated glycosides **2**–**4** and in C_5D_5N for free glycosides **5** and **6** as 0.02–0.04 M solutions on a Bruker Avance-500 spectrometer (500 and 125 MHz, respectively) and in $CDCl_3$ for **1** and in C_5D_5N for **1** and **7** on a Bruker Avance-700 spectrometer (700 and 175 MHz, respectively) (TMS internal

standard). Chemical shifts are given on the δ-scale relative to TMS. Multiplicities of ^{13}C resonances were established from DEPT-135 experiments using the standard method. Homonuclear 2D COSY-45 spectra for proton–proton correlations and heteronuclear 2D HSQC and HMBC correlation spectra were also measured using standard methods. HMBC experiments were optimized for $^{n}J_{HC}$ = 5 Hz. NMR spectra were analyzed on personal computers using Bruker TopSpin V.2.1.0 and Advanced Chemistry Development, Inc. (ACD/Labs) ACD/NMR Processor Academic Edition V.12.0.1 programs. TLC used Sorbfil plates (Russia). Column chromatography was carried out over SiO_2 using solvent systems hexane:acetone from 40:1 to 5:1and C_6H_6:MeOH from 15:1 to 5:1. Specific rotation to an accuracy of ±1° was determined on a Perkin—Elmer 343 polarimeter using monochromatic light from a sodium lamp (D-line), 1-mL cuvettes (concentration in g/mL), and optical pathlength 100 mm in $CHCl_3$ or C_5H_5N solutions. Reaction mixtures for glycosylation were stirred on a magnetic stirrer at room temperature.

Betulin [3*β*,28-dihydroxy-20(29)-lupene] was isolated as before [17] from the acetone extract of the outer bark of *Betula platyphylla* Sukacz. [*B. mandshurica* (Regel) Nakai] collected in the vicinity of Artem, Primorskii Krai. The acetone extract for partial purification was filtered through a layer of basic Al_2O_3 and evaporated to dryness. The resulting crystalline solid was recrystallized from EtOH with added NaOH (to hydrolyze betulin esters) and then from pure alcohol to afford betulin, mp 247–248°C (EtOH), lit. [18] mp 250–251°C.

Oxidation of Betulin by RuO_4 ($RuO_2{\cdot}xH_2O$–$NaIO_4$) in Biphasic EtOAc–H_2O. A solution of $Ru(OH)Cl_3$ (103.2 mg, 0.45 mmol) and a suspension of betulin (5.06 g, 11.43 mmol) in EtOAc (200 mL) was stirred vigorously, treated dropwise over 9 h with a solution of $NaIO_4$ (15.3 g, 71.5 mmol) in H_2O (150 mL), and left undisturbed overnight. The aqueous layer was separated and extracted with EtOAc (3 × 25 mL). The combined EtOAc solution was stirred, treated with *i*-PrOH (5 mL) to decompose the excess of oxidant, and filtered to remove black $RuO_2{\cdot}xH_2O$. The filtrate was dried over anhydrous $CaCl_2$ and evaporated to dryness in a rotary evaporator. The resulting colored semi-crystalline precipitate was dissolved in C_6H_6 (200 mL) and filtered through a Schott filter (pore size 16) to remove black RuO_2. Then, colloidal RuO_2 was removed by filtration through a 1-cm-thick layer of Al_2O_3 in another Schott filter. The resulting C_6H_6 filtrate was stirred and treated with KOH (10 mL, 10%). The C_6H_6 solution was decanted from the resulting resinous precipitate of triterpene acid salts. The precipitate was dissolved in EtOH (100 mL) and treated with HCl (100 mL, 15%) and H_2O (300 mL). The separated resinous product was dissolved in EtOH (100 mL) and partially decolorized by treatment with activated carbon, filtered, and evaporated to dryness. The resulting precipitates (2.1 g) were chromatographed over a SiO_2 column with elution by hexane:acetone (30:1)→ (5:1) to isolate 3,20-dioxo-30-norlupan-28-oic acid (1.4 mmol or 12.3%), mp 223–224°C (EtOH), $[\alpha]_D^{25}$ +31° (*c* 0.01, $CHCl_3$); lit. [3] data: Me-ester, mp 162–164°C, $[\alpha]_D$ +2° (*c* 1.04, $CHCl_3$); and 3*β*-hydroxy-20-oxo-30-norlupan-28-oic (platanic) acid (**1**, 800 mg, 1.75 mmol or 15%), $C_{29}H_{46}O_4$. Mass spectrum $[M + Na]^+$ 481.3285, mp 280-282°C (EtOH), $[\alpha]_D^{25}$ –31° (*c* 0.01, $CHCl_3$), $[\alpha]_{578}$ –29.4° (*c* 0.014, $CHCl_3$); lit. [8] data: mp 279–282°C, $[\alpha]_D^{25}$ –38° (*c* 0.62, Py). IR spectrum ($CDCl_3$, ν, cm^{-1}): 3700, 3613.5, 3514.1, 3014.8, 2948.1, 2871.8, 1738.9, 1700.7, 1602.4, 1453.3, 1389.7, 1378.5, 1357.1, 1279.1, 1243.3, 1193.8, 1169.1, 1107.4, 1042.5, 1029.6 (Table 2 presents the chemical shifts in the ^{13}C NMR spectrum).

Condensation of 1 with ABG in the Presence of Ag_2O with Various Ratios of Reagents in Various Solvents: Py, Py:CH_2Cl_2, and CH_2Cl_2. General Method. A solution of **1** (~1 mmol) in Py, Py:CH_2Cl_2 (1:1), or CH_2Cl_2 (10–20 mL) was stirred on a magnetic stirrer, treated with the required amount of Ag_2O and then ABG, stirred continuously for from 3–5 h to 2 d at room temperature until ABG disappeared (TLC monitoring), diluted with $CHCl_3$, and filtered to remove insoluble silver compounds. The solvent was distilled completely at reduced pressure. The solid was chromatographed over a column of silica gel with elution by hexane:acetone (40:1)→(5:1) to isolate the acetylated glucosides and the unreacted excess of starting **1**.

Reaction in Py with 1:ABG Mole Ratio 1:3. A mixture of **1** (460 mg, 1.0 mmol), Ag_2O (695 mg, 3 mmol), and ABG (1234 mg, 3 mmol) in Py (10 mL) was stirred for 3 h and worked up by the general method to isolate chromatographically pure **2** (49.2%) and an unidentified resinous compound.

Reaction in Py:CH_2Cl_2 (1:1) with 1:ABG Mole Ratio 1:3. A mixture of **1** (460 mg, 1.0 mmol), Ag_2O (695 mg, 3 mmol), and ABG (1235 mg, 3 mmol) in Py:CH_2Cl_2 (1:1, 10 mL) was stirred for 5 h and worked up by the general method to isolate chromatographically pure **2** (663 mg, 84%).

Reaction in CH_2Cl_2 with 1:ABG Mole Ratio (1:4). A mixture of **1** (460 mg, 1.0 mmol), Ag_2O (926 mg, 4 mmol), and ABG (1645 mg, 4 mmol) in CH_2Cl_2 (15 mL) was stirred for 48 h and worked up by the general method to isolate **2** (41 mg, 5.2%), **3** (663 mg, 67.5%), **4** (78 mg, 7.0%), and starting **1** (57 mg, 12.4%).

Reaction in CH_2Cl_2 with 1:ABG Mole Ratio (1:6). A mixture of **1** (460 mg, 1.0 mmol), Ag_2O (1.39 g, 6 mmol), and ABG (2.47 g, 6 mmol) in CH_2Cl_2 (20 mL) was stirred for 48 h and worked up by the general method to isolate **2** (91 mg, 11.5%), **3** (272 mg, 34.5%), **4** (199 mg, 17.8%), and starting **1** (47 mg, 10.2%).

Repeated Glycosylation of 28-Glucoside 2 in CH_2Cl_2 with 2:ABG Mole Ratio 1:3. A mixture of **2** (565 mg, 0.72 mmol), Ag_2O (500 mg, 2.16 mmol), and ABG (888 mg, 2.16 mmol) in CH_2Cl_2 (10 mL) was stirred for 48 h and worked up by the general method to isolate chromatographically pure **4** (458 mg, 57.2%) and starting **2** (157 mg, 27.8%).

2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl-3β-hydroxy-20-oxo-30-norlupan-28-oate (2), $C_{43}H_{64}O_{13}$, MW 788.97, $[M + Na]^+$ 811.4243, mp 205–206°C (EtOH), $[\alpha]_D^{27}$ –20° (*c* 0.020, $CHCl_3$). IR spectrum ($CDCl_3$, ν, cm^{-1}): 3700, 3611, 3015, 2948, 2872, 1757, 1708, 1602, 1453, 1368, 1249, 1198, 1167, 1070, 1038. Tables 2–4 present the NMR spectral data.

3β-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-20-oxo-30-norlupan-28-oic acid (3), $C_{43}H_{64}O_{13}$, MW 788.97, $[M + Na]^+$ 811.4245, mp 157–160°C (EtOH), $[\alpha]_D^{27}$ –19.7° (*c* 0.020, $CHCl_3$). IR spectrum ($CDCl_3$, ν, cm^{-1}): 3700, 3610, 3514, 3013, 2952, 2873, 1755, 1703, 1602, 1454, 1368, 1248, 1171, 1038. Tables 2-4 present the NMR spectral data.

2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl-3β-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-20-oxo-30-norlupan-28-oate (4), $C_{57}H_{82}O_{22}$, MW 1119.26, $[M + Na]^+$ 1141.5199, mp 251–253°C (EtOH), $[\alpha]_D^{27}$ –16.8° (*c* 0.020, $CHCl_3$). IR spectrum ($CDCl_3$, ν, cm^{-1}): 3700, 3610, 2950, 2874, 1756, 1708, 1602, 1454, 1368, 1249.9, 1195, 1169, 1068, 1038. Tables 2–4 present the NMR spectral data.

General Method for Deacetylation of Acetylated Glucosides 2 and 4. Glucoside (~1 mmol) was dissolved in MeOH (30 mL), treated with MeONa in MeOH (1 mL, 0.1 N), held for 3–4 h at room temperature (TLC monitoring), neutralized with KU-2-8 ion-exchange resin (H^+-form), and evaporated to afford ~95% free glucoside **5** or **6**, respectively.

β-D-Glucopyranosyl-3β-hydroxy-20-oxo-30-norlupan-28-oate (5), $C_{35}H_{56}O_9$, MW 620.82, $[M + Na]^+$ 643.3815, amorph., $[\alpha]_D^{27}$ –26.1° (*c* 0.020, Py). IR spectrum (KBr, ν, cm^{-1}): 3421, 2941, 2871, 2360, 1742, 1690, 1633, 1455.1, 1385, 1362, 1320, 1250, 1072, 1041, 988, 896, 862. Tables 2–4 present the NMR spectral data.

β-D-Glucopyranosyl-3β-(β-D-glucopyranosyloxy)-20-oxo-30-norlupan-28-oate (6), $C_{41}H_{66}O_{14}$, MW 782.87, $[M + Na]^+$ 805.4344, amorph., $[\alpha]_D^{27}$ –19.3° (*c* 0.0075, Py). IR spectrum (KBr, ν, cm^{-1}): 3425, 2942, 2874, 1741, 1698, 1632, 1454, 1385, 1358, 1167, 1075, 896. Tables 2–4 present the NMR spectral data.

Preparation of the 3-Monoglucoside (7). A solution of KOH in MeOH (10 mL, 10%) was treated with acetylated diglucoside **4** (200 mg, 0.18 mmol). The resulting solution was stirred on a magnetic stirrer at room temperature for 7 d (until **4** disappeared according to TLC monitoring), diluted with H_2O (30 mL), neutralized with HCl solution (10%), and extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and evaporated at reduced pressure. The solid was chromatographed over a column of SiO_2 with elution by C_6H_6:MeOH from 15:1 to 5:1 to isolate chromatographically pure 3β-(β-D-glucopyranosyloxy)-20-oxo-30-norlupan-28-oic acid (**7**, 94 mg, 75.9%), $C_{35}H_{56}O_9$, MW 620.82, $[M + Na]^+$ 643.3817, amorph., $[\alpha]_D^{27}$ –35.6° (*c* 0.0136, Py). IR spectrum (KBr, ν, cm^{-1}): 3434, 2944, 2871, 1699, 1632, 1454, 1385, 1358, 1245, 1167, 1077, 1019. Tables 2–4 present the NMR spectral data.

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