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SYNTHESIS OF FLUORINE-CONTAINING BETULIN ESTERS

T. S. Khlebnikova,[1*] Yu. A. Piven′,[1] V. A. Nikolaevich,[1]
A. V. Baranovskii,[1] F. A. Lakhvich,[1]
and Nguen Van Tuen[2]

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28-O-*Fluoroacylbetulins were synthesized via acylation of betulin by fluorocarboxylic (perfluorobutyric, perfluorooctanoic, 3-fluorobenzoic, perfluorobenzoic) acid chlorides in $CHCl_3$ in the presence of Py. 3,28-Di*-O-*fluoroacylbetulins were prepared by treatment of betulin with fluorocarboxylic (trifluoroacetic, pentafluoropropionic) acid anhydrides in Py in the presence of 4-dimethylaminopyridine. 3,28-Di*-O-*(3-fluorobenzoyl)betulin was obtained from acylation of betulin by 3-fluorobenzoic acid in CH_2Cl_2 in the presence of N,N′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine whereas acylation by perfluorobutyric acid under the same conditions gave 28*-O-*perfluorobutanoylbetulin.*

Keywords: betulin, acylation, fluorine-containing betulin esters.

Betulin is a triterpene alcohol that is isolated from birch bark and many other plants. It possesses antiviral (in particular, against herpes virus HSV-1), anti-inflammatory, and antituberculosis activity and other types of biological activity [1, 2]. Therefore, the development of approaches to chemical modification of betulin and the synthesis of effective biologically active compounds based on it are undoubtedly timely. Investigations of the pharmacological properties of betulin esters revealed their hepatoprotective, anti-inflammatory, antitumor, and antiviral properties [3, 4].

Synthetic methods for various fluorine-containing polyfunctional compounds of interest as potential drugs and plant protection agents are currently widely studied [5-9]. Introduction of F atoms and fluoroalkyl groups into a structure affect considerably the physical and chemical properties, reactivity, and biological activity of the compounds [5–9].

We studied the acylation of betulin by fluorocarboxylic acids and their anhydrides and chlorides in order to prepare new fluorine-containing betulin esters.

Betulin (**1**) has two secondary hydroxyls on C-3 and C-28 that can undergo acylation. Depending on the acylation conditions, mono- or diacylated products are formed [3]. Monoacylation of **1** was observed upon treatment with fluorocarboxylic (perfluorobutyric, perfluorooctanoic, 3-fluorobenzoic, perfluorobenzoic) acid chlorides at 18–20°C in anhydrous $CHCl_3$ in the presence of an equivalent amount of Py. The 28-*O*-acylbetulins **2a**–**d** were obtained in 55–83% yield. Diacylation of **1** occurred by reaction with a 12-fold excess of fluorocarboxylic (trifluoroacetic, pentafluoropropionic) acid anhydrides in Py in the presence of 4-dimethylaminopyridine at 18–20°C. 3,28-Di-*O*-fluoroacylbetulins **3a** and **3b** were isolated in 82 and 78% yields. Treatment of **1** in $CHCl_3$ with a 12-fold excess of trifluoroacetic anhydride at 18–20°C produced an inseparable mixture of mono- and diacylated products. Acylation of **1** by 3-fluorobenzoic acid in CH_2Cl_2 in the presence of *N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine at 0°C for 10 min and then for 72 h at room temperature gave diester **3c** in 85% yield whereas acylation of **1** by perfluorobutyric acid under the same conditions gave monoester **2a**.

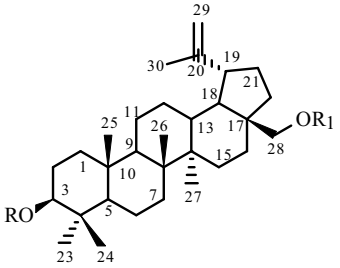
3,28-Di-*O*-trifluoroacetylbetulin (**3a**) was isolated earlier via oxidation of **1** by DMSO activated with trifluoroacetic anhydride [10] and also by reaction of **1** with an excess of trifluoroacetic anhydride in CCl_4 at 0–10°C [11].

The structures of products **2a**–**d** and **3a**–**c** were established and confirmed using IR, PMR, ^{19}F NMR and ^{13}C NMR spectroscopy, and elemental analysis.

1) Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 220141, Minsk, ul. Akad. Kuprevicha, 5/2, e-mail: khlebnicova@iboch.bas-net.by; 2) Chemistry Institute of the Viet Nam Academy of Sciences and Technology, Hanoi, Viet Nam. Translated from Khimiya Prirodnykh Soedinenii, No. 6, November–December, 2011, pp. 807–810. Original article submitted April 27, 2011.

TABLE 1. PMR and ^{13}C NMR Spectra of **3a** and **3b**

C atom	δ_C, ppm, J_{CF}/Hz		H atom	δ_H, ppm	
	3a	**3b**		**3a**	**3b**
1	38.37	38.42	1α	1.02	1.01
2	23.41	23.42	1β	1.75	1.74
3	86.39	86.85	2α	1.70	1.72
4	38.22	38.23	2β	1.75	1.72
5	55.40	55.48	3α	4.67	4.71
6	18.23	18.25	5α	0.81	0.81
7	34.17	34.22	6	1.54	1.54
8	41.04	41.10	6	1.43	1.42
9	50.36	50.43	7 (2)	1.42	1.42
10	37.20	37.25	9α	1.31	1.31
11	20.93	20.98	11α	1.43	1.42
12	25.22	25.30	11β	1.25	1.25
13	37.91	38.01	12α	1.08	1.06
14	42.90	42.95	12β	1.68	1.66
15	27.08	27.08	13β	1.65	1.65
16	29.50	29.50	15	1.10	1.10
17	46.76	46.83	15	1.68	1.66
18	48.93	49.03	16α	1.33	1.32
19	47.82	47.84	16β	1.83	1.79
20	149.65	149.62	18α	1.65	1.65
21	29.50	29.53	19β	2.43	2.42
22	34.36	34.31	21α	1.46	1.46
23	27.93	27.87	21β	1.98	1.98
24	16.38	16.30	22α	1.16	1.15
25	16.28	16.26	22β	1.77	1.75
26	16.14	16.16	23α	0.890	0.88
27	14.91	14.95	24β	0.895	0.88
28	67.04	67.43	25β	0.88	0.88
29	110.44	110.43	26β	1.05	1.05
30	19.24	19.28	27α	1.00	0.99
CF_3	114.83 (q, ^1J = 285)	117.90 (qt, J = 286, 34)	28	4.14	4.15
CF_3	114.89 (q, ^1J = 285)	117.97 (qt, J = 286, 34)	28	4.57	4.60
$2CF_2$	–	106.12 (tq, J = 264, 39)	29α	4.63	4.62
CO	157.57 (q, ^2J = 42)	158.33 (t, ^2J = 29)	29β	4.71	4.71
CO	158.12 (q, ^2J = 42)	158.94 (t, ^2J = 29)	30	1.69	1.69



1, 2a - d, 3a - c

1: R = R_1 = H; **2a:** R = H, R_1 = COC_3F_7; **2b:** R = H, R_1 = COC_7F_{15}
2c: R = H, R_1 = COC_6H_4-(3F); **2d:** R = H, R_1 = COC_6F_5
3a: R = R_1 = $COCF_3$; **3b:** R = R_1 = COC_2F_5; **3c:** R = R_1 = COC_6H_4-(3F)

Taking into account the varied and contradictory data regarding the assignment of proton resonances in the NMR spectra of betulin esters [12], we performed a series of two-dimensional experiments in order to refine the resonances and assign them completely in the PMR and ^{13}C NMR spectra. The experiments (HSQC, COSY, HMBC, TOCSY, NOESY) were performed with **3a** and **3b**. This allowed the C and H chemical shifts and in most instances the steric position of the protons

to be determined. The configurations of the C-6 and C-15 protons could not be established because of overlap of the cross peaks. Table 1 presents the analysis of the spectra.

As expected, differences in the structures of the carboxylic acids had a minimal effect on the chemical shifts of nuclei near the hydroxyls. The substituents did have an effect on the C-3, C-19, C-24, C-26, and C-28 protons in PMR spectra of betulin itself and its acetate [13]. The resonances for C-2, C-3, and C-28 differed by greater than 1 ppm in ^{13}C NMR spectra of betulin and esters **3a** and **3b** and by about 1 ppm for C-17, C-18, C-19, and C-24.

Thus, we synthesized fluorine-containing esters of betulin. Acylation of betulin by fluorocarboxylic (perfluorobutyric, perfluorooctanoic, 3-fluorobenzoic, perfluorobenzoic) acid chlorides in $CHCl_3$ in the presence of Py formed 28-*O*-fluoroacylbetulins whereas acylation of betulin by fluorocarboxylic (trifluooracetic, pentafluoropropionic) acid anhydrides in Py in the presence of 4-dimethylaminopyridine gave 3,28-di-*O*-fluoroacylbetulins. Use of 3-fluorobenzoic acid in the presence of *N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine in CH_2Cl_2 as the acylating agent formed 3,28-di-*O*-(3-fluorobenzoyl)betulin whereas use of perfluorobutyric acid under the same conditions gave 28-*O*-perfluorobutanoylbetulin.

EXPERIMENTAL

NMR spectra were recorded in $CDCl_3$ (if not otherwise specified) with TMS internal standard for PMR spectra (500 MHz) and ^{13}C NMR spectra (125 MHz) and α,α,α-trifluorotoluene with a conversion to CCl_3F for ^{19}F NMR (470 MHz) on a Bruker Avance-500 spectrometer. All NMR experimental data were obtained and processed using XWIN-NMR 3.5 programs. IR spectra were recorded in KBr pellets on a Bomem Michelson 100 instrument. Mass spectra were measured in an HPLC Accela system with an LCQ-Fleet mass detector (three-dimensional ion trap) in chemical ionization mode at atmospheric pressure (APCI). Melting points were determined on a Boetius stage. The course of reactions and purity of products were monitored by TLC on Silufol UV-254 plates (EtOAc:hexane). Column chromatography was carried out over silica gel (EtOAc:hexane). Solvents were evaporated *in vacuo*. Elemental analyses of all compounds agreed with those calculated.

Acylation of Betulin by Fluorocarboxylic Acid Chlorides. A solution of **1** (0.1 mmol) in anhydrous $CHCl_3$ (7 mL) was stirred at 18–20°C; treated with Py (0.1 mmol) and dropwise with fluorocarboxylic acid chloride (0.1 mmol) in anhydrous $CHCl_3$ (5 mL); and stirred for 1 h for perfluorobutyric and perfluorooctanoic acid chlorides, 48 h for 3-fluorobenzoic acid chloride, and 8 h for perfluorobenzoic acid chloride. The solvent was removed. Column chromatography isolated 28-*O*-acyl betulin derivatives **2a**–**d**.

28-*O*-Perfluorobutanoylbetulin (2a). Yield 70%, mp 79–83°C (EtOH), $C_{34}H_{49}F_7O_3$. IR spectrum (KBr, ν, cm^{-1}): 1780 (C=O), 1640 (C=C), 1220 (C–F).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.68 (1H, d, ^3J = 9.4, H-5), 0.76 (3H, s, CH_3), 0.83 (3H, s, CH_3), 0.97 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.69 (3H, s, CH_3), 0.87-2.00 (4H, m, CH, CH_2), 2.41 (1H, m, H-19), 3.19 (1H, dd, J = 11.3, 4.7, H-3), 4.13 (1H, d, ^2J = 11.0, H-28), 4.59 (1H, d, ^2J = 11.0, H-28), 4.61 (1H, s, H-29), 4.70 (1H, s, H-29).

^{19}F NMR spectrum ($CDCl_3$, δ, ppm): –127.13 (2F), –119.4 (2F), –81.0 (3F).

Mass spectrum (APCI): 621 $[M - 18]^+$.

28-*O*-Perfluorooctanoylbetulin (2b). Yield 73%, mp 69–72°C (EtOH), $C_{38}H_{49}F_{15}O_3$. IR spectrum (KBr, ν, cm^{-1}): 1780 C=O), 1640 (C=C), 1220 (C–F).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.68 (1H, d, ^3J = 9.4, H-5), 0.76 (3H, s, CH_3), 0.83 (3H, s, CH_3), 0.97 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.69 (3H, s, CH_3), 0.87-2.00 (4H, m, CH, CH_2), 2.41 (1H, m, H-19), 3.19 (1H, dd, J = 11.3, 4.7, H-3), 4.13 (1H, d, ^2J = 11.0, H-28), 4.58 (1H, d, ^2J = 11.0, H-28), 4.61 (1H, s, H-29), 4.70 (1H, d, H-29).

^{19}F NMR spectrum ($CDCl_3$, δ, ppm): –126.4 (2F), –122.0 (2F), –122.8 (2F), –122.3 (2F), –121.9 (2F), –118.5 (2F), –80.0 (3F).

Mass spectrum (APCI): 821 $[M - 18]^+$.

28-*O*-(3-Fluorobenzoyl)betulin (2c). Yield 55%, mp 190–192°C (EtOH), $C_{37}H_{53}FO_3$. IR spectrum (KBr, ν, cm^{-1}): 1725 (C=O), 1640 (C=C), 1270 (C–F).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.69 (1H, d, ^3J = 9.7, H-5), 0.76 (3H, s, CH_3), 0.84 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.71 (3H, s, CH_3), 0.88-2.07 (4H, m, CH, CH_2), 2.51 (1H, m, H-19), 3.19 (1H, dd, J = 11.4, 4.7, H-3), 4.01 (1H, d, ^2J = 11.0, H-28), 4.53 (1H, d, ^2J = 11.0, H-28), 4.61 (1H, s, H-29), 4.72 (1H, s, H-29), 7.43 (1H, m, H_{arom}), 7.72 (1H, d, ^3J = 9.2, H_{arom}), 7.76 (1H, m, H_{arom}), 7.84 (1H, d, ^3J = 7.7, H_{arom}).

^{19}F NMR spectrum ($CDCl_3$, δ, ppm): –112.6.

28-*O*-Pentafluorobenzoylbetulin (2d). Yield 83%, mp 105–108°C (EtOH), $C_{37}H_{49}F_5O_3$. IR spectrum (KBr, ν, cm^{-1}): 1740 (C=O), 1640 (C=C), 1230 (C–F).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.69 (1H, d, ^3J = 9.4, H-5), 0.76 (3H, s, CH_3), 0.84 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.70 (3H, s, CH_3), 0.87-2.05 (4H, m, CH, CH_2), 2.47 (1H, m, H-19), 3.19 (1H, dd, J = 11.4, 4.7, H-3), 4.15 (1H, d, ^2J = 11.0, H-28), 4.59 (1H, d, ^2J = 11.0, H-28), 4.61 (1H, s, H-29), 4.71 (1H, s, H-29).

^{19}F NMR spectrum ($CDCl_3$, δ, ppm): –160.66 (2F), –148.9 (1F), –138.2 (2F).

Acylation of Betulin by Fluorocarboxylic Acid Anhydrides. A solution of betulin (0.1 mmol) and 4-dimethylaminopyridine (0.2 mmol) in Py (5 mL) at 18–20°C was treated with fluorocarboxylic (trifluoroacetic, pentafluoropropionic) acid anhydride (1.2 mmol). The mixture was stirred for 1.5 h at room temperature, diluted with EtOAc (20 mL) and washed with HCl solution (20%, 3 × 40 mL) and H_2O. The organic layer was dried over anhydrous $MgSO_4$. The solvent was removed to isolate 3,28-di-*O*-acyl derivatives of betulin **3a** and **3b** as colorless crystalline compounds. Table 1 presents the PMR and ^{13}C NMR data for **3a** and **3b**.

3,28-Di-*O*-trifluoroacetylbetulin (3a). Yield 82%, mp 172–175°C (EtOH), $C_{34}H_{48}F_8O_4$. IR spectrum (KBr, ν, cm^{-1}): 1780 (C=O), 1640 (C=C), 1220 (C–F). ^{19}F NMR spectrum ($CDCl_3$, δ, ppm): –75.6, –75.2.

3,28-Di-*O*-pentafluoropropanoylbetulin (3b). Yield 78%, mp 54–57°C (EtOH), $C_{36}H_{48}F_{10}O_4$. IR spectrum (KBr, ν, cm^{-1}): 1780 (C=O), 1640 (C=C), 1220 (C–F). ^{19}F NMR spectrum ($CDCl_3$, δ, ppm): –121.8 (2F), –121.6 (2F), –83.1 (3F), –83.0 (3F).

Acylation of Betulin by Fluorocarboxylic Acids. A solution of **1** (0.1 mmol), fluorocarboxylic acid (perfluorobutyric, 3-fluorobenzoic) (0.4 mmol), and 4-dimethylaminopyridine (catalytic amount) in CH_2Cl_2 (10 mL) at 0°C was treated with *N*,*N*′-dicyclohexylcarbodiimide (0.4 mmol), stirred at 0°C for 10 min and at 18–20°C for 72 h, and filtered to remove the precipitate. The solvent was removed. Column chromatography isolated as colorless crystalline compounds 28-*O*-perfluorobutanoylbetulin (**2a**) in 70% yield for perfluorobutanoic acid and the 3,28-di-*O*-acylderivative of betulin **3c** using 3-fluorobenzoic acid.

3,28-Di-*O*-(3-fluorobenzoyl)betulin (3c). Yield 89%, mp 192–195°C (EtOH), $C_{44}H_{56}F_2O_4$. IR spectrum (KBr, ν, cm^{-1}): 1720 (C=O), 1715 (C=O), 1640 (C=C), 1270 (C–F).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.87 (1H, d, ^3J = 7.1, H-5), 0.91 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.56 (3H, s, CH_3), 1.0-2.07 (4H, m, CH, CH_2), 2.52 (1H, m, H-19), 4.09 (1H, d, ^2J = 11.5, H-28), 4.54 (1H, d, ^2J = 11.5, H-28), 4.62 (1H, s, H-29), 4.71 (1H, dd, J = 11, 5.4, H-3), 4.72 (1H, s, H-29), 7.26 (2H, m, H_{arom}), 7.42 (2H, m, H_{arom}), 7.71 (2H, m, H_{arom}), 7.84 (2H, m, H_{arom}).

^{19}F NMR spectrum ($CDCl_3$, δ, ppm): –112.8, –112.6.

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