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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF BETULIN ESTERS

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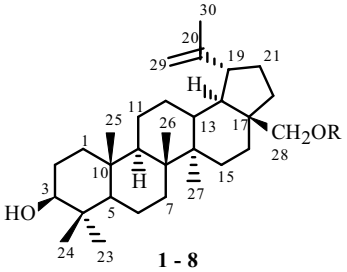
Betulin isonicotinate, 6-chloronicotinate, 2-chlorocinnamate, and benzoates were synthesized by the reaction of betulin and the acid chlorides. It was shown that the 3,4-difluorobenzoate exhibited antibacterial activity against Ochrobactrum anthropi*,* Stenotrophomonas maltophilia*, and* S. nitritireducens *whereas the 3,5-dinitrobenzoate was active against the last two microorganisms.*

Keywords: betulin, esters, antibacterial activity.

The outer part of the bark of several white birch species is a rich source of pentacyclic lupane triterpenoids [1]. The most famous of these is betulin [2]. The high content, simple isolation methods, and presence of reactive functional groups make it an available and convenient starting material for synthesizing new biologically active compounds [3–5]. Compounds with anti-inflammatory, antioxidant, antibacterial, hepatoprotective, antiviral, antitumor, and immunostimulant properties were found among synthesized betulin derivatives [6, 7]. The increasing number of publications in this area indicates that active research is continuing.

Herein we present results from the synthesis of betulin esters and data on their antibacterial activity.

Betulin (**1**) was obtained by extraction of birch (*Betula platyphylla*) bark collected in Chungbuk Province (South Korea). The structure of **1** was confirmed by comparison of its PMR and ^{13}C NMR spectra with those published [8]. Esters **2**–**8** were prepared by reaction of **1** with the acid chlorides in Py (1:1.2 mole ratio) in the presence of dimethylaminopyridine (1.2 equivalents). The 28-*O*-monosubstituted betulin derivatives were isolated in 56–69% yields.



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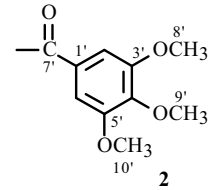

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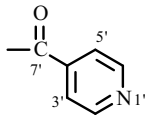

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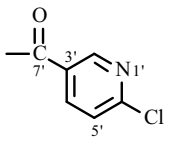

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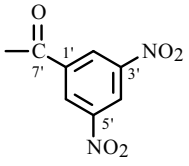

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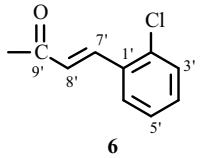

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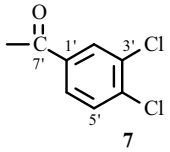

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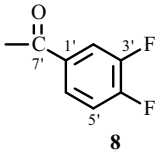

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The structures of the esters were elucidated using IR and NMR spectra and comparison of them with those of analogous compounds [5, 9, 10]. ^{13}C NMR spectra of synthesized **2**–**8** exhibited resonances for the betulin part of the molecule and the corresponding number of C resonances for the acyl components (Table 1). A weak-field shift of the C-28 resonances and the appearance of resonances at 163.1–167.9 ppm indicated that the ester bonds had formed. This was also confirmed by the appearance of absorption bands at ~1700 cm^{-1} in the IR spectra of these compounds.

Synthesized **2**–**8** were checked for antibacterial activity that was assessed using the diameter of the colony growth inhibition zone around paper disks with the applied compound (values are given below after the name of the bacteria). Betulin 3,4-difluorobenzoate (**8**) exhibited activity against *Ochrobactrum anthropi* (12 mm), *Stenotrophomonas nitritireducens* (13 mm), and *S. maltophilia* (11 mm) whereas the 3,5-dinitrobenzoate (**5**) inhibited growth of the last two test cultures (11 and 10 mm, respectively).

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TABLE 1. ^{13}C NMR Spectra of Betulin (**1**) and Esters **2**–**8** (δ, ppm, J/Hz)

C atom	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	38.89	39.07	39.07	39.07	39.07	39.08	39.07	39.05
2	27.58	27.61	27.60	27.60	27.58	27.62	27.61	27.59
3	79.18	79.15	79.12	79.14	79.14	79.18	79.16	79.14
4	39.06	38.92	38.92	38.92	38.91	38.93	38.92	38.90
5	55.47	55.51	55.50	55.50	55.49	55.52	55.51	55.49
6	18.49	18.47	18.48	18.48	18.48	18.51	18.49	18.47
7	34.41	34.40	34.40	34.39	34.40	34.41	34.40	34.38
8	41.10	41.11	41.10	41.10	41.11	41.11	41.11	41.09
9	50.58	50.58	50.57	50.56	50.54	50.60	50.58	50.56
10	37.34	37.36	37.36	37.36	37.36	37.37	37.36	37.34
11	21.01	20.99	20.98	20.98	20.98	21.01	20.99	20.98
12	25.39	25.41	25.40	25.38	25.38	25.43	25.41	25.39
13	37.49	37.87	37.92	37.92	37.99	37.84	37.90	37.88
14	42.91	42.97	42.96	42.96	42.99	42.95	42.97	42.95
15	27.23	27.37	27.30	27.29	27.30	27.34	27.32	27.29
16	29.36	30.36	30.08	30.09	30.13	30.10	30.14	30.10
17	47.98	46.96	46.90	46.90	46.95	46.80	46.90	46.87
18	48.94	49.05	49.06	49.06	49.09	49.06	49.07	49.04
19	48.00	48.03	47.95	47.95	47.95	47.96	47.95	47.94
20	150.67	150.29	150.09	150.07	149.89	150.36	150.16	150.15
21	29.93	29.88	29.76	29.75	29.74	29.86	29.80	29.77
22	34.16	34.99	34.84	34.84	34.87	34.87	34.88	34.85
23	28.18	28.18	28.19	28.19	28.19	28.21	28.19	28.18
24	15.56	15.57	15.58	15.58	15.58	15.58	15.58	15.56
25	16.31	16.30	16.31	16.31	16.32	16.32	16.31	16.30
26	16.17	16.26	16.27	16.25	16.26	16.27	16.26	16.25
27	14.95	15.02	15.02	15.02	15.04	15.01	15.03	15.00
28	60.75	63.76	64.41	64.31	65.78	63.29	64.21	64.05
29	109.90	110.17	110.27	110.28	110.43	110.11	110.23	110.20
30	19.21	19.35	19.35	19.35	19.34	19.38	19.37	19.34
1′		125.69			134.31	135.16	131.66	126.72
2′		117.74	150.80	155.85	129.58	132.93	130.50	119.07 (d, J = 15.1)[a]
3′		153.15	137.82	151.35	148.90	127.84	133.15	150.28 (dd, J = 196.4, 15.5)[a]
4′		142.44	123.06	139.75	122.56	131.22	137.73	153.72 (dd, J = 211.5, 9.6)[a]
5′		153.15	137.82	125.49	148.90	127.27	128.85	117.55 (d, J = 14.6)[a]
6′		117.74	150.80	124.40	129.58	130.38	130.75	127.67
7′		166.72	165.64	164.96	163.09	140.64	165.29	167.87
8′		56.46				121.09		
9′		61.13				167.14		
10′		56.46						

[a]Splitting constants (Hz) of C resonances by F atoms are given in parentheses.

These microorganisms are Gram-negative pathogenic bacteria. *S. maltophilia* causes absess–septic infections [11]. *S. nitritireducens* can cause various types of biological damage [12]. *O. anthropi* causes sepsis in people with weakened immunity [13]. Compounds that exhibit activity against *Bacillus subtilis*, *Escherichia coli*, *Enterrococcus faecalis*, *Micrococcus luteus*, *Staphylococcus aureus*, *S. epidermidis* [14, 15], and *Klebsiella pneumoniae* [16] are known among betulin derivatives. This list of bacteria sensitive to betulin compounds can be extended to the three microorganisms noted above as a result of the present work.

EXPERIMENTAL

^{13}C NMR and PMR spectra were recorded in $CDCl_3$ on a Varian Unity Inova 600 spectrometer at operating frequencies 125 and 600 MHz, respectively, using TMS as an internal standard. IR spectra were recorded on an FT-IR spectrometer

(JASCO FT-IR 4100) using attenuated total internal reflectance (ATIR). Elemental analysis was performed on an EA1110-FISONS instrument (ThermoQuest Italia SPA, CE Instruments). Melting points were determined on a Fisher-Jones instrument (Fisher Scientific Co.). All acid chlorides were purchased from Sigma-Aldrich.

Preparative reversed-phase HPLC was carried out on an OptimaPaK C_{18} column (250 × 21.1 mm, 10 μm particle size) with isocratic elution by $CH_3CN:H_2O$ (86:14, vol%) at flow rate 7 mL/min. Absorbance of effluents was estimated at 212 nm using a UV detector (UV730D, Young Lin Instrument, Korea).

Isolation of Betulin from Birch Bark. Dried and ground bark from *B. platyphylla* (265.0 g) was soaked in EtOAc and refluxed for 2 h. The yield of dry extract after double extraction was 53.1 g (20.0%). The resulting extract was recrystallized twice from $CHCl_3$:EtOH (82:18, vol%). Small impurities of polar compounds were removed over a column of Al_2O_3 to afford pure **1** (99.0%, 32.5 g, HPLC analysis).

General Method for Synthesizing 2–8. A solution of **1** (2.0 g, 4.5 mmol), acid chloride (5.4 mmol), and dimethylaminopyridine (0.64 g, 5.4 mmol) in anhydrous Py (35 mL) was stirred at 40°C under a N_2 atmosphere for 24 h. The mixture was diluted with EtOAc, washed several times with HCl solution (5%) and then H_2O until neutral, and dried over anhydrous Na_2SO_4. The solvent was vacuum distilled. The solid was purified by preparative HPLC to afford highly pure esters **2**–**8** in 56–69% yields.

Antibacterial activity was determined using paper disks as described previously [17]. Disks of diameter 8 mm (Advantec Toyo Roshi Kaisha, Ltd., Japan) were treated with a solution (60 μL) of the compound in DMSO (1 mg/mL) and placed on the surface of inoculated agar in Petri dishes. All tests were performed in triplicate. Pure solvent was used as a negative control; gentamicin, as a positive control. The test cultures *Ochrobactrum anthropi* KCTC 22833^T and *Stenotrophomonas maltophilia* KCTC 1773^T were obtained from the Korean Collection for Type Culture (KCTC); *Stenotrophomonas nitritireducens* KACC 10891^T, from the Korean Agricultural Culture Collection (KACC).

Betulin 28-*O*-3,4,5-trimethoxybenzoate (2), $C_{40}H_{60}O_6$, 61% yield, white crystalline compound, 97.9% pure (HPLC analysis), mp 223–224°C. IR spectrum (ATIR, ν, cm^{-1}): 3410 (br), 2939, 2866, 1701, 1640, 1588, 1462, 1415, 1334, 1227, 1125, 1009, 874, 768, 732.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76, 0.84, 0.97, 1.01, 1.07 (15H, 5s, CH_3-23, 24, 25, 26, 27), 1.71 (3H, s, CH_3-30), 2.54 (1H, td, J = 11.4, 5.4, H-19), 3.19 (1H, dd, J = 11.4, 4.8, H-3), 3.90 (6H, s, CH_3-8′, 10′), 3.91 (3H, s, CH_3-9′), 4.09 and 4.53 (2H, both d, J = 10.8, H-28), 4.61 and 4.72 (2H, both br.s, H-29), 7.31 (2H, s, H-2′, 6′) (for all compounds, only characteristic proton resonances are given).

Betulin 28-*O*-isonicotinate (3), $C_{36}H_{53}NO_3$, 69% yield, white crystalline compound, 98.3% pure (HPLC analysis), mp 221–222°C. IR spectrum (ATIR, ν, cm^{-1}): 3420 (br), 2936, 2868, 1715, 1639, 1599, 1560, 1455, 1406, 1377, 1347, 1325, 1287, 1255, 1120, 1066, 1046, 1013, 970, 915, 883, 858, 849, 841, 831, 822, 810, 760, 708, 675.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.77, 0.84, 0.97, 1.01, 1.06 (15H, 5s, CH_3-23, 24, 25, 26, 27), 1.71 (3H, s, CH_3-30), 2.52 (1H, td, J = 11.4, 5.4, H-19), 3.19 (1H, dd, J = 11.4, 4.8, H-3), 4.13 and 4.57 (2H, both d, J = 10.8, H-28), 4.62 and 4.73 (2H, both br.s, H-29), 7.85 (2H, d, J = 4.8, H-3′, 5′), 8.79 (2H, d, J = 4.8, H-2′, 6′)

Betulin 28-*O*-6-chloronicotinate (4), $C_{36}H_{52}ClNO_3$, 56% yield, white crystalline compound, 98.5% pure (HPLC analysis), mp 193–194°C. IR spectrum (ATIR, ν, cm^{-1}): 3430 (br), 2936, 2868, 1720, 1638, 1586, 1455, 1389, 1367, 1288, 1269, 1122, 1105, 1045, 1019, 971, 879, 766.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.77, 0.84, 0.97, 1.01, 1.06 (15H, 5s, CH_3-23, 24, 25, 26, 27), 1.71 (3H, s, CH_3-30), 3.18 (1H, dd, J = 11.4, 4.8, H-3), 4.13 and 4.57 (2H, both d, J = 10.8, H-28), 4.62 and 4.72 (2H, both br.s, H-29), 7.43 (1H, m, H-5′), 8.25 (1H, m, H-4′), 9.01 (1H, m, H-2′).

Betulin 28-*O*-3,5-dinitrobenzoate (5), $C_{37}H_{52}N_2O_7$, 65% yield, white crystalline compound, 98.2% pure (HPLC analysis), mp 239–240°C. IR spectrum (ATIR, ν, cm^{-1}): 3420 (br), 2932, 1717, 1640, 1542, 1458, 1386, 1340, 1279, 1177, 1074, 1044, 984, 922, 884, 773 721.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.77, 0.84, 0.97, 1.02, 1.08 (15H, 5s, CH_3-23, 24, 25, 26, 27), 1.71 (3H, s, CH_3-30), 2.52 (1H, td, J = 10.2, 5.4, H-19), 3.19 (1H, dd, J = 11.4, 4.8, H-3), 4.24 and 4.68 (2H, both d, J = 10.8, H-28), 4.63 and 4.74 (2H, both br.s, H-29), 9.15 (2H, s, H-2′, 6′), 9.23 (1H, s, H-4′).

Betulin 28-*O*-2-chlorocinnamate (6), $C_{39}H_{55}ClO_3$, 59% yield, white crystalline compound, 98.2% pure (HPLC analysis), mp 200–201°C. IR spectrum (ATIR, ν, cm^{-1}): 3410 (br), 2941, 2868, 1703, 1638, 1454, 1385, 1323, 1267, 1185, 1107, 1087, 1039, 1017, 977, 887, 766.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76, 0.83, 0.97, 0.99, 1.06 (15H, 5s, CH_3-23, 24, 25, 26, 27), 1.70 (3H, s, CH_3-30), 2.50 (1H, td, J = 10.8, 5.4, H-19), 3.19 (1H, dd, J = 11.4, 4.8, H-3), 4.00 and 4.42 (2H, both d, J = 10.8, H-28),

4.60 and 4.71 (2H, both br.s, H-29), 6.45 (1H, d, J = 16.2, H-8′), 7.29 (2H, m, H-4′, 5′), 7.42 (1H, d, J = 7.8, H-3′), 7.63 (1H, d, J = 7.2, H-6′), 8.10 (1H, d, J = 16.2, H-7′).

Betulin 28-*O*-3,4-dichlorobenzoate (7), $C_{37}H_{52}Cl_2O_3$, 63% yield, white crystalline compound, 98.4% pure (HPLC analysis), mp 240–241°C. IR spectrum (ATIR, ν, cm^{-1}): 3420 (br), 2930, 2867, 1704, 1644, 1456, 1387, 1284, 1272, 1237, 1147, 1113, 1045, 1031, 981, 884, 759.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76, 0.83, 0.97, 1.00, 1.06 (15H, 5s, CH_3-23, 24, 25, 26, 27), 1.70 (3H, s, CH_3-30), 2.51 (1H, td, J = 10.8, 6.0, H-19), 3.19 (1H, dd, J = 11.4, 4.8, H-3), 4.10 and 4.53 (2H, both d, J = 10.8, H-28), 4.63 and 4.72 (2H, both br.s, H-29), 7.53 (1H, d, J = 8.4, H-5′), 7.86 (1H, dd, J = 8.4, 1.8, H-6′), 8.10 (1H, d, J = 1.8, H-2′).

Betulin 28-*O*-3,4-difluorobenzoate (8), $C_{37}H_{52}F_2O_3$, 60% yield, white crystalline compound, 98.5% pure (HPLC analysis), mp 200–201°C. IR spectrum (ATIR, ν, cm^{-1}): 3410 (br), 2935, 2867, 1702, 1649, 1519, 1436, 1315, 1197, 1115, 1092, 1047, 973, 887, 833, 777, 766.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76, 0.83, 0.97, 1.00, 1.06 (15H, 5s, CH_3-23, 24, 25, 26, 27), 1.70 (3H, s, CH_3-30), 2.51 (1H, td, J = 10.8, 5.4, H-19), 3.19 (1H, dd, J = 11.4, 4.8, H-3), 4.09 and 4.52 (2H, both d, J = 10.8, H-28), 4.61 and 4.72 (2H, both br.s, H-29), 7.23 (1H, m, H-5′), 7.84 (2H, m, H-2′, 6′).

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REFERENCES

1. P. A. Krasutsky, *Nat. Prod. Rep.*, **23**, 919 (2006).
2. G. A. Tolstikov, E. E. Shults, L. A. Baltina, T. G. Tolstikova, and O. B. Flekhter, *Chem. Sustainable Develop.*, **13**, 1 (2005).
3. I. A. Tolmacheva, A. V. Nazarov, O. A. Maiorova, and V. V. Grishko, *Khim. Prir. Soedin.*, 491 (2008) [*Chem. Nat. Comp.*, **44**, 606 (2008) (Engl. transl.)].
4. N. V. Uzenkova, N. I. Petrenko, M. M. Shakirov, E. E. Shults, and G. A. Tolstikov, *Khim. Prir. Soedin.*, 571 (2005) [*Chem. Nat. Comp.*, **41**, 692 (2005) (Engl. transl.)].
5. R. C. Santos, J. A. R. Salvador, S. Marin, and M. Cascante, *Bioorg. Med. Chem.*, **17**, 6241 (2009).
6. T. G. Tolstikova, I. V. Sorokina, G. A. Tolstikov, A. G. Tolstikov, and O. B. Flekhter, *Russ. J. Bioorg. Chem.*, **32**, 37 (2006).
7. S. Alakurtti, T. Makela, S. Koskimies, and J. Yli-Kauhaluoma, *Eur. J. Pharm. Sci.*, **29**, 1 (2006).
8. S. A. Ayatollahi, A. Shojaii, F. Kobarfard, M. Nori, M. Fathi, and M. I. Choudhari, *J. Med. Plant Res.*, **3**, 660 (2009).
9. O. B. Flekhter, L. T. Karachurina, V. V. Poroikov, L. R. Nigmatullina, L. A. Baltina, F. S. Zarudii, V. A. Davydova, L. V. Spirikhin, I. P. Baikova, F. Z. Galin, and G. A. Tolstikov, *Bioorg. Khim.*, **26**, 215 (2000).
10. M. Kvasnica, J. Sarek, E. Klinotova, P. Dzubak, and M. Hajduch, *Bioorg. Med. Chem.*, **13**, 3447 (2005).
11. M. E. Falagas, P. E. Valkimadi, Y. T. Huang, D. K. Matthaiou, and P. R. Hsueh, *J. Antimicrob. Chemother.*, **62**, 889 (2008).
12. H.-L. Alakomi, A. Paananen, M.-L. Suihko, I. M. Helander, and M. Saarela, *Appl. Environ. Microbiol.*, **72**, 4695 (2006).
13. S. A. Vaidya, D. M. Citron, M. B. Fine, G. Murakami, and E. J. Goldstein, *J. Clin. Microbiol.*, **44**, 1184 (2006).
14. P. A. Krasutsky and R. M. Carlson, WO Pat. 026762 (2002).
15. J. Yli-Kauhaluoma, S. Koskimies, S. Alakurtti, T. Makela, and P. Tammela, WO Pat. 141389 (2007).
16. O. B. Kazakova, G. V. Giniyatullina, G. A. Tolstikov, N. I. Medvedeva, T. M. Utkina, and O. L. Kartashova, *Russ. J. Bioorg. Chem.*, **36**, 383 (2010).
17. M. L. Delignette-Muller and J. P. Flandrois, *J. Antimicrob. Chemother.*, **34**, 73 (1994).