# New Caryophyllene Derivatives from *Betula litwinowii*

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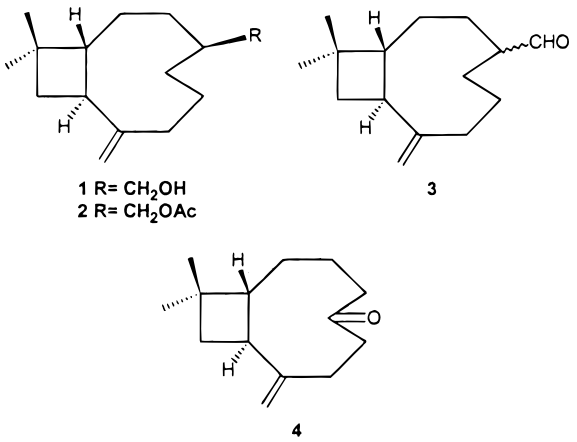
Four new caryophyllene derivatives−14-hydroxy-4,5-dihydro-$\beta$-caryophyllene (**1**), 14-acetoxy-4,5-dihydro-$\beta$-caryophyllene (**2**), 4,5-dihydro-$\beta$-caryophyllene-14-al (**3**), and caryophylla-8(14)-en-5-one (**4**)—are reported from the essential oil of *Betula litwinowii*. Compounds **1**−**4** were characterized by MS and 1D and 2D NMR analyses and chemical transformations. The antibacterial and antifungal activity of **1** is also reported.

*Betula* sp. (Betulaceae), commonly known as birch, are trees and tree-like shrubs that are widespread in temperate regions of the world. A characteristic feature of *Betula* species is their white bark. Five *Betula* species can be found in the north eastern and eastern regions of Turkey. *B. litwinowii* Doluch. is distributed in Turkey and the Caucasus at altitudes ranging from 1700 to 2800 m and has a characteristic pinkish white trunk.[2,3] The medicinal and cosmetic applications of *Betula* leaves, tar, and essential oils have been known since ancient times, and the essential oils obtained from *Betula* species have been the subject of many investigations.[4−7]

Our research on *Betula* species growing in Turkey has resulted in isolation of the new caryophyllene derivatives (**1**−**4**) from *B. litwinowii* The biological activities of the main essential oil component, 14-hydroxy-4,5-dihydro-$\beta$-caryophyllene (**1**), are also discussed.




## Results and Discussion

Water distillation of semi-fresh buds of *B. litwinowii* yielded a colorless essential oil with a characteristic odor. The oil was subjected to MPLC, which provided compound **1** in 25% yield from the essential oil mixture.

The HRES−FTMS of **1** provided a molecular ion $[M - H]^+$ at *m*/*z* 221.1906, suggesting the molecular formula $C_{15}H_{26}O$ and three degrees of unsaturation. The $^{13}C$ NMR and DEPT spectra contained 15 carbon signals; an exocyclic double bond, two tertiary methyls, seven methylenes (one bearing oxygen), three methines, and one quaternary carbon atom. These data, along with other spectral data, indicated the close similarity of **1** to $\beta$-caryophyllene.[8] The methyl carbons resonating at $\delta$ 22.3 and 30.3 were assigned C-12 and C-13, respectively (Table 1). These assignments were based on $^3J$-HMBC correlations between $H_3$-12 and -13 with C-1 and C-10. The geminal location of both C-12 and C-13 methyl carbons on C-11 was deduced from their $^3J$-HMBC correlation to each other and their $^2J$-HMBC correlation to the same quaternary carbon at $\delta$ 33.4 (C-11). The proton H-9 ($\delta$ 2.50, Table 1) displayed strong COSY couplings to H-1 ($\delta$ 1.68) and $H_2$-10 ($\delta$ 1.66 and 1.77). H-9 also showed a $^3J$-HMBC correlation with C-11, while H-1 was HMBC-correlated with C-10, which confirmed the assignments of the cyclobutane ring. The $\Delta^{8,15}$ system was assigned on the basis of the $^3J$-HMBC correlations between the exomethylene olefinic singlets at $\delta$ 4.82 and 4.88 ($H_2$-15) and C-7 and C-9. Both $H_2$-15 signals also displayed a $^2J$-HMBC correlation to the quaternary olefinic carbon at $\delta$ 153.9 (C-8). The downfield oxygenated doublet of doublets at $\delta$ 3.37 and 3.45 ($H_2$-14) displayed COSY coupling with H-4 ($\delta$ 1.50). $H_2$-14 also showed $^3J$-HMBC correlations with both C-3 and C-5 ($\delta$ 32.1 and 28.4, respectively). Both $H_2$-3 signals also displayed a $^3J$-HMBC correlation to C-1, confirming the assignments of the macrocycle C-1/C-9. The relative stereochemistry of **1** was resolved by comparison of $^{13}C$ NMR chemical shift values with literature,[9] molecular modeling study (Spartan 5.0), and analysis of NOESY data (Figure 1). The α-oriented H-9 in **1** (Figure 1) displayed NOESY correlation with the proton multiplet at $\delta$ 2.00 (H-7a), suggesting a similar stereo orientation. The proton H-7a also displayed a NOESY correlation with H-4, which indicated the $\beta$-orientation of the hydroxymethylene C-14. The assignment of the relative stereochemistry of C-12 and C-13 methyl groups was primarily based on comparison of their $^{13}C$ NMR chemical shift values with other known caryophyllenes.[9] Thus, the structure of **1** was established as 14-hydroxy-4,5-dihydro-$\beta$-caryophyllene, a new natural product.

Compound **2** displayed a molecular ion peak $[M]^+$ at *m*/*z* 264, suggesting the molecular formula $C_{17}H_{28}O_2$. The IR spectrum of **2** displayed strong bands at 1761 and 1231 $cm^{-1}$, suggesting the presence of an ester moiety. The GC retention data and $^1H$ and $^{13}C$ NMR data of **2** (Table 1) suggested the close similarity to **1**, with one additional acetate moiety. The proton singlet resonating at $\delta$ 2.04 correlated with the methyl carbon at $\delta$ 21.0 and was assigned to the 14-*O*-acetate group. The carbonyl carbon absorbed at $\delta$ 171.3 completed the acetate functionality. Hence, compound **2** was proved to be 14-acetoxy-4,5-

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**Table 1.** $^{13}C$ and $^{1}H$ NMR Spectral Data of Compounds **1**−**4**[a]

| | **1**[b] | | **2** | | **3** | | **4**[b] | |
|---|---|---|---|---|---|---|---|---|
| C# | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 55.9 | 1.68, m | 55.4 | 1.65, 1H, m | 55.7 | 1.65, 1H, m | 54.6 | 1.70, m |
| 2 | 30.1 | 1.69, 2H, m | 28.1 | 1.73, 2H, m | 26.9 | 1.70, 2H, m | 28.0 | 1.72, m |
| 3 | 32.1 | 1.07, m (Ha); 1.78, m (Hb) | 29.4 | 1.12, m | 26.3 | 1.19, m | 24.8 | 1.76, m (Ha); 2.02, m (Hb) |
| 4 | 40.9 | 1.50, m | 37.1 | 2.43, m | 43.9 | 2.20, m | 43.7 | 2.40, m |
| 5 | 28.4 | 1.34, 2H, m | 31.7 | 1.26, m | 27.4 | 1.25, m | 215.2 | |
| 6 | 27.6 | 1.59, 2H, m | 27.1 | 1.43, 2H, m | 25.1 | 1.57, 2H, m | 41.5 | 2.36, m; 2.57, m |
| 7 | 35.5 | 2.00, m (Ha); 2.36, m (Hb) | 35.1 | 2.30, m | 29.6 | 2.20, m | 34.9 | 1.80, m (Ha); 2.36, m (Hb) |
| 8 | 153.9 | | 153.4 | | 153.8 | | 152.7 | |
| 9 | 46.3 | 2.50, dd (8.8, 9.3) | 45.8 | 2.45, m | 49.1 | 2.35, m | 45.7 | 2.29, dd (9.4, 9.8) |
| 10 | 37.7 | 1.66, m (Ha); 1.77, m (Hb) | 37.4 | 1.63,2H, m | 38.6 | 1.61, 2H, m | 39.1 | 1.68, m |
| 11 | 33.4 | | 33.5 | | 33.5 | | 33.5 | |
| 12 | 22.3 | 1.00, 3H, s | 21.9 | 0.98, 3H, s | 21.6 | 0.95, 3H, s | 22.3 | 1.01, 3H, s |
| 13 | 30.3 | 1.00, 3H, s | 29.9 | 0.98, 3H, s | 29.8 | 0.91, 3H, s | 30.3 | 1.04, 3H, s |
| 14 | 68.7 | 3.37, dd (4.3, 10.4) 3.45, dd (6.1, 10.4) | 69.4 | 3.86, 2H, d (6.4) | 205.2 | 9.47, s | 111.6 | 4.91, s; 4.99, d (1.9) |
| 15 | 109.8 | 4.82, s; 4.88, s | 109.6 | 4.84, 2H, d (4.3) | 109.7 | 4.80, 2H, m | | |
| 14-*O*Ac | | | 21.0 | 2.04, 3H, s | | | | |
| 14-O*Ac* | | | 171.3 | | | | | |

[a] In $CDCl_3$, 22.4 MHz for $^{13}C$ NMR and 90 MHz for $^{1}H$ NMR. Coupling constants ($J$) are in Hz. [b]At 125 MHz for $^{13}C$ NMR and 500 MHz for $^{1}H$ NMR.

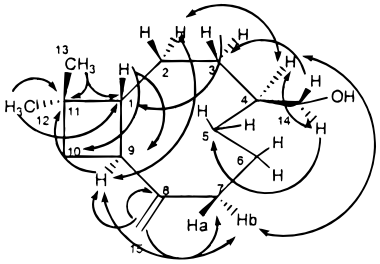


**Figure 1.** Important HMBC (plain arrow) and NOESY (two-sided arrow) correlations for compound **1**.

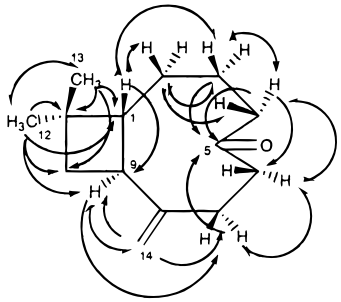


**Figure 2.** Important $^{1}H-^{1}H$ COSY (two sided arrow) and HMBC (plain arrow) correlations for compound **4**.

dihydro-$\beta$-caryophyllene, which was also present naturally in the essential oil compared by GC−MS.

Compound **3** was purified by MPLC of the essential oil. The molecular formula ($C_{15}H_{24}O$) was deduced from GC−MS and $^{13}C$ NMR. The IR spectrum indicated a saturated aldehyde with the absorptions at 2807, 2697, and 1739 $cm^{-1}$. Compound **3** displayed spectral data closely similar to those of **1** (Table 1). The $^{1}H$ NMR spectrum of **3** contained a signal characteristic for an aldehyde at $\delta$ 9.48, which correlated with the downfield methine carbon resonating at $\delta$ 205.2 (C-14). Kaiser and Lamparsky[11] reported the occurrence of the related caryophyllen-14-al (=α-betulenal) in lavender oil and its synthesis. The instability of **3** hindered any attempts to determine the stereochemistry of C-4. Hence, compound **3** was assigned as 4,5-dihydro-$\beta$-caryophyllen-14-al.

Compound **4** was isolated from the oil using the same procedure as explained above. The GC−FTIR spectrum indicated a characteristic ketone band at 1720 $cm^{-1}$. The HRES−FTMS showed a molecular ion $[M + H]^+$ at $m/z$ 207.1740 ($C_{14}H_{22}O$). The MS data supported the proposed formula, as did the NMR data, which were similar to those of **1**. The downfield ketone carbon resonating at $\delta$ 215.2 was assigned to C-5 (Table 1). This was based on its $^{3}J$-HMBC correlations with $H_2$-3 ($\delta$ 2.02 and 1.76) and $H_2$-7 ($\delta$ 2.36 and 1.80). Also H-4 ($\delta$ 2.40) and H-6a ($\delta$ 2.36) correlated with C-5, along with supporting COSY correlations in two different spin systems (see Figure 2). Hence, compound **4** was assigned as the new natural product caryophylla-8(14)-en-5-one (**4**). This compound was previously reported as a synthetic product from $\beta$-caryophyllene along with other derivatives by Matsubara et al.[12] However, the provided data and stereochemistry were tentative.

**Table 2.** Antimicrobial Activity of Compound 1[a]

| microorganism | standard MIC $\mu$g/mL | **1** MIC $\mu$g/mL |
|---|---|---|
| *E. coli* | 250[b] | 250 |
| *S. aureus* | 7.81[b] | 250 |
| *M. luteus* | 125[b] | 250 |
| *P. aeruginosa* | 62.5[b] | 250 |
| *B. cereus* | 125[b] | 125 |
| *C. glabrata* | 62.5[c] | 125 |

[a] Using broth susceptibility assay.[14] [b]Chloramphenicol. [c]Ketoconazole.

Compound **1** induced 100% inhibition of the plant pathogenic fungi *Cephalosporium aphidicola* and *Rhizoctonia cerealis* at 200 $\mu$g/mL. Compound **1** was less effective against *Drechslera sorokinianse* and *Fusarium solani* by displaying 75 and 59% inhibition, respectively, as compared to the 100% inhibition induced by the standard antifungal ketoconazole.

Antimicrobial activity of **1** against several human pathogens is presented in Table 2. Compound **1** was as active as the antibacterial standard chloramphenicol against *Bacillus cereus*, with a MIC value of 125 $\mu$g/mL, but was less active against *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Compound **1** displayed moderate antifungal activity against *Candida glabrata*, with a MIC value of 125 $\mu$g/mL, compared with ketoconazole (62.5 $\mu$g/mL).

## Experimental Section

**General Experimental Procedures.** TLC plates were prepared using Si gel 60G (Merck 7731) + Si gel $60GF_{254}$ (Merck 7730) in equal proportions. Compounds were visualized with UV light (365 and 254 nm) and developed with 1%

vanillin−5% $H_2SO_4$ in EtOH followed by heating. Separations were performed using a Büchi B-680 MPLC system. Si gel 60G (Merck 7734) was used as packing material, filled wet with hexane (column size: 26 × 460 mm). Hexane−diethyl ether was gradient eluted with a flow rate of 10 mL/min, at 14 bar pressure. Optical rotations were measured on an Oriel Pol S-2. IR were measured using a GC−FTIR Perkin-Elmer Spectrum 2000. $^1$H and $^{13}$C NMR spectra were recorded on a JEOL JNM-EX90A at 90 and 22.4 MHz, respectively, in $CDCl_3$. Tetramethylsilane (TMS) at 0.0 ppm was referenced as an internal standard. Additionally, 1D and 2D NMR data were obtained using Bruker Avance DPX-400 and 500 spectrometers. Spectra were measured and reported in parts per million (ppm) by using the residual solvent peak as an internal standard. MS were recorded using a HP G1800A GCD system. Innowax FSC column (60 m × 0.25 mm i.d., with 0.25 $\mu$m film thickness) was used with helium (1 mL/min) as carrier gas. GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, then kept constant at 220 °C for 10 min to 240 °C at rate of 1 °C/min. Mass range was recorded from *m*/*z* 35 to 425. MS were measured at 70 eV. Relative percentages of the individual compounds were calculated automatically from peak areas of the total ion chromatogram. ES−FTMS analyses were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron HRHPLC−FTMS spectrometer by direct injection into an electrospray interface.

**Plant Material.** *B. litwinowii* was collected in May 1998, from Artvin, Hatila, 2050 m. Voucher specimens are kept at the Faculty of Pharmacy Herbarium at Anadolu University in Eskişehir, Turkey (ESSE 12757).

**Distillation and Isolation.** Air-dried buds of *B. litwinowii* were water distilled for 4 h using a Clevenger-type apparatus to yield 6.34% essential oil on a dry-weight basis. *B. litwinowii* bud essential oils were used for MPLC separations yielding the following compounds.

**14-Hydroxy-4,5-dihydro-*β*-caryophyllene (1):** essential oil (0.6 g) subjected to MPLC over Si gel, eluted with hexane−diethyl ether (7:3) to yield **1** (150 mg); $[\alpha]^{25}_D$ −11.6° (*c* 2.1, EtOH), GC−FTIR $\nu_{max}$ (cm$^{-1}$) 3669 (OH) 3082, 1634 and 890 (C=$CH_2$), 1374 [$C(CH_3)_2$]; $^1$H NMR and $^{13}$C NMR ($\delta$, ppm), see Table 1; EIMS *m*/*z* 222 $[M]^+$ (2), 207 (7), 191 (23), 147 (10), 135 (35), 121 (31), 109 (48), 95 (100), 79 (74), 67 (61), 55 (44), 41 (76); HRES−FTMS *m*/*z* 221.1906 $[M - H]^+$ (calcd for $C_{15}H_{26}O$: 221.1905).

**14-Acetoxy-4,5-dihydro-*β*-caryophyllene (2):** acetylation of **1** (26 mg) in pyridine (1 mL) with acetic anhydride (1 mL) yielded **2** (22 mg); $[\alpha]^{25}_D$ −10.2° (*c* 2.0, EtOH); GC−FTIR $\nu_{max}$ (cm$^{-1}$) 3082, 1633 and 891 (C=$CH_2$), 1761 (C=O), 1231 and 1035 (OAc); $^1$H NMR and $^{13}$C NMR ($\delta$, ppm), see Table 1; EIMS *m*/*z* 264 $[M]^+$ (1.0), 249 (1), 204 (9), 176 (13), 161 (22), 147 (16), 133 (40), 119 (32), 108 (54), 93 (55), 82 (79), 79 (53), 67 (41), 55 (27), 43 (100).

**4,5-Dihydro-*β*-caryophyllen-14-al (3):** essential oil (0.6 g) subjected to MPLC over Si gel, eluted with hexane−diethyl ether (96:4) to yield **3** (10 mg); $[\alpha]^{25}_D$ −6.0° (*c* 2.0, EtOH); GC−FTIR $\nu_{max}$ (cm$^{-1}$) 3079, 1634 and 899 (C=$CH_2$), 2807, 2697 and 1739 (CHO), 2937; $^1$H NMR and $^{13}$C NMR ($\delta$, ppm) see Table 1; EIMS *m*/*z* 220 $[M]^+$ (2.0), 205 (4), 177 (5), 135 (15), 121 (18), 107 (26), 95 (33), 82 (100), 79 (43), 67 (43), 55 (25), 41 (53).

**Caryophylla-8(14)-en-5-one (4):** essential oil (0.6 g) subjected to MPLC over Si gel, eluted with hexane−diethyl ether (95:5) to yield an oil, **4** (19 mg); $[\alpha]^{25}_D$ −14.5° (*c* 1.0, EtOH); UV ($CHCl_3$) $\lambda_{max}$ (log $\epsilon$) 243 (3.11); GC−FTIR $\nu_{max}$ (cm$^{-1}$) 3079, 1634 and 892 (C=$CH_2$), 1720 (C=O), 2956, 2874, 1452; $^1$H NMR and $^{13}$C NMR ($\delta$, ppm), see Table 1; EIMS *m*/*z* 206 $[M]^+$ (2.0), 191 (15), 189 (15), 163 (15), 145 (38), 124 (64), 82 (92), 79 (88), 55 (97), 41 (100); HRES−FTMS *m*/*z* 207.1740 $[M + H]^+$ (calcd $C_{15}H_{26}O$: 207.1749).

**Antifungal Bioassay**. Antifungal activity was determined using an agar tube dilution technique. Stock solution of **1** was freshly prepared in DMSO to reach a final concentration of 200 $\mu$g/mL using sterile molten SDA. Test tubes were kept at room temperature for solidification. Medium containing only DMSO was used as negative control. Fungal cultures were cut to 4 × 4 mm from 1-week grown cultures and then inoculated onto the slant. After an incubation period of 7−10 days at 29 °C, tubes were examined for growth inhibition. Ketoconazole was used as a reference antifungal drug. Media growth with the compound was determined by measuring linear growth (mm) of fungal culture. Growth inhibition (%) was calculated with reference to the negative control.[13] The plant pathogenic fungi was obtained from the culture collection of the Biology Department at the Faculty of Science and Letters, Anadolu University.

**Antimicrobial Bioassay.** A stock solution of pure compound was prepared in DMSO and serially diluted from 1000 $\mu$g/mL to 0.97 $\mu$g/mL in sterile distilled water. Freshly grown bacterial suspensions in double strength Mueller-Hinton broth and yeast suspension of *C. glabrata* in yeast medium were standardized to 108 CFU/mL. Each dilution of the antimicrobial agent (100 $\mu$L) was transferred to a 96-well microtiter plate. Sterile distilled water served as growth control. Each microbial suspension (100 $\mu$L) was then added to each well. The last row, containing only the serial dilutions of antimicrobial agent without microorganism, was used as negative control. After incubation at 37 °C for 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC).[14] Human pathogens used for this assay were obtained from the culture collection of Osmangazi University, Medical Faculty, Microbiology Department; *E.coli*, *S. aureus*, *M. luteus*, *P. aeruginosa*, *B. cereus* and the fungus *C. glabrata* (Table 2).

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## References and Notes

(1) Part of the Ph.D. Dissertation of B.D.: *The Essential Oil Compositions of* Betula *Species Growing in Turkey*, Anadolu University: Eskişehir, Turkey, 1999.
(2) Browicz, K. *Flora of Turkey and the East Aegean Islands*; Davis, P. H., Ed.; University Press: Edinburgh, 1972; Vol. 7, pp 688−691.
(3) Yaltirik, F. *Angiospermae*, Lecture Book; Istanbul University: Istanbul, 1988; Ist. Uni. Pub. No: 3509, Faculty Forestry No: 390, pp 68−82.
(4) Diener, H. Fachlexicon Abc: *Arzneipflanzen und Drogen*; Verlag Harri Deutsch: Thun, 1989; 2. Edition; pp 97−99.
(5) Kaneko, N.; Ishii, H.; Sato, A.; Kanisawa, T.; Watanabe, S. *12th International Congress of Flavors, Fragrances and Essential Oils. Proceedings*; Woidich, H., Buchbauer, G., Eds.; Fachzeitschriftverlag-GmbH: Vienna, 1992; pp 53−63.
(6) Treibs, W.; Lossner, G. *Justus Liebigs Ann. Chem.* **1960**, *634*, 124−131.
(7) Stepen, R. A.; Khan, V. A.; Vershnyak, V. M.; Peryshkina, G. I. *Chem. Nat. Comp.* **1987**, *6*, 666−668.
(8) Joulain, D.; König, W. A. *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*; E. B. Verlag: Hamburg, 1998.
(9) Barrero, A. F.; Molina, J.; Oltra, J. E.; Altarejos, J.; Barragan, A.; Lara, A.; Segura, M. *Tetrahedron* **1995**, *51*, 3813−3822.
(10) Manns, D.; Hartmann, R. *Planta Med.* **1992**, *58*, 442−444.
(11) Kaiser, R.; Lamparsky, D. *Helv. Chim. Acta* **1983**, *66*, 1843−1849.
(12) Matsubara, Y.; Uchida, T.; Takenokuchi, H. *Nippon Nogei Kagaku Kaishi* **1985**, *59*, 19−24.
(13) Paxton, J. D. *Methods in Plant Biochemistry*, Hostettmann, K., Ed. Academic Press: London, 1991; Vol. 6, pp 37−53.
(14) Koneman, E. W.; Allen, S. D.; Janda, W. M.; Schreckenberger, P. C.; Winn W. C. *Color Atlas and Textbook of Diagnostic Microbiology*; Lippincott-Raven: Philadelphia, 1997; pp 785−856.

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