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6-HYDROXYISOCARYOPHYLLENE AND ISOCARYOPHYLLENIC ACID FROM BIRCH VEGETATIVE BUDS

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The new isocaryophyllene derivatives: 6-hydroxyisocaryophyllene [(1R,4Z,6R,9S)-8-methylene-11,11-dimethyl-bicyclo[7.2.0]undec-4-ene-6-ol], epoxide of 6-hydroxyisocaryophyllene [(6R)-hydroxy-(4R,5S)-epoxyisocaryophyllene [(1R,4R,5S,6R,9S)-4.5-epoxy-8-methylene-11,11-dimethylbicyclo[7.2.0]undeco-4-ene-6-ol], (6R)-acetoxyisocaryophyllene [(6R)-acetoxy-(1R,4Z,9S)-8-methylene-11,11-dimethylbicyclo[7.2.0]undec-4-ene], isocaryphyllenic acid [(1R,4E,9S)4-carboxy-8-methylene-11,11-dimethylbicyclo[7.2.0]undeco-4-ene] were first detected in the birch vegetative buds. 6-Hydroxyisocaryophyllene and epoxide of 6-hydroxyisocaryophyllene are isolated by from the *Betula pendula* Roth. birch buds etheric extract by chromatography on silica gel. (6R)-Acetoxyisocaryophyllene was synthesized. The structure of 6-hydroxyisocaryophyllene and epoxide of 6-hydroxyisocaryophyllene isolated from the buds were determined by NMR spectroscopy. Caryophyllenic acids are isolated from the ether extract with an aqueous solution of alkali. Caryophyllenic acids are separated by chromatography on silica gel. The structures of caryophyllenic acid and isocaryphyllenic acid isolated from the *Betula grandifolia* Litv., *B. albo-sinensis* Burk., *B. fusca* Pall.ex Georg, *B. obscura* A. Kotula, *B. litwinowii* Doluch., *B. hallii* Howell, *B. grandifolia* Litv. birch buds were determined by X-ray diffraction analysis. The physico-chemical characteristics and NMR data of 6-hydroxyisocaryophyllene, epoxide of 6-hydroxyisocaryophyllene and all the isolated acids are given. The obtained mixtures of compounds were analyzed by gas chromatography – mass spectrometry (GC-MS). The gas chromatographic retention indices of all identified compounds were determined.

Keywords: 6-hydroxyisocaryophyllene, epoxide of 6-hydroxyisocaryophyllene, isocaryophyllenic, caryophyllenic acid, vegetative buds of birch, NMR spectroscopy, X-ray diffraction analysis, gas chromatography – mass spectrometry.

Introduction

We have earlier reported the hydrocarbon extract sesquiterpene alcohols composition of pendent white birch (*Betula pendula* Roth.) vegetative buds. The fraction contains 6-hydroxycaryophyllene, 6-hydroxyhumulene, 14-hydroxycarophyllene [1]. The main components of the investigated birch 14-hydroxy-caryophyllene, 6-hydroxycarophyllene and their esters. But on the GC-MS chromatogram, there was an intense peak of an unknown compound whose mass spectrum was similar to the mass spectrum of 6-hydroxycaryophyllene. The same pattern was observed in the analysis the birch buds *Betula grandifolia* Litv., *B. albo-sinensis* Burk., *B. fusca Pall.*ex Georg, *B. obscura* A. Kotula, *B. litwinowii* Doluch. , birch of golden, bog birch *B. hallii* Howell, *B. grandifolia* Litv. extract obtained by extraction with ether. Two peaks of other compounds were present on chromatograms. Whose mass spectra were little distinguishable. The aim of this work was to establish the structure of compounds that give similar mass spectra.

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Experimental

Vegetative buds of pendent white birch *Betula pendula* (100 g (65 g absolutely dry buds) of buds) were collected in April 2016 in the Kirishi district of the Leningrad Region. Buds of bog birch *B. hallii* (28 g (17.4 g a.d.b.)) were harvested in March 2017 in the arboretum of St. Petersburg Forestry University. The wet, crushed buds were extracted with methyl tert-butyl ether (MTBE) in a Soxhlet apparatus. The yield of extract from a.d.b.of pendent white birch was 41%, of the bog birch buds – 42%. The extracts were treated with a 1% aqueous solution of NaOH to remove acids. The yield of acids from the pendent white birch extract was 33%, the acid yield from the bog birch extract – 87%. The obtained neutral substances of the extract (17.8 g) of pendent white birch buds and the acids of bog birch extract (6.36 g) were separated by preparative liquid chromatography on silica gel with gradient elution using petroleum benzine (PE) (boiling range 40–70 °C) as an eluent with the addition of up to 30% MTBE. The fraction of sesquiterpene alcohols (8.35% of the MTBE extract) eluted from the PE column supplemented with 5% MTBE. The fraction was re-chromatographed into individual compounds. Unknown sesquiterpene alcohol (0.120 g) (1) (eluted earlier than other sesquiterpene alcohols: 6-hydroxycaryophyllene and 6-hydroxy-gumulene). The fraction of oxides of sesquiterpene alcohols eluted with the addition of 7% MTBE.

Sesquiterpene acids eluted PE with an addition of 25 to 30% MTBE. The acid (3) (2.21 g) was eluted before the acid (4) (2.42 g). The acid fractions were methylated with diazomethane before analyzing by gas chromatography-mass spectrometry (GLC-MS) since the acids did not appear as peaks in the chromatogram.

The chromatographic analysis was carried out using a 6850A Agilent chromato-mass spectrometer (Agilent Technologies, Inc.) with a model G2629A gas chromatograph equipped with a model G2577A HP5973 Network selective mass spectrometry detector. The flow rate of carrier gas (He) was 1mL per min. Samples of 2 μL were injected in the split mode at a ratio of 20 : 1. The injector temperature was 270 °C. The transfer line were kept at 270 °C. The quadrupole temperature was 270 C. The ion source was kept at 230 °C. The ionizing energy was 70 eV. Mass range was from 40 to 550 m/z.

To fractionate samples, a HP-5MS quartz column (30 m × 0.25 mm; film thickness 0.25 μm) with a 5% phenylmethyl-siloxane stationery phase was used. The thermostat temperature was programmed to increase from 100 to 270 °C at a rate of 5 °C min^{-1}. The oven was held at this temperature for 30 min.

The gas chromatographic retention indices (RI) of the analyzed substances were determined using the retention indices of *n*-alkanes (C_{16}- C_{19}) as standard compounds (Sigma-Aldrich)). The standard compounds were chosen so that the retention times of the studied substances fell between those of the reference alkanes. Retention indices were calculated following the determination of the coefficients of the following equation: $RI = a\tau^2 + b\tau + c$, where RI and τ represent the retention index and retention time, respectively. All calculations were performed using an Advanced Grapher program (version 2.08).

High resolution mass spectrum (HR-ESI) was recorded on a Bruker-micrOTOF instrument using an electrospray method. The scanning interval is 50–1200 m / z. The ion polarity is positive, the voltage of the ion source capillary is 4500 V, the gas pressure at spraying is 0.4 bar, and the dry gas flow rate is 4.0l / min. The solvent was methanol.

NMR spectra were recorded using a NMR spectrometer Jeol ECX-400A (400 and 100 MHz for ^{1}H and ^{13}C spectra, respectively) and $CDCl_3$ as a solvent (δ-scale). As internal standards residual signals $CHCl_3$ (δ H 7.25 ppm) were used.

IR spectra were recorded on a FTIR-8400S Shimadzu instrument using the FT-IR reflection technique.

Optical rotation angle was determined on the device: Automatic Polarimeter AA65. Solvent-chloroform. The length of the cuvette is 3 cm.

Thin layer chromatography was performed on Merck Silica gel 60 F254 plates. The spots on the TLC plates were sprayed with a 10% solution of sulfuric acid in ethanol with the addition of vanillin.

For single crystal X-ray diffraction experiment crystals were fixed on a micro mount and placed on an Agilent Technologies SuperNova (4), (5) using CuKα and MoKα monochromated radiation diffractometers, respectively. All of crystals were measured at a temperature of 100K. The structures were solved by the Direct methods and refined by means of the SHELXL program [2] incorporated in the OLEX2 program package [3]. The crystallographic data and some parameters of refinement are placed in Table 1. Empirical absorption correction was applied in CrysAlisProprogram complex [4] using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. Supplementary crystallographic data for this paper have been deposited at Cambridge Crystallographic

Data Centre (CCDC 1583801. 1583802 for *isocaryophyllenic acid* (4) and *caryophyllenic acid* (5), respectively) and can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

Physic-chemical and Spectral Characteristics of the Extracted Compounds

(6R)-Hydroxyisocaryophyllene [(1R,4Z,6R,9S)-8-methylene-11.11-dimethylbicyclo[7.2.0]undec-4-ene-6-ol] (1). Oil, RI= 1642. IR-spectrum (ν, sm^{-1}): 3385(OH), 3057(=CH), 2953(CH_2), 2923(CH_2), 2851(CH_2), 1713, 1708, 1633(C=CH_2), 1462(-C($\underline{CH_3}$)=CH-), 1378, 1367, 1265, 1025(CH-OH), 894(C=CH_2), 740, 704, 581.

Mass-spectrum (m/z): 220 (1), 205 (7), 187 (11), 177(11), 149 (21), 131 (43), 121 (41), 109(74), 107(45), 105 (46), 95(58), 93(54), 91(69), 79(81), 69(79), 41(100).

High resolution mass spectrometry (HR-ESI): m/z 243.1720(100) [M+Na]+, 244.1752(16.3) [M+Na+1]+, 245.1782(1.3) [M+Na+2]. Mass calculation $C_{15}H_{24}O$: m/z 243.1725(100), 244.1758(16.2), 245.1792(1.2).

(6R)-Acetoxyisocaryophyllene [(6R)-acetoxy-(1R,4Z,9S)-8-methylene-11.11-dimethylbicyclo[7.2.0]-undec-4-ene] (2). Oil, RI= 1742. $[\alpha]_D^{20}$ +32.8° (*c*= 0.945. $CHCl_3$). IR-spectrum (ν, $см^{-1}$): 2950, 2929, 2859, 1737 (C=O), 1630.1451. 1368.1242 (O-C=O), 1020. 964. 888(C=CH_2), 850.

Mass-spectrum (m/z): 262 (<1), 247(<1), 220(3), 202(17), 189 (21), 159(31), 145(23), 133(76), 131(61), 119(32), 109(46), 105(51), 91(63), 79(51), 67(21), 65(14), 55(23), 43(100).

(6R)-hydroxy-(4R,5S)-epoxyisocaryophyllene [(1R,4R,5S,6R,9S)-4.5-epoxy-8-methylene-11.11-dimethyl-bicyclo[7.2.0]undeco-4-ene-6-ol] (3). Oil, RI=1808.

Mass-spectrum (m/z): 236 (<1), 221(2), 205(3), 203(6), 193(4), 189(5), 175(8), 161(12), 147(13), 135 (14), 121(45), 109(61), 95(76), 82(50), 79(78), 69(100), 55(47), 41(94), 29(18).

High resolution mass spectrometry (HR-ESI): m/z 259.1674(100) [M+Na]+; 260.1708(16.5) [M+Na+1]+, 261.1741(2.0) [M+Na+2]+. Mass calculation $C_{15}H_{24}O_2$ +Na: m/z 259.1674(100), 260.1708(16.2), 261.1741(1.2).

^{1}H NMR in $CDCl_3$ (δ): 0.99 (3H, s, H-12), 1.00 (3H, s, H-13), 1.52 (3H, s, H-14), 1.55 (1H, dd, $J_{9\text{-}10\alpha}$=9.2, H-10α), 1.56 (2H, m, H-2), 1.67 (1H,m, H-3α), 1.76 (1H, dd, $J_{10\alpha\text{-}10\beta}$=10.5, $_{9\text{-}10\beta}$=9.2. H-10β), 1.77 (1H, ddd, $J_{1\text{-}9}$=9.2, $_{1\text{-}2\alpha}$=11.7, $_{1\text{-}2\beta}$=3.5, H-1) 1.91 (1H, dd, $J_{6\text{-}7\alpha}$=9.4, $_{7\alpha\text{-}7\beta}$=12.0, H-7α), 1.96 (1H, dd, $J_{6\text{-}7\beta}$=3.4. H-7β), 2.04 (1H, m, H-3β), 2.50 (1H, ddd, $J_{9\text{-}10\alpha}$=8.9; $J_{9\text{-}10\beta}$=10.0, H-9), 2.86 (1H, d, $J_{5\text{-}6}$=4.1, H-5), 4.30 (1H, ddd, $J_{6\text{-}7\beta}$=6.5, H-6), 5.03 (1H, br.s, H-15β), 5.15 (1H, br.s H-15α).

^{13}C NMR in $CDCl_3$ (δ): 16.9 (q, C-14), 22.4 (q, C-13), 27.6 (t, C-2), 29.9 (q, C-12), 32.9 (s, C-11), 40.00 (t, C-3), 40.2 (t, C-10), 41.4 (t, C-7), 46.9 (d, C-9), 49.3 (s, C-4), 55.4 (d, C-1), 64.2(d, C-6), 67.6 (d, C-5), 147.7 (s, C-8), 114.0(t, C-15).

Isocaryophyllenic acid [1R,4E,9S)4-carboxy-8-methylene-11.11-dimethylbicyclo [7.2.0]undeco-4-ene] (4). Tm = 103–104 °C (from hexane). RI of methyl ester – 1726. $[\alpha]_D^{26}$ –46.1° (*c*=1.447. $CHCl_3$). IR-spectrum (ν, $см^{-1}$): 2947, 2920, 2854, 2650, 2565, 1674(C=C-$\underline{CO}$OH), 1635(C=C), 1450, 1423(HRC=CH_2), 1307, 1288, 1257, 1226, 1199, 1188, 941, 922, 887, 771, 740, 532.

Mass-spectrum of methyl ester (m/z): 248(6), 233(18), 216(6), 201(11), 189(35), 179(63), 173(25), 165(13), 156(2), 147(88.5), 133(76), 119(57), 105(60), 91(100), 79(61), 69(79), 59(14), 53(28).

High resolution mass spectrometry (HR-ESI): m/z 235.1697(100) [M+H]+, 236.1740(16.6) [M+H+1]+, 237.1774(1.3) [M+H+2]+. Mass calculation $C_{15}H_{22}O_2$ +H: m/z 235.1698(100), 236.1732(16.2), 237.1765 (1.2).

TLC: system: hexane-MTBE=10:3. R_f=0.55 (pink).

Caryophyllenic acid [1R,4Z,9S)4-carboxy-8-methylene-11.11-dimethylbicyclo [7.2.0]undeco-4-ene] (5). Tm = 90–91 °C (from hexane). RI of methyl ester – 1688. $[\alpha]_D^{26}$ –31.5 (*c*=1.642. $CHCl_3$). IR-spectrum (ν, $см^{-1}$): 3070, 3016, 2924, 2854, 2781, 2604, 1670(C=C-$\underline{CO}$OH), 1628(C=C), 1439, 1419(HRC=CH_2), 1369, 1303, 1269, 1200, 1169, 956, 933, 887, 783, 624, 574.

Mass spectrum of methyl ester (m/z): 248(2), 233(16), 216(6), 201(9), 189(31), 179(60), 173(25), 165(14), 156(3), 147(100), 145(30), 133(62), 119(72), 105(57), 91(96), 79(80), 69(75), 59(14), 53(28).

High resolution mass spectrometry (HR-ESI): m/z 235.1697(100) [M+H]+, 236.1740(16.6) [M+H+1]+, 237.1774(1.3) [M+H+2]+. Mass calculation $C_{15}H_{22}O_2$ +H: m/z 235.1698(100), 236.1732(16.2), 237.1765(1.2).

TLC: system: hexane-MTBE=10:3. R_f=0.63 (lilac).

Caryophyllenic acid pyrazoline methyl ester кислоты (6). $T_{пл}$=113–115 °C (from hexane). RI=1731. IR-spectrum (ν, $см^{-1}$): 2950, 2861, 1737, 1639, 1463, 1438, 1373, 1248, 1208, 1171, 1047, 891.

High resolution mass spectrometry (HR-ESI): m/z 291.2067 (100) [M+H]+; 292.2100(19.6) [M+H+1]+; 293.2133(2) [M+H+2]+. Mass calculation: $C_{17}H_{26}N_2O_2$+H: m/z 291.2103 (100); 292.2134(18.4); 293.2094(1.6);

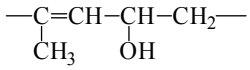
main peaks: m/z 313.1886 (100) [M+Na]+; 314.1919(20.0) [M+Na+1]+; 315.1952(2.0) [M+Na+2]+. Mass calculation: $C_{17}H_{26}N_2O_2$+Na: m/z 313.1894 (100); 314.1928(18.4); 315.1961(1.6).

Mass spectrum (m/z): 262 (10), 247(10), 234(5), 219(13), 215(13), 205(10), 203(34), 193(8), 187(20), 173(11), 161(39), 147(47), 133(66), 119(33), 109(20),107(100), 93(57), 91(87), 79(85), 69(39), 41(59).

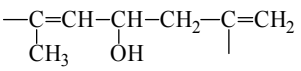
^{13} C NMR in $CDCl_3$ (δ): 21.9 q (C-13) 29.8, q (C-12), 23.9 t (C-6), 25.9 t (C-2), 34.5 s (C-11), 36.3 t(C-3), 36.4 t (C-7), 36.9 t (C-10),36.9 d (C-9), 42.7d (C-1) 52.0 q (OCH_3), 58.8 d (C-5), 83.6 t ($\underline{C}H_2$-N=N), 98.4 s (C-4), 111.2 t (C-15), 151.2 s (C-8), 168.0 s (C-14).

Results and discussion

The structure of compound (1) was established on the basis of two-dimensional NMR spectra and analysis of the spin-spin interaction constants. We were compared the NMR spectra of the two compounds, since the mass spectrum (1) practically coincides with the mass spectrum of (6R) -hydroxycaryophyllene [(1R,6R,4E,9S)-8-methylene-11,11-dimethylbicyclo[7.2.0]undeco-4-ene-6-ol, RI = 1670]. In the ^{13}C NMR and ^{13}C NMR spectra of DEPT 135. a similar set of carbon signals associated with different numbers of hydrogen atoms was present, as in the spectrum of 6-hydroxycaryloryllene [5]. In the ^{1}H NMR spectrum, proton signals of three methyl groups with centers at 0.98; 0.97; 1.62 ppm were observed (Table 1). The last signal belongs to the protons of the CH_3-group, which is bonded to the carbon atom (C-4) of the double bond. The structure also contained an exomethylene group, as evidenced by proton signals at 4.92 and 4.85 ppm, trisubstituted double bond (proton signal at 1H at 5.20 ppm (H-C5)) and adjacent secondary alcohol group (proton signal at 1H at 4.55 ppm (H-C6)). The proton signal at the carbon atom (C-6) bound to the OH group was noted as a triplet of doublets, and the proton signal at the double bond carbon atom in the cyclic structure gave the signal as a doublet. Thus, the structure of the compound should have the following fragment:



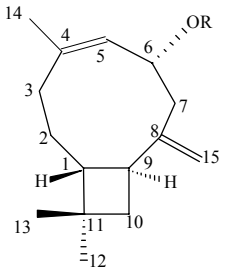
Two doublets of doublets with the intensity of one proton with centers at 2.55 and 2.42 ppm on the ^{1}H NMR spectrum refer to the protons of the methylene group next to the carbon atom with the secondary hydroxyl. The second side of the methylene group should contain a completely substituted carbon atom (C-8) (the absence of the protons interaction of the methylene group with other protons). The displacement of the signals of the protons of the methylene group into a weaker field suggests a close proximity of the exomethylene group. That is, in the structure of the molecule of the compound there is a fragment:



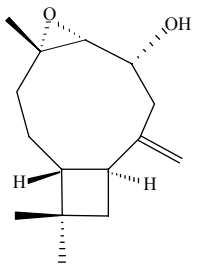
The isolated compound is a derivative of caryophyllene, since on the ^{1}H NMR spectrum, the signals of methyl groups in the form of singlets with centers of 0.97 and 0.98 ppm (3H-C13, 3H-C12) proton signals at carbon of two double bonds are visible. One combination of atoms refers to the exomethylene group, and another combination of atoms belongs to the group with trisubstituted carbons, which is characteristic of caryophyllene.

A secondary hydroxyl group is present in the structure of the compound. The hydroxyl group should be located next to the double bond. Two-dimensional COSY NMR spectrum was obtained to confirm the existence of the above assumed fragments, which showed interactions between neighboring hydrogen atoms. Two-dimensional spectrum of C-H was obtained to assign hydrogen signals to specific carbon atoms. The fragments found were similar to the (6R)-hydroxycaryophyllene fragments, but chemical shifts and spin-spin interaction constants differed. Thus, J_{5-6} for hydroxy-caryophyllene is 10.4 Hz, and for the isolated compound it is 7.7. which makes the signal narrower. It was assumed that the isolated compound is (6R)-hydroxyisocaryophyllene.

Two-dimensional NMR spectrum of NOESY was analyzed to confirm the structure. The cross peaks that appeared on the spectrum corresponded to the structure of (6R)-hydroxy-isocaryophyllene (Fig.1). Earlier it was reported that 14-hydroxyisocaryophyllene and isocaryophyllene-14-al were found in essential oils of various birches, including pendent white birch [6].



R=H (1)
R=$COCH_3$ (2)



(3)

The isolated alcohol was proacetylated with a mixture of acetic anhydride and pyridine and the obtained product (2) was found on the chromatogram of the hydrocarbon extract of the buds, since acetates of sesquiterpene alcohols are present in the buds of birch [7].

The epoxide (3) corresponding to the alcohol (1) is also present in the birch bud extract, and was isolated by preparative chromatography. The epoxy of 6-hydroxycaryophyllene is also present in the extract of the birch buds [7].

J/Hz: for (**1**): 1-9=9.1; 1-2α=11.9; 1-2β=3.7; 2α-3β=8.9; 2β-3β=4.0; 2α-2β=13.8; 2β-3α=4.5; 3α-2α=4.6; 3α-3β=13.6; 3β-14=1.1; 15α-15β=1.5; 5-14=2.0; 5-6=7.7; 6-7β=3.6; 6-7α=8.4; 7α-7β=13.0; 9-10α =9.1; 9-10β= 9.6; 10α-10β =10.6;

for (**2**): 1-9=9.1; 1-2α=11.0; 1-2β=4.0; 2α-3β =8.9; 2β-3β=4.0; 2α-3β=8.9; 2α-2β=13.6; 2β-3α=4.5; 2α-3α=4.9; 3α-3β=13.2; 3β-14=1.1; 6-7α =8.4; 6-7β=3.6; 6-5=7.7; 7α-7β =4.0; 9-10α =9.1; 9-10β =10.2; 10α-10β =10.6; 15β-7α =1.2

The isolated compounds of the bog birch buds are acids, since the compounds were shown on GC-MS chromatograms only after preliminary methylation with diazomethane, dissolved in an aqueous-alkaline solution when extracting the etheric extract of the buds. Further, the structure of the isolated acids was established by X-ray structural analysis. The analysis showed that both compounds crystallize in PE at + 4 °C (Table 2) and form crystals consisting of two conformers in a 1 : 1 ratio (Fig.2. 3).

Two-dimensional spectra of ^{1}H-^{1}H ROESY, COSY, HMQC, HMBC were taken to establish the correspondence of carbon and hydrogen atoms to the signals on the NMR spectra, The results are shown in Table 3 below and Figures 4 and 5.

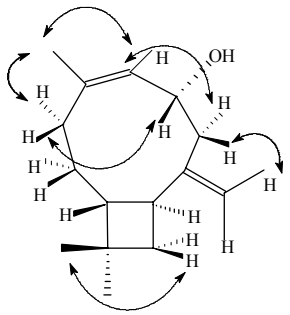


Fig. 1. Selected NOE correlations of the compound (1)

Table 1. Data on the ^{1}H NMR and ^{13}C NMR spectra of the isolated compounds (1) and (2) (δH, δC)

C atom	1		2	
	δC, ppm	δH, ppm	δC, ppm	δH, ppm
1	49.4 d	1.69 ddd	50.1 d	1.68 ddd
2	25.4 t	α 1.62 m	25.5 t	α1.58 m
		β 1.52 m		β1.54 m
3	29.2 t	α 1.83 m	29.2 t	α1.94 m
		β 2.30 m		β 2.44 m
4	136.0 s	–	138.0 s	–
5	130.0 d	5.20 d	125.5 d	5.14d
6	70.9 d	4.55 ddd	72.3 d	5.60ddd
7	45.4 t	α 2.55 dd	42.5 t	α 2.39 dd
		β 2.42 dd		β 2.56 dd
8	151.9 s	–	151.0 s	–
9	40.3 d	2.44 ddd	39.8 d	2.47 ddd
10	39.7 t	α 1.53 dd	39.7 t	α1.56 dd
		β 1.72 dd		β1.72 dd
11	33.0 s	–	33.0 s	–
12	29.9 q	0.98 s	30.1q	0.98 s
13	23.0 q	0.97 s	23.2 q	0.99s
14	22.8 q	1.62 s	22.9 q	1.63 s
15	112.3 t	α 4.92 br.s	113.2 t	α 4.91 br.s
		β 4.85 br.s		β 4.97 br.s
$\underline{C}H_3COO$	–	–	21.6 q	2.04 s
$CH_3\underline{C}OO$	–	–	170.5 s	–

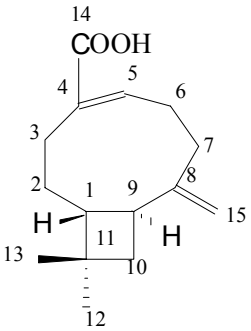
Acids (4) and (5) are geometric isomers that crystallize, forming conformers

The ^{1}H NMR spectrum of compound (5) was similar to the spectrum given for caryophyllenic acid isolated from extracts of a plant of the genus *Lichnofora* [8].

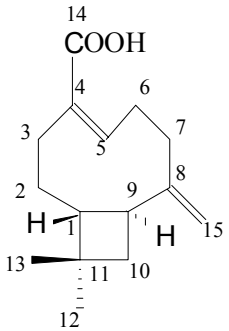
Table 2. Summary crystallographic data for (4) and (5)

Compound	(4)	(5)
Empirical formula	$C_{15}H_{22}O_2$	$C_{15}H_{22}O_2$
Formula weight	Orthorhombic	Monoclinic
Temperature/K	6.1029(3)	9.2537(2)
Crystal system	13.0180(5)	11.4795(3)
Space group	34.244(2)	13.4148(4)
a/Å	90	90
b/Å	90	104.500(3)
c/Å	90	90
α/°	2720.6(2)	1379.64(6)
β/°	234.32	234.32
γ/°	$P2_12_12_1$	$P2_1$
Volume/Å^3	0.580	0.073
Z	100(2)	100(2)
ρ_{calc}g/cm^3	8	4
μ/mm^{-1}	1.144	1.128
F(000)	0.15 × 0.05 × 0.05	0.8 × 0.3 × 0.15
Crystal size/mm^3	CuKα	MoKα
Radiation	12799	30724
Angle range 2θ (°)	5365	6308
Total reflections	7.264–144.970	5.998–55.000
Unique reflections	4386	6133
Reflections with I≥2σ(I)	0.0721	0.0401
R_{int}	0.0736	0.0265
R_{sigma}	0.0747	0.0324
S	0.1916	0.0856
R_1 [I≥2σ (I)]	0.0926	0.0335
wR_2 [I≥2σ (I)]	0.2156	0.0867
R_1 [all data]	1.081	1.058
wR_2 [all data]	0.46; -0.30	0.20; -0.18
ρ_{max}, ρ_{min}(e/Å^3)/ e Å^{-3}		
CCDC Number		

$R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$;
$w = 1/[\sigma^2(F_o^2)+(aP)^2 + bP]$, where где $P = (F_o^2 + 2F_c^2)/3$; $s = \{\Sigma[w(F_o^2 - F_c^2)]/(n-p)\}^{1/2}$,
where n is the number of reflections and p is the number of refinement parameters.



(4)



(5)

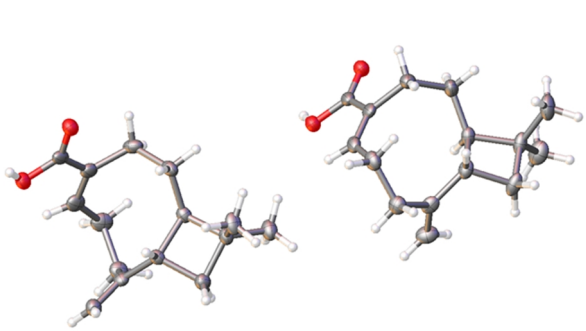


Fig. 2. Conformers (4) according to X-ray diffraction

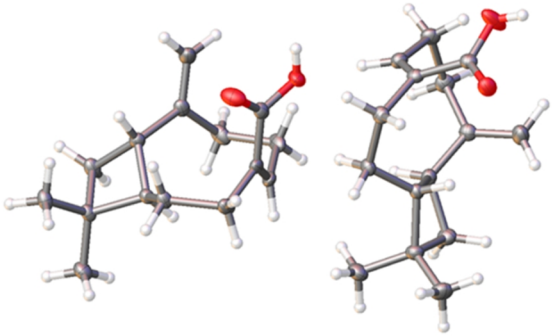


Fig. 3. Conformers (5)

Fig. 4. Selected NOE and HMBC correlations of the compound (4)

Fig. 5. Selected NOE and HMBC correlations of the compound (5)

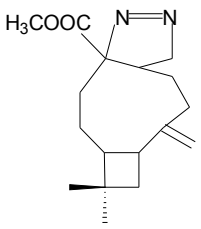
Table 3. Data on the ^{1}H NMR and ^{13}C NMR spectra of the compounds (4) and (5)* (δH, δC, ppm)

C atom	(4)		(5)	
	δC, ppm	δH, ppm	δC, ppm	δH, ppm
1	52.1d	1.81 ddd	51.6 d	1.63 ddd
2	27.4 t	α 1.48m	30.0 t	α 1.66m
		β 1.66m		β 1.85m
3	23.9 t	α 2.45m	34.8 t	α 2.68m
		β 2.30 m		β 2.63 m
4	132.2 s	–	130.2 s	–
5	144.9 d	7.00 dd	147.0 d	6.23 br. t
6	28.7 t	α 2.49 m	31.2 t	α 2.23 m
		β 2.41 m		β 3.22 m
7	34.0 t	α 2.41m	33.5 t	2.19 m
		β 2.29 m		
8	154.6s	–	151.8 s	–
9	40.2 d	2.50 ddd	48.9 d	2.46 ddd
10	40.3 t	α 1.74 dd	40.3 t	1.59 dd
		β 1.56 dd		1.78 dd
11	33.4 s	–	33.9s	–
12	30.1 q	0.99 s	30.2 q	1.00 s
13	23.0 q	0.96 s	22.5 q	0.98 s
14	173.7s	–	174.3 s	–
15	111.6 t	α 4.87 d	114.3 t	α 5.01 d
		β 4.80 d		β 4.86 d

* ^{1}H and ^{13}C NMR spectra were obtained at 50 ° C.

J, Hz: for (4): 1-2α=9.4; 1-2β=4.0; 2α-3β=11.5; 2β-3β=4.2; 2β-3α=12.6; 2α-2β=13.8; 3α-2α=5.3; 3α-3β=13.7; 15-15'=1.5; 5-6β=9.3; 5-6α=6.1; 6-7β=4.0; 6-7α=8.9; 7α-7β=13.0; 1-9=9.7; 9-10α =2.0; 9-10β= 9.1; 10α-10β=10.4.

J, Hz: for (5): 1-9=8.9; 1-2α=11.0; 1β-2β=1.4; 2β-3α=2.5; 3α-2α=7.0; 2β-2α=9; 2α-3β =13.0; 2β-3β =2.0; 3α-3β =13.5; 5-6β=7.7; 5-6α=7.6; 7α-6α=4.0; 7β-6β=7.0; 6β-7α=10.0; 7β-7α=11.0; 6β-6α=13.0; 9-10α =8.5; 9-10β= 9.6; 10α -10β=10.8.



(6)

Analysis of caryophyllenic acids fractions after methylation with diazomethane often gave not two peaks, but 4 by the GLC-MS method. At the same time, the corresponding peak to the methyl ester of caryophyllenic acid (5) decreased. The compound to which the peak corresponded on a chromatogram with a retention index of 1731 was separated from the methyl ester of caryophyllenic acid by column chromatography on silica gel, using PE as the eluent with up to 3% diethyl ether.

The spectral data of the by-product of methylation coincided with the spectral data of pyrazoline methyl ester of caryophyllenic acid (5) [8]. On the chromatogram obtained by the GLC-MS method, as well as the peak of the compound with the retention index of 1747. the corresponding mass spectrum, which completely corresponds to the mass spectrum (6), but the intensity of this peak is 10 times smaller. Presumably, this is the peak of the methyl ester of isocaryophyllenic acid.

It is assumed that the presence of compound (5) in the extracts of plants of the genus Lychnophorinae leads to the development of antitumor, antinociceptive and anti-inflammatory activities. The alcohol extract of this plant, containing (5) inhibits the activity of xanthine oxidase [9].

Conclusions

6-Hydroxyisocaryophyllene was isolated from *Betula pendula* birch buds. Structure was determined by NMR spectroscopy. Caryophyllenic acid and isocaryphyllenic acid were discovered in the Betula grandifolia, B. albo-sinensis, B. fusca, B. obscura, B. litwinowii, B. hallii, B. grandifolia vegetative buds. The structure of the acids were determined by X-ray diffraction analysis. Thus, in the birch buds, together with the (E) isomers, there are also (Z) isomers.

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