New, Sesquiterpenoid-Type Bicyclic Compounds from the Buds of *Betula pubescens* − Ring-Contracted Products of β-Caryophyllene?

Karel D. Klika,*[a] Betül Demirci,[b] Juha-Pekka Salminen,[c] Vladimir V. Ovcharenko,[a] Satu Vuorela,[d] K. Hüsnü Can Başer,[b] and Kalevi Pihlaja[a]

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The essential oils obtained from the buds of *Betula pubescens* ssp. *pubescens* and *B. pubescens* ssp. *czerepanovii* were analyzed both by GC and GC/MS and, of the 31 compounds identified, 14-acetoxy-β-caryophyllene was determined to be the main component in both oils in addition to 25 other previously known compounds. Three of the compounds were isolated from the oils and further characterized by NMR spectroscopy and mass spectrometry, including a new bicyclic aldehyde (4,8,8-trimethyl-2-methylenebicyclo[5.2.0]nonane-4-carbaldehyde, birkenal), a new tricyclic lactone (1,4,4,8-tetramethyl-10-oxatricyclo[6.2.1.$0^{2,5}$]undecan-9-one, hushinone), and the recently described 6-hydroxycaryophyllene. The isolation of birkenal also enabled identification of its corresponding alcohol {(4,8,8-trimethyl-2-methylenebicyclo[5.2.0]non-4-yl)methanol, birkenol} and the acetate of this alcohol {(4,8,8-trimethyl-2-methylenebicyclo[5.2.0]non-4-yl)methyl acetate, birkenyl acetate}, both of which are also novel compounds and are present in the two essential oils. These new compounds, bearing evident sesquiterpenoid traits, potentially arise biosynthetically as ring-contracted products of β-caryophyllene or a derivative thereof. The fifth novel compound present in the oils was determined to be the acetate of 6-hydroxycaryophyllene. The preferred conformations of the seven- or nine-membered rings in these structures were also determined.

Introduction

Betula sp. (Betulaceae), commonly known as birch, are trees or tree-like shrubs that are widespread in temperate regions of the northern hemisphere from Canada to Japan, and a characteristic feature of *Betula* species is their papery, white bark, which peels off easily.[1] Birch, in particular *B. pendula*, has long been medicinally important in many countries and cultures and practically all of the plant parts have been utilized for these purposes, but especially the leaves, tar, and essential oils have been used in traditional medicine to treat a wide variety of maladies, including skin and hair problems, infections, inflammations, rheumatism, arthritis, urinary-tract disorders etc.[2−4] Birch essential oils have also been used in cosmetic and hair products[5−7] and have been the subject of numerous investigations[4,5,8−13] over the years. As an extension of our ongoing investigation into the essential oils derived from birch species native to Turkey,[4,11−13] we have now investigated two species native to Finland, the mountain birch [*Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti] and the white birch (*Betula pubescens* ssp. *pubescens* Erhr.). The wood of both these tree species is utilized extensively as firewood, and white birch, to a limited degree, for furniture manufacture, whilst the leafy branchlets are traditionally employed as vihta (or vasta) in the Finnish sauna ritual. Birch essential oils, unadulterated or mixed with other essential oils, are also often used to create a more conducive atmosphere in this aforementioned practice by sprinkling them directly onto the hot stones or by their addition as a dispersion to the water which is splashed onto the stones. Birch essential oils are also used in a similar vein in aromatherapy and other analogous practices which have achieved recent widespread popularity in Western culture. Thus, the exposure of man to birch compounds can be considerable either through direct contact or inhalation. Moreover, in the search for functional foods or food additives with desirable properties, particularly antioxidant properties, many plants or their extracts, including species not normally considered as food crops, have come under close scrutiny.[14]

Herein we report the compositions in terms of the compounds present in the essential oils of the early-season buds

[a] Structural Chemistry Group, Department of Chemistry, University of Turku,
Vatselankatu 2, 20014 Turku, Finland
[b] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University,
26470 Eskişehir, Turkey
[c] Laboratory of Environmental Chemistry, Department of Chemistry, University of Turku,
Vatselankatu 2, 20014 Turku, Finland
[d] Food Chemistry, Department of Applied Chemistry and Microbiology, University of Helsinki,
Latokartanonkaari 11, 00014 Helsinki, Finland

of mountain and white-birch trees obtained by hydrodistillation. As a result, four new compounds, an aldehyde (birkenal), an alcohol (birkenol), the acetate of this alcohol (birkenyl acetate), and a lactone (hushinone), were identified which were all structurally related to one another by possession of the same carbon framework. These appeared to be, in principle, derived from the ring contraction of β-caryophyllene. Another new compound, the acetate of 6-hydroxycaryophyllene, was also identified. The isolation of birkenal (the most abundant component of this set of structurally unique compounds), 6-hydroxycaryophyllene, and hushinone from the oils, together with the subsequent semi-synthetic preparation of birkenol by the reduction of birkenal, provided samples for the structural elucidation of these compounds based on NMR spectroscopic and mass spectrometric analyses. The structural determination of the acetates was based on MS analyses and confirmed by acetylation of the corresponding alcohols.

Results and Discussion

Collection, Identification, and Isolation

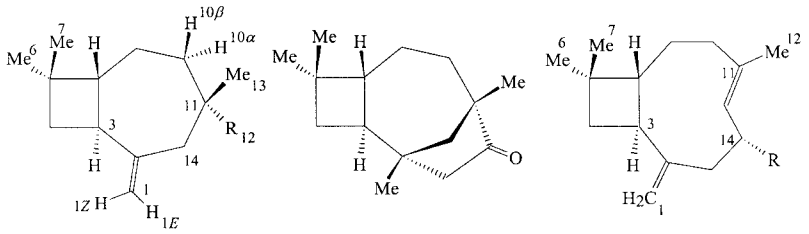
The buds of both mountain birch and white birch were collected during the month of April, 2002, from the lower branches of mature (> 20 years old) trees growing in South-West Finland, although the seed material originated from Northern Finland (mountain birches) and Southern Finland (white birches). The time of collection was selected based on the seasonal abundance of buds and the presumption of a high oil content being present in the bud material. This proved true by the high yields of the essential oils (5−8%) afforded by the buds and facilitated the ready collection by hydrodistillation, and which to some degree offset the tedious manual collection of the plant material. Whether a high oil content has evolved to assist in protection against the often still severe weather conditions prevailing at the time or as an inhibition against herbivorous predation is open to conjecture.

The collected oils were subsequently subjected to analysis by GC and GC/MS and a combined total of 26 known compounds (see Table 1) for both species were identified on the basis of both MS and relative retention index (RRI) data. Most of these 26 compounds are commonly occurring and all have been identified[4,12] previously in *Betula* species. 14-Acetoxy-β-caryophyllene was determined to be the main component in both oils in contrast to its usual presence[4,11,12] as only a minor component (often less than 1%) in other *Betula* species, although very often 14-hydroxy-β-

Table 1. Essential oil compositions (%) of mountain birch [*Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti] and white birch (*Betula pubescens* ssp. *pubescens* Erhr.)

Compound	RRI[a]	White birch[b]	Mountain birch[b]
Hexanol	1360	–	tr
α-Copaene	1497	0.1	0.1
Linalool	1553	tr	tr
6-Methyl-3,5-heptadien-2-one	1602	tr	–
β-Caryophyllene (**5**)	1612	0.3	0.7
α-Humulene	1687	tr	tr
α-Terpineol	1706	tr	tr
β-Selinene	1742	tr	tr
δ-Cadinene	1773	tr	tr
Birkenal (**1**)	1823	11.7	10.8
Caryophyllene oxide	2008	3.1	3.5
Birkenyl acetate (**2a**)	2009	0.1	0.1
Humulene epoxide I	2045	–	tr
Humulene epoxide II	2071	0.4	0.5
Humulene epoxide III	2081	–	0.2
Heneicosane	2100	1.3	0.4
Birkenol (**2**)	2149	0.4	0.6
β-Betulenal (**7**)	2193	1.1	1.7
Hushinone (**3**)	2209	0.7	0.2
6-Acetoxycaryophyllene (**4a**)	2210	5.0	1.0
14-Acetoxy-β-caryophyllene	2272	32.5	30.0
Tricosane	2300	1.7	2.6
Caryophylla-2(12),6(13)-dien-5β-ol (caryophylladienol I)	2316	1.2	1.3
Caryophylla-2(12),6(13)-dien-5α-ol (caryophylladienol II)	2324	5.2	5.8
14-Acetoxy-α-humulene	2329	1.2	0.7
6-Hydroxycaryophyllene (**4**)	2346	11.7	15.1
14-Hydroxy-β-caryophyllene	2357	1.7	3.5
Pentacosane	2500	0.3	0.4
14-Acetoxy-4,5-epoxy-β-caryophyllene (βα)	2617	1.3	1.8
14-Hydroxy-4,5-epoxy-β-caryophyllene (βα)	2663	–	0.2
Heptacosane	2700	0.3	0.4
Total		81.3	81.6

[a] Relative retention indices calculated against *n*-alkanes. [b] tr = trace (< 0.1%).



1, R = CHO (birkenal)
2, R = CH_2OH (birkenol)
2a, R = CH_2OCOCH_3 (birkenyl acetate)

3, hushinone

4, R = OH (6-hydroxycaryophyllene)
4a, R = CH_2OCOCH_3
5, R = H (β-caryophyllene)

Figure 1. The structures of **1**−**4** together with the numbering system in use; in this unconventional numbering, C-14 equates to C-6 in more commonly used systems, hence the name 6-hydroxycaryophyllene for **4**; the usual convention of denoting hydrogen atoms or substituents protruding above the idealized plane of the rings (i.e. towards the reader) as β and as α for the opposite case is followed; although the absolute stereochemistry as indicated for **1**−**4** has not been proven, it is highly probable in all cases

caryophyllene has been reported[4,11,12] as the major component in the essential oils of *Betula* sp. (here, by contrast, it was present in only minor amounts). Also present in the oils, identified by various strategies, were five new compounds (depicted in Figure 1) — birkenal (**1**), birkenol (**2**), birkenyl acetate (**2a**), hushinone (**3**), and the acetate of 6-hydroxycaryophyllene (**4a**) — amounting to a total of 31 identified compounds.

The structural similarities of birkenal (**1**), birkenol (**2**), and birkenyl acetate (**2a**) were apparent at a very early stage by GC/MS analysis providing similar fragmentation patterns and the appropriate mass distinctions (together with their RRIs) giving clue to their functionalities (aldehyde, alcohol, and ester, respectively). Due to the low content of **2** and **2a** in the essential oils, it was deemed judicious to isolate only the structurally related **1** by column chromatography and to obtain **2** from **1** by reduction and then **2a** from **2** by acetylation. The identity of the naturally occurring compounds **2** and **2a** in the oils was then unequivocally established by co-elution (TLC and GC/MS) with their semisynthetic counterparts. A complete structural determination, of course, was necessary by NMR spectroscopy and mass spectrometry (vide infra) to determine the full structures, but this was performed only on the isolated sample of **1** and the semisynthetically prepared **2**. Regarding the relative configurations of **1** and **2**, it is a safe assumption that the relative configuration of C-11 in the semisynthetic sample of **2** is the same as that of natural **2** (the two samples co-eluted), therefore the relative configurations of **1** and natural **2** are the same.

The lactone hushinone (**3**) was considered very interesting on the basis of its apparent distinctiveness by MS from the other compounds and, despite its low concentration, its isolation was persevered with. Only after examination by NMR spectroscopy (vide infra) was its structural similarity to birkenal (**1**) and birkenol (**2**) realized. Indeed, the close structural similarity of **3** to **1** and **2** compels one to question the likely natural presence of hushinone (**3**) in the plant or whether it is an artifact of the extraction process itself, albeit a comparatively mild one. All that would be required in the latter case is the oxidation of **1** (or **2**) to an acid coupled with hydration of the double bond (quite feasible given that a tertiary carbocation would be the intermediate), followed by spontaneous lactonization. We believe, however, that hushinone (**3**) is truly present as a natural product in the plant but there is also the further question of whether it is the lactone itself which is present or the hydroxy acid (vide infra).

Of the known compounds, only for the relatively recently reported[5,15,16] 6-hydroxycaryophyllene (**4**) was it considered necessary to isolate the compound by column chromatography to confirm its structure by comprehensive NMR analysis due to a lack of available data (viz. RRI). Since the acetate of 6-hydroxycaryophyllene (**4a**) was also not present in sufficient amounts to permit convenient isolation, it too was prepared semisynthetically by the acetylation of **4**, the structural similarity of **4** and **4a** again being evident by MS and the appropriate mass distinction and RRIs giving a clue to the functionalities of **4** and **4a**. As before, the identity of the naturally occurring compound **4a** was unequivocally established by co-elution (TLC and GC/MS) with its semisynthetic counterpart. Although 6-hydroxycaryophyllene (**4**) has apparently been identified[15] previously in the essential oil of *Betula* sp. (but with unspecified stereochemistry for the carbon atom bearing the hydroxy group) and seemingly with a (*Z*) configuration for the endocyclic double bond, the strength of both these conclusions is doubtful. Definitive identification[5] of **4**, i.e. with an (*E*) configuration of the endocyclic double bond, in *Betula pubescens* buds has been made by Japanese workers. The relative stereochemistry of the compound was subsequently shown, by ^{13}C NMR spectroscopy, to be the same as a sample of **4** obtained from the fruiting bodies of a Basidiomycete (after hydrolysis of the stearate ester in which form it is present in the organism) and for which a complete stereochemical analysis had been performed.[16] However, it is clear that the acetate of 6-hydroxycaryophyllene (**4a**) is a new natural product.

Structural Analyses of 1−4

On the expectation of a β-caryophyllene skeleton, and therefore using it as an initial base, it was evident by NMR

spectroscopy (^{1}H, ^{13}C, and subsequent 2-D experiments) that most of the structural features of the carbon skeleton of β-caryophyllene remained intact for the isolated compounds birkenal (**1**), hushinone (**3**), and 6-hydroxycaryophyllene (**4**) and the semisynthetically prepared birkenol (**2**). For example, the presence of a *trans*-fused cyclobutane ring bearing geminal methyl groups, an exocyclic double bond adjacent to this cyclobutane ring (except **3** for this feature), and an (*E*)-configured C-11=C-13 double bond also bearing a methyl group (only **4** for this feature) were all clearly evident, quickly established, and, furthermore, consistent with literature values for partial structures. It was thus a straightforward exercise using the standard combination of COSY, CHDEC/HMQC, and HMBC spectra to trace out the framework and, together with the preconceived notions of the functional groups present (duly confirmed by appropriate signals in the ^{1}H and ^{13}C NMR spectra), to arrive at the finalized gross structures for **1**−**4**. Stereochemical determinations (and some geminal ^{1}H NMR assignments) were based on NOE difference experiments, and to a degree supported also by comparison with literature values (available completely for **4** and partially for **1**−**3**). In some cases, geminal proton assignments were also evident by the presence of large *trans*-diaxial couplings to result in the complete assignment of all spins for **1**−**4**. Compounds **1**−**3** are unusual in that they represent loss of a methylene group and ring contraction in comparison to their presumed parent, β-caryophyllene (**5**). This was apparent initially from the low-resolution MS and subsequently indicated by the elemental composition provided by HRMS and consistent with the nuclei counts in the ^{1}H and ^{13}C NMR spectra. The full ^{1}H and ^{13}C NMR spectroscopic data for compounds **1**−**4** are presented in Tables 2−4. Of note are the large number of ^{1}H−^{1}H couplings extending over four bonds (Table 3). Some of these are realized via the structural constitution (e.g. allylic, cyclobutyl moieties) and are unexceptional in that sense and no doubt others arise from favorable W-type geometries. Such W-type geometries also enabled the observation of some $^4J_{H,C}$ correlations, notably that between 12-H(*pro-R*) and C-9 in birkenol (**2**) which, together with the observed NOEs, enabled the stereochemical distinction between the H-12 methylene protons in this compound.

The relative stereochemistry at C-11 for **1** and **2** — β for the methyl and α for the carbaldehyde (hydroxymethyl in the latter compound) groups — was easily ascertained on the basis of NOEs between (the) 12-H(s) and 3-H and between 13-H and 8-H. From examination of a Dreiding model it was ascertained that birkenal (**1**) and birkenol (**2**) could adopt one of three conformations — a $^{2,14}C_9$, an $^8S_{10}$, or a $B_{2,3,10}$ conformation (see Scheme 1). The most favored conformation based on the observed ^{1}H,^{1}H vicinal coupling constants of the C-8−C-9−C-10 segment was indicated to be a distorted $^{2,14}C_9$ conformation in both cases (all corresponding $J_{H,H}$ couplings are essentially the same for **1** and **2** thus indicating that their conformational behavior is essentially indifferent). The $^{2,14}C_9$ conformer appeared to be essentially quite rigid with only slight, presumably rapidly oscillating, deformations of the seven-membered ring seemingly possible. In particular, the observed values for $J_{9\text{-H}\alpha,10\text{-H}\beta}$ and $J_{9\text{-H}\beta,10\text{-H}\alpha}$, as they should have very large and moderately small values, respectively, in the $^{2,14}C_9$ conformation or conversely for the $^8S_{10}$, and $B_{2,3,10}$ conformations, indicate that there can only be a minor contribution from these latter two conformations. However, based on the observed NOEs — $\eta_{8\text{-H},13\text{-H}}$, $\eta_{10\text{-H}\beta,13\text{-H}}$, and

Table 2. ^{1}H NMR chemical shifts (in ppm) and multiplicities for compounds **1**−**4** in $CDCl_3$; the temperature was varied as necessary to shift the water signal away from the signals of interest; this did not result in any appreciable change to the NMR parameters (overlapped)

	Birkenal (**1**)[a]	Birkenol (**2**)[b][c]	Hushinone (**3**)[a]	**4**[c][d]
1-H(*E*)[e]	4.777 (qd)	4.711 (dq)	1.332 (s)	4.891 (≈ q)
1-H(*Z*)	4.566 (≈ qt)	4.570 (m)	−	4.997 (≈ t)
3-H	2.311 (qm)	2.569 (qm)	2.189 (td)	2.274 (≈ q)
4-Hα	1.824 (≈ ddd)	1.780 (≈ ddd)	1.732 (over dd)	1.786 (ddd)
4-Hβ	1.589 (over ≈ t)	1.595 (≈ t)	1.300 (≈ dd)	1.565 (over)
6-H	0.979 (br. s)	1.009 (br. s)	1.007 (br. s)	0.961 (br. s)
7-H	0.995 (s)	1.002 (s)	1.018 (s)	0.971 (s)
8-H	1.635 (≈ tdd)	1.723 (≈ ddd)	1.689 (over td)	1.427 (ddt)
9-Hα	1.497 (over m)	1.402 (over m)	1.258 (over dddd)	1.665 (m)
9-Hβ	1.547 (over m)	1.513 (m)	1.641 (over ddt)	1.592 (over m)
10-Hα	2.080 (dtd)	1.370 (over m)	2.005 (≈ dddd)	2.532 (br. dd)
10-Hβ	1.401 (≈ dddd)	1.654 (m)	1.519 (≈ ddd)	1.564 (over m)
12-H[f]	9.574 (d)	3.463 (br. d, *pro-R*); 3.326 (br. d, *pro-S*)	−	1.625 (≈ t)
13-H	1.044 (s)	0.923 (s)	1.265 (s)	5.262 (dquint)
14-Hα	2.610 (dquint)	2.129 (br. d)	1.788 (dd)	−
14-Hβ	2.463 (dq)	2.294 (dq)	2.574 (d)	4.601 (td)
15-Hα	−	−	−	1.938 (ddd)
15-Hβ	−	−	−	2.780 (dd)

[a] Spectrum acquired at 18 °C. [b] Spectrum acquired at 33 °C. [c] The OH proton signal was not observed. [d] Spectrum acquired at 19 °C. [e] 1-H is methyl group protons in the case of **3**. [f] 12-H is an aldehydic proton in the case of **1**, a pair of methylene protons in the case of **2**, and methyl group protons in the case of **4**.

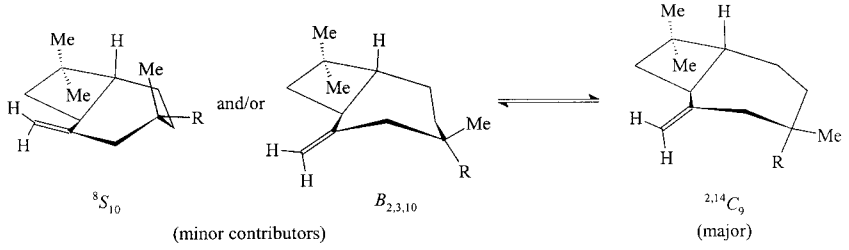
Table 3. $J_{H,H}$ couplings (in Hz) for compounds **1**−**4** in $CDCl_3$ extracted using Perch[a] iteration software; the temperature was varied as necessary to shift the water signal away from the signals of interest; this did not result in any appreciable change to the NMR parameters; ne: not extracted by Perch;[17] pd: presence of coupling determined but not extracted by Perch

	1[b]	**2**[c]	**3**[b]	**4**[d]		**1**[b]	**2**[c]	**3**[b]	**4**[d]
$J_{1\text{-H}(E),1\text{-H}(Z)}$	−1.84	−2.16	−[e]	−1.42	$J_{9\text{-H}\alpha,10\text{-H}\alpha}$	4.80	5.12	4.84	7.05
$J_{1\text{-H}(E),3\text{-H}}$	−2.27	−1.97	ne[e]	−1.12	$J_{9\text{-H}\alpha,10\text{-H}\beta}$	11.37	10.15	12.62	13.63[f]
$J_{1\text{-H}(E),14\text{-H}\alpha}$	−1.21	−1.32	ne[e]	ne[g]	$J_{9\text{-H}\beta,10\text{-H}\alpha}$	4.71	5.73	2.11	2.60
$J_{1\text{-H}(E),14\text{-H}\beta}$	−2.08	−1.56	ne[e]	−0.87[g]	$J_{9\text{-H}\beta,10\text{-H}\beta}$	4.54	4.84	5.55	1.85[f]
$J_{1\text{-H}(Z),3\text{-H}}$	−2.32	−2.11	−[e]	ne	$J_{10\text{-H}\alpha,10\text{-H}\beta}$	−14.25	−13.63	−13.95	−13.93
$J_{1\text{-H}(Z),14\text{-H}\alpha}$	−0.76	−0.74	−[e]	−1.24[g]	$J_{10\text{-H}\beta,12\text{-H}}$	−0.72	pd	−	−0.87[h]
$J_{1\text{-H}(Z),14\text{-H}\beta}$	−2.16	−1.21	−[e]	ne[g]	$J_{10\text{-H}\alpha,13\text{-H}}$	ne	ne	ne	−1.67
$J_{3\text{-H},4\text{-H}\alpha}$	7.76	7.65	7.88	8.19	$J_{10\text{-H}\alpha,14\text{-H}\alpha}$	−1.45	ne	−1.19	−
$J_{3\text{-H},4\text{-H}\beta}$	9.85	9.93	11.51	10.04	$J_{10\text{-H}\alpha,14\text{-H}\beta}$	pd	−0.17	ne	ne
$J_{3\text{-H},8\text{-H}}$	11.08	10.34	11.99	9.16	$J_{10\text{-H}\beta,14\text{-H}\alpha}$	ne	−0.73	ne	−
$J_{3\text{-H},14\text{-H}\alpha}$	−0.73	−0.76	ne	ne[g]	$J_{12\text{-H}(proR,S)}$	−	−10.91	−	−
$J_{3\text{-H},14\text{-H}\beta}$	−1.73	−1.26	ne	ne[g]	$J_{12\text{-H},13\text{-H}}$	pd	pd	−	−1.54
$J_{4\text{-H}\alpha,4\text{-H}\beta}$	−10.25	−10.12	−9.43	−11.00	$J_{12\text{-H},15\text{-H}\beta}$	−	−	−	−0.21
$J_{4\text{-H}\alpha,8\text{-H}}$	−0.65	−0.59	ne	−0.84	$J_{13\text{-H},14\text{-H}\beta}$	pd	pd	ne	10.24
$J_{4\text{-H}\beta,6\text{-H}}$	−0.53	−0.56	ne	−0.58	$J_{14\text{-H}\beta,15\text{-H}\alpha}$	−	−	−	9.76
$J_{8\text{-H},9\text{-H}\alpha}$	12.11	11.63	11.75	10.76	$J_{14\text{-H}\beta,15\text{-H}\beta}$	−	−	−	6.67
$J_{8\text{-H},9\text{-H}\beta}$	3.87	5.41	1.46	1.39	$J_{14\text{-H}\alpha,14\text{-H}\beta}$	−16.09	−14.72	−12.93	−
$J_{9\text{-H}\alpha,9\text{-H}\beta}$	−13.79	−13.84	−13.67	−8.18[f]	$J_{15\text{-H}\alpha,15\text{-H}\beta}$	−	−	−	−11.54

[a] For Perch iteration, the signs of the couplings were assumed (^{even}J = negative, ^{odd}J = positive). [b] Spectrum acquired at 18 °C. [c] Spectrum acquired at 33 °C. [d] Spectrum acquired at 19 °C. [e] 1-H is methyl group protons. [f] The region of the spectrum containing these spins was of very high order and due to spectral overlap this region could not be satisfactorily simulated. Therefore, the reliability of these couplings wholly contained within the region must be treated with caution. [g] 15-H instead of 14-H. [h] $J_{10\text{-H}\alpha,12\text{-H}}$.

Table 4. ^{13}C NMR chemical shifts (in ppm) for compounds **1**−**4** in $CDCl_3$ at 25 °C

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15
1	107.24	150.42	40.76	38.42	34.56	21.91	30.26	50.43	24.89	36.18	48.72	206.29	25.51	44.50	−
2	107.41	152.06	40.51	37.98	34.87	21.63	30.32	50.72	23.58	36.38	39.09	71.76	24.45	47.33	−
3	22.30	85.99	48.80	37.76	35.49	19.77	30.26	50.81	26.61	39.36	46.65	181.85	28.08	46.56	−
4	112.41	150.07	48.93	42.21	32.73	21.84	29.83	55.46	31.21	34.73	136.88	22.97	127.87	70.68	49.18



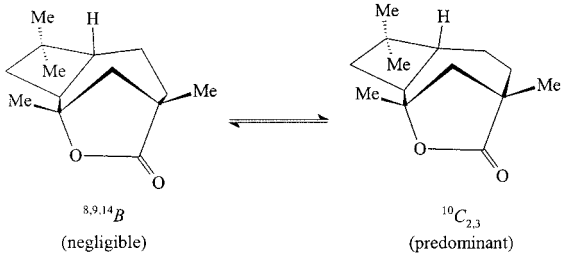
Scheme 1. The conformational equilibrium for birkenal (**1**, R = CHO) and birkenol (**2**, R = CH_2OH)

$\eta_{12\text{-H}/12\text{-H}(proS),10\text{-H}\beta}$ — there must be some, although minimal, contribution from one or the other or both of the $^8S_{10}$ and $B_{2,3,10}$ conformations (these two conformations being related by a flip of the methylene group which is β in the former conformation). Since only one set of NMR signals was observed at 18 °C, fast exchange must necessarily be in effect.

Though the functional group distinctions for hushinone (**3**) in comparison to **1** and **2** were readily apparent — the lack of a terminal C=C double bond, the extra methyl group, and C-2 now singly bound to an oxygen atom — there still existed a structural problem regarding whether the isolated sample was really the lactone as indicated or was in fact the corresponding hydroxy acid, as the distinction is not necessarily straightforward by NMR spectroscopy. For EIMS analysis by direct insertion, the observation of a mass of only 222 amu does not carry much weight as it can just as easily represent the $M^{+\cdot}$ ion of the lactone as the highest observed ion of the hydroxy acid following facile loss of water. The observation of only an ion of mass of 222 amu and never an ion of mass 240 amu ($M^{+\cdot}$ ion for the hydroxy acid) is therefore inconclusive. For GC/MS analysis, although a hydroxy acid would be unlikely to elute from the polar column that was in use and, as such, the observed peak is quite probably the lactone irrespective of what may be occurring in the subsequent MS region (the

only high mass peak observed was 222 amu), lactonization could also be readily occurring in the injection port of the GC or even on the column. However, given the method of isolation (hydrodistillation) the isolated sample should be the lactone, as indicated, rather than the hydroxy acid, as the former should be hydrodistillable whilst the latter is unlikely to be. Given the method of isolation, however, it therefore remains something of an open question as to whether the natural product is the hydroxy acid or the lactone, or even a complete artifact as considered above.

The relative stereochemistry of hushinone (**3**) was readily established as being the same at C-11 as for **1** and **2** given the observed NOE between 14-Hβ and 8-H, thus indicating that the bridging C-14 methylene group is orientated in an upward (β) manner. Hence, due to geometric constraints, the lactone bridge is α, resulting in a β configuration for C-2 as well as the same previous relative configuration at C-11 (methyl group β). Despite its tricyclic nature, hushinone (**3**) could, by examination of a Dreiding model, also be conformationally mobile with respect to the seven-membered ring and adopt either a $^{10}C_{2,3}$ conformation or an $^{8,9,14}B$ conformation (see Scheme 2). Based on the observed NOE between 10-Hβ and 8-H and essentially all of the $^{3}J_{H,H}$ couplings of the C-8−C-9−C-10 segment, but especially $J_{8\text{-}H,9\text{-}H\beta}$, $J_{9\text{-}H\alpha,10\text{-}H\beta}$, and $J_{9\text{-}H\beta,10\text{-}H\beta}$ where opposing extreme values (i.e. conversely either large or small) were to be expected in each conformer, the chair conformation was clearly favored over the boat conformation and indeed the contribution of the boat conformation can only be negligible at best.



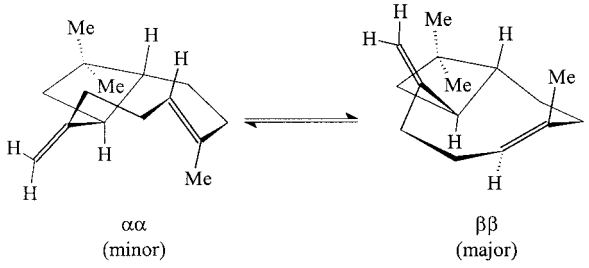
$^{8,9,14}B$ (negligible) $^{10}C_{2,3}$ (predominant)

Scheme 2. The conformational equilibrium for hushinone (**3**)

The structure and relative configuration of 6-hydroxycaryophyllene (**4**) was firmly established based on the comparison of the ^{1}H and ^{13}C NMR parameters with literature values.[16] The ^{13}C chemical shifts all agreed to within 0.2 ppm whilst on the whole excellent agreement was found for the ^{1}H NMR parameters (chemical shifts to within 0.01 ppm or better and all available $J_{H,H}$ couplings within 10%). Some small discrepancies in the ^{1}H NMR parameters were observed for 9-Hβ, 9-Hα, 10-Hβ, and 4-Hβ due to the fact that these resonances were of high order and/or suffered from spectral overlap. Confidence, though, is placed in the ^{1}H chemical shifts obtained here from Perch[17] simulation for these resonances as they agreed within experimental limitations to the chemical shifts observed in the CHDEC experiment.

Although the relative configuration of 6-hydroxycaryophyllene (**4**) obtained in this work is the same as previous isolates[5,16] based on the matching of the ^{13}C NMR spectroscopic data, we, like the Japanese workers[5] who had difficulties due to lack of material, also encountered difficulties in this work due to impurities present in the sample which diminished the reliability of the optical measurement {this work: $[\alpha]_D^{23} = -15.8$ ($c = 0.126$, EtOH); ref.:[16] $[\alpha]_D = -51$ ($c = 0.4$, $CHCl_3$)} and hence a clear conclusion regarding the absolute configuration or optical purity of the isolated material. However, for β-caryophyllenes (i.e. the *trans*-fused isomers) it seems that only stereoisomers comprising (*S*) and (*R*) configurations for C-3 and C-8, respectively (as indicated in Figure 1), have been encountered in nature.[16] Assuming this to hold for **1**−**3**, the C-11 stereochemistry is thus (*S*) in accordance with the results discussed above; for **3**, the C-2 stereochemistry is, by geometric constraints, also (*S*); and for **4** (and hence also **4a**), the C-14 stereochemistry is (*R*) based on literature results.[16,18]

For 6-hydroxycaryophyllene (**4**) the contrast between it and β-caryophyllene (**5**) with regards to the conformational mobility of the nine-membered ring is noteworthy. β-Caryophyllene (**5**) displays an observable dynamic exchange in the ^{1}H NMR spectrum between a number of conformations at room temperature,[18,19] whilst 6-hydroxycaryophyllene (**4**) displays only one set of signals under the same conditions. The assignment of configuration in **4** was compounded by a potential interchange between conformers,[16,19] hence the intense examination previously afforded to this structure[16,18] (**4**) and β-caryophyllene (**5**), in particular where several re-evaluations of its conformational constitution have been performed.[16,18,19] Nevertheless, for **4** it was concluded[18] that the conformational preference was heavily biased towards the ββ conformer (see Scheme 3) and that there was essentially no contribution from either the βα or the αα conformers, with the αβ conformer having been fully discounted on energetic grounds. However, there must be some contribution from the αα conformer to a dynamic equilibrium as an NOE was observed between 13-H and 8-H (4.3%, cf. $\eta_{3\text{-}H,8\text{-}H}$ 1.5−2.6%) in this work, this NOE not being observed between these two protons in the previous study.[16] Since only one set of NMR signals was observed at 19 °C, fast exchange must necessarily therefore be in effect. The smaller than expected magnitudes of $J_{14\text{-}H\beta,15\text{-}H\alpha}$ (9.76 Hz) and $J_{13\text{-}H,14\text{-}H\beta}$ (10.24 Hz) in



αα (minor) ββ (major)

Scheme 3. The conformational equilibrium for 6-hydroxycaryophyllene (**4**)

6-hydroxycaryophyllene (**4**) lend support to this notion after taking into consideration electronegativity effects (primarily O-14).

Antioxidant Activities

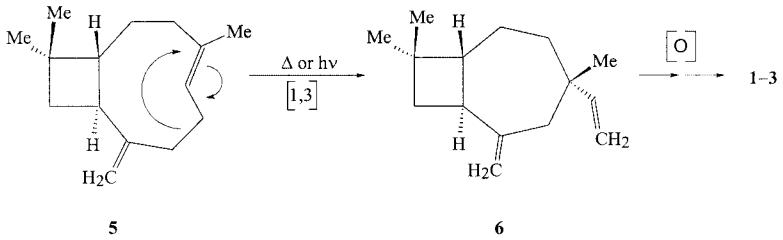
The antioxidant activities of the essential oils from both species and the compounds available in the pure state and in sufficient quantities [birkenal (**1**), birkenol (**2**), hushinone (**3**), and 6-hydroxycaryophyllene (**4**)] were assessed by measuring their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The test was performed on the samples at concentrations of 0.5 and 1.0 mg·mL^{-1} but significant scavenging of the radicals was not realized (the percentage of radicals scavenged varied between 1.5 and 2% and was basically independent of concentration). In contrast, the percentage of radicals scavenged by the reference compound pyrogallol was 92%. This test is normally considered to be a good preliminary screening test for evaluating the potential antioxidant properties of new compounds.[20]

Biosynthetic Speculation

Concerning the biosynthetic pathway to **1**−**3**, one can speculate on the manner in which these apparently corrupted caryophyllene structures arise. Scheme 4 shows a plausible route for the biosynthesis of structures **1**−**3**, assuming that they emanate by ring contraction from β-caryophyllene (**5**), a commonly occurring[4,12] compound in *Betula* sp. (and found here in minor amounts). The [1,3]-sigmatropic rearrangement of β-caryophyllene (**5**) to the alkene **6** has been reported to occur in vitro by thermolysis[21] or by photolysis.[22] Compound **6** has in fact even been previously reported[23] to occur as a natural product from mango fruit after removal of the pedicel, although the configuration at C-11 was not determined. Furthermore, it seems readily plausible that a structure such as **6** could easily be transformed into structures such as the aldehyde **1** by familiar biochemical transformations. It is noteworthy that the C-12 methyl group in β-caryophyllene (**5**) would appear to remain intact in **1**−**3** if this was the actual biosynthetic route.

Experimental Section

General Experimental Procedures: NMR spectra were acquired with a JEOL Alpha 500 NMR (or JEOL Lambda 400) spectrometer [11.75 (9.40) T] equipped with either a 5-mm normal-configuration tunable ($^{13}C\{^1H\}$) probe or a 5-mm inverse *z*-axis field-gradient ($^1H\{X\}$) probe operating at 500.16 (399.78) MHz for ^1H and 125.78 (100.54) MHz for ^{13}C. Spectra were acquired at 25 °C, unless otherwise stated, in $CDCl_3$ and both ^1H and ^{13}C spectra were referenced internally to TMS (δ = 0.00 ppm for both). Spectral widths of 2-D spectra were appropriately selected from the 1-D spectra and acquired with an adequate level of resolution. All experiments were performed using standard, vendor-supplied pulse sequences. 1-D ^1H NMR spectra were processed with a double exponential to effect resolution enhancement prior to spin analysis which was performed using Perch[17] iteration software for the extraction of ^1H chemical shifts and $J_{H,H}$ coupling constants. Since the reliable extraction of small couplings approaching the linewidth is heavily dependent on whether they are to a degree resolvable on at least one spin for Perch to reliably extract them, only those couplings reliably extracted by Perch are reported whilst couplings buried in the linewidth on both interacting spins are not reported (i.e., not extracted) even if their likely presence is probable or is evident from homodecoupling experiments or COSY experiments. NOE difference measurements were acquired with saturation times of 7−12 s and signal enhancement was integrated relative to the intensity of the irradiated signal set to −100%. Prior to NOE measurements, samples were deoxygenated by effusion with nitrogen. For EXSY spectra, mixing times of 700 ms were utilized. For all ^1H,^{13}C correlation experiments, $^1J_{H,C}$ was optimized on a value of 145 Hz whilst the HMBC correlations were optimized for a long-range $^nJ_{H,C}$ coupling of 8 Hz. GC analyses were performed with a Hewlett Packard 6890 system equipped with an HP-Innowax FSC column (60 m × 0.25 mm i.d., film thickness 0.25 μm) using N_2 as the carrier gas (1 mL min^{-1}). Temperature programming: 60 °C held for 10 min followed by ramping to 220 °C at 4 °C min^{-1} and holding for 10 min and then a second ramp to 240 °C at a rate of 1 °C min^{-1}. *n*-Alkanes were used as references for the calculation of relative retention indices (RRI). GC/MS analyses were performed using a Hewlett−Packard GCD system equipped with the same column and using the same conditions, except that He was used as the carrier gas. Masses were scanned over the range of *m*/*z* = 35−425 amu. Library searches were conducted using both a commercially available Wiley GC/MS Library and an in-house Başer Library of Essential Oil Constituents. High-resolution mass measurements were performed with a VG Analytical ZabSpec sector instrument using a direct insert probe by ESA voltage scanning using PFK as a reference substance at a resolution of 8000−10000 (at 10% peak height). Due to extreme sample volatility at reduced pressure, measures were taken to start the acquisition as soon as the probe was introduced into the high-vacuum area, hence voltage scanning was used in preference to peak matching. Optical rotations were measured using a Perkin−Elmer 241 polarimeter. UV spectra were recorded using a Varian UV/Vis Cary 50 Bio spectrophoto-



5 **6**

Scheme 4. In this proposed biosynthetic scheme, β-caryophyllene (**5**) is transformed by a [1,3]-sigmatropic rearrangement into the ring-contracted structure **6**; oxidation and other common metabolic processes could then potentially give rise to the set of ring-contracted products that were isolated (**1**−**3**)

meter in spectroscopic grade EtOH. IR spectra were measured using a GC/FT-IR Perkin−Elmer Spectrum 2000 spectrophotometer.

DPPH Radical Scavenging Assay: The assay was carried out essentially according to the procedure of Kähkonen and Heinonen[20] using a Perkin−Elmer Lambda 15 UV/Vis spectrophotometer on methanolic solutions of the samples at concentrations of either 0.5 or 1 mg·mL^{-1}. All samples were measured as triplicates at both concentrations and pyrogallol (0.5 mg·mL^{-1}) was used as a reference.

Plant Material: Buds of mountain birch [*Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti] and white birch (*Betula pubescens* ssp. *pubescens* Erhr.) were collected in April 2002 from the lower branches of ten individual, mature (> 20 years old) trees of both species located in the Botanical Garden of the University of Turku (SW Finland). The seed material of the mountain birches originated from Utsjoki, Kevo (Northern Finland) and that of the white birches from Punkaharju (Southern Finland). Voucher specimens of the buds have been deposited in the Turku University Herbarium under the following accession codes: TUR 573171 (white birch) and TUR 573172 (mountain birch).

Isolation of the Essential Oils: The air-dried buds were hydrodistilled for 3 h using a Clevenger-type apparatus to yield 5.0% (white birch) and 7.8% (mountain birch) of essential oils on a dry-weight basis after drying over anhydrous Na_2SO_4.

(1*S*,4*S*,7*R*)-4,8,8-Trimethyl-2-methylenebicyclo[5.2.0]nonane-4-carbaldehyde (Birkenal, 1): The essential oil (0.4 g) was subjected to column chromatography on silica gel with *n*-hexane/diethyl ether (97:3) as eluent to provide **1** as a colorless oil; yield 2.8% (11 mg). R_f = 0.81 (silica; *n*-hexane/acetone, 5:1). $[\alpha]_D^{23}$ = +25 (*c* = 0.004, EtOH). IR (GC-FTIR): $\tilde{\nu}$ = 3082, 1641 and 889 (C=CH_2), 1741 (CHO) cm^{-1}. UV (EtOH): λ_{max} = 205 nm. ^{1}H and ^{13}C NMR: see Tables 2−4. EIMS (70 eV): *m*/*z* (%) = 206 (6) [M]$^+$, 191 (12), 177 (16), 163 (29), 149 (22), 135 (45), 121 (64), 107 (97), 93 (100), 79 (70), 69 (79), 55 (39), 41 (97). HREIMS (70 eV): calcd. for $C_{14}H_{22}O$ 206.1671; found 206.1678.

Reduction of 1 to {(1*S*,4*S*,7*R*)-4,8,8-Trimethyl-2-methylenebicyclo-[5.2.0]non-4-yl}methanol (Birkenol, 2): The aldehyde **1** (9 mg) was dissolved in dry methanol (1 mL) and treated with $NaBH_4$ (2 mg) at room temperature until TLC showed the reaction to be complete. Water (5 mL) was then added to the reaction mixture and the resulting solution extracted with *n*-hexane to yield **2** (7 mg) as a white solid. R_f = 0.41 (silica; *n*-hexane/acetone, 5:1). $[\alpha]_D^{23}$ = −12.5 (*c* = 0.04, EtOH). IR (GC-FTIR): $\tilde{\nu}$ = 3670 (OH), 3079, 1640 and 888 (C=CH_2), 1377 [$C(CH_3)_2$] cm^{-1}. UV (EtOH): λ_{max} = 210 nm. ^{1}H and ^{13}C NMR: see Tables 2−4. EIMS (70 eV): *m*/*z* (%) = 208 (2) [M]$^+$, 193 (3), 177 (56), 162 (13), 147 (25), 133 (19), 121 (100), 105 (73), 93 (74), 79 (70), 69 (76), 55 (46), 41 (87). HREIMS (70 eV): calcd. for $C_{14}H_{24}O$ 208.1827; found 208.1833; calcd. for $C_{13}H_{21}O$ [M − CH_3]$^+$ 193.1592; found 193.1598. Compound **2** was shown to be naturally present in the essential oils at low concentrations and identified as such by co-elution (TLC and GC/MS).

Acetylation of 2 to {(1*S*,4*S*,7*R*)-4,8,8-Trimethyl-2-methylenebicyclo-[5.2.0]non-4-yl}methyl Acetate (Birkenyl Acetate, 2a): An analytical amount of the alcohol **2** in pyridine (0.5 mL) was treated with acetic anhydride (0.5 mL) yielding **2a** by GC/MS analysis. EIMS (70 eV): *m*/*z* (%) = 250 (1) [M]$^+$, 190 (20), 175 (32), 162 (16), 147 (58), 134 (51), 119 (79), 105 (80), 91 (62), 79 (48), 69 (40), 55 (25), 43 (100). Compound **2a** was shown to be naturally present in the essential oils at low concentrations and identified as such by co-elution (TLC and GC/MS).

(1*S*,2*S*,4*S*,7*R*)-1,4,4,8-Tetramethyl-10-oxatricyclo[6.2.1.0^{2,5}]-undecan-9-one (Hushinone, 3): The essential oil (0.4 g) was subjected to column chromatography on silica gel with *n*-hexane/diethyl ether (85:15) as eluent to provide **3** as colorless needle-like crystals; yield 0.8% (3 mg). R_f = 0.54 (silica; *n*-hexane/acetone, 5:1). ^{1}H and ^{13}C NMR: see Tables 2−4. EIMS (70 eV): *m*/*z* (%) = 222 (1) [M]$^+$, 207 (4), 194 (2), 179 (5), 167 (100), 164 (68), 149 (19), 140 (26), 125 (45), 121 (76), 109 (37), 107 (99), 95 (70), 94 (85), 93 (61), 81 (57), 79 (40), 69 (27), 67 (37), 43 (86) 41 (83). HREIMS (70 eV): calcd. for $C_{14}H_{22}O_2$ 222.1620; found 222.1620.

(1*R*,6*R*,9*S*)-6-Hydroxycaryophyllene (4): The essential oil (0.4 g) was subjected to column chromatography on silica gel with *n*-hexane/diethyl ether (70:30) as eluent to provide **4** as a colorless oil, yield 7% (28 mg). R_f = 0.34 (silica; *n*-hexane/acetone, 5:1). $[\alpha]_D^{23}$ = −15.8 (*c* = 0.126, EtOH). IR (GC-FTIR): $\tilde{\nu}$ = 3651 (OH), 3076, 1635, 1379 [$C(CH_3)_2$] and 892 (C=CH_2) cm^{-1}. UV (EtOH): λ_{max} = 215 nm. ^{1}H and ^{13}C NMR: see Tables 2−4. EIMS (70 eV): *m*/*z* (%) = 220 (1) [M]$^+$, 205 (9), 187 (11), 177 (15), 164 (11), 149 (23), 137 (24), 131 (27),121 (43), 109 (76), 95 (60), 81 (76), 79 (63), 69 (100), 55 (22), 41 (90). HREIMS (70 eV): calcd. for $C_{15}H_{24}O$ 220.1827; found 220.1838. The spectroscopic data are consistent with those reported in the literature.[16]

Acetylation of 4 to (1*R*,6*R*,9*S*)-6-Acetoxycaryophyllene (4a): 6-Hydroxycaryophyllene (**4**) was acetylated as above. EIMS (70 eV): *m*/*z* (%) = 247 (0.5) [M − CH_3]$^+$, 220 (3), 202 (17), 187 (20), 177 (4), 159 (28), 145 (24), 133 (72), 121 (25), 109 (39), 91 (37), 79 (39), 69 (54), 55 (22), 43 (100). Compound **2a** was shown to be naturally present in the essential oils at low concentrations and identified as such by co-elution (TLC and GC/MS).

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