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SYRINGETIN 3-*O*-(6″-ACETYL)-β-GLUCOPYRANOSIDE AND OTHER FLAVONOLS FROM NEEDLES OF NORWAY SPRUCE, *PICEA ABIES*

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Key Word Index—*Picea abies*; Pinaceae; Norway spruce; needles; flavonols; acetylation; syringetin 3-*O*-(6″-acetyl)-β-glucopyranoside; spectral data.

Abstract—The novel flavonol, syringetin 3-*O*-(6″-acetyl)-β-glucopyranoside, has been isolated from needles of Norway spruce (*Picea abies*) together with the 3-*O*-(6″-acetyl)-β-glucopyranosides of isorhamnetin and kaempferol, the 3-*O*-(6″-α-rhamnopyranosyl)-β-glucopyranosides of laricitrin, isorhamnetin, myricetin, quercetin and kaempferol and the 3-*O*-β-glucopyranosides of laricitrin, isorhamnetin, myricetin, quercetin and kaempferol. Most of the flavonols have been isolated for the first time from Norway spruce. Kaempferol 3-*O*-(6″-acetyl)-β-glucopyranoside has previously been isolated from *Senecio aureus*, but without determination of the binding site of the acetyl group. Structure determination of the flavonols was achieved from TLC, ^{1}H NMR and UV shift reagent data, and, in most cases, ^{13}C NMR and MS.

INTRODUCTION

Norway spruce [*Picea abies* (L.) Karst., Pinaceae] is a characteristic species of the northern sub-arctic forests of Europe, but it is also found more widely in Europe and in northern Asia. The species is an economically important lumber source, but such added-value products as Jura Turpentine and Burgundy Pitch are also derived from it [1]. Much of the distribution of the species is in economically sensitive areas and relevant phytochemical studies are thus of great interest. Studies of phenolics are particularly apt as the importance of these compounds in mediating a wide range of interactions between species is documented [2]. In the case of Norway spruce, it is known that soil under stands of these trees contains persistently high levels of phenolics which continue to inhibit herbal growth for years after the trees have been cut [3]. Roots of *P. abies* infected with *Heterobasidon annosum* show increased levels of increasingly toxic phenolic glucosides in front of the hyphal attack zone [4]. Changes in levels of phenolics have been documented in needles on stands suffering novel forest decline [5]. The present paper describes an investigation into flavonols in newly burst buds of *P. abies* and forms part of a sustained effort to elucidate factors of importance for sylviculture of this species.

While the present investigation reports all 15 flavonols (**1**–**15**) (Fig. 1), earlier work indicated the presence of only eight flavonols in needles of Norway spruce: the aglycones kaempferol and quercetin, and kaempferol 3-glucoside (**11**), kaempferol 7-glucoside (**8**), kaempferol 3,4′-diglucoside (**1**), quercetin 3-glucoside (**5**), isorhamnetin 3-glucoside (**12**) and myricetin 3,4′-diglucoside [6–8]. The present investigation, while failing to find the aglycones and myricetin 3,4′-diglucoside, confirmed the presence of a further 10 flavonol glycosides for the first time in this species.

Although acylated flavonol glycosides have previously been reported in Pinaceae species (*Cedrus*, *Larix*, *Picea*, *Pinus* and *Pseudolarix*) [9], none has previously been detected in *Picea abies*. We now report the presence of three such acylated flavonol glucosides (**13**–**15**), one of which is a novel compound (**14**).

RESULTS AND DISCUSSION

The compounds **1**–**15** were isolated from the aqueous methanolic extract of Norway spruce buds by a combination of chromatographic techniques; ion exchange resin, centrifugal partition chromatography, gel filtration and preparative high performance liquid chromatography. The compounds are numbered in order of increased elution time during HPLC.

A heteronuclear coupling-modulated spin-echo NMR-experiment (SEFT) (Table 3) showed **13** to contain 23 carbon atoms. The downfield region in this experiment contained 15 signals including a flavonoid C_{15} skel-

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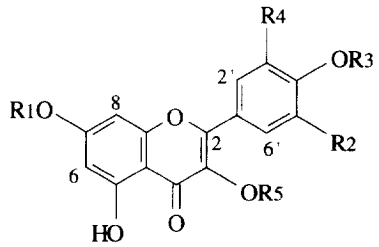

	R1	R2	R3	R4	R5
1	H	H	Glc	H	Glc
2	H	OH	H	OH	Rut
3	H	OH	H	OH	Glc
4	H	H	H	OH	Rut
5	H	H	H	OH	Glc
6	H	OH	H	OMe	Rut
7	H	OH	H	OMe	Glc
8	Glc	H	H	H	H
9	H	H	H	H	Rut
10	H	H	H	OMe	Rut
11	H	H	H	H	Glc
12	H	H	H	OMe	Glc
13	H	H	H	H	(Ac)Glc
14	H	OMe	H	OMe	(Ac)Glc
15	H	H	H	OMe	(Ac)Glc

Fig. 1. Structures of flavonols **1–15**.

eton and an anomeric sugar carbon. Both the ^{13}C–^{1}H heteronuclear shift correlation (HSC) and the SEFT spectra showed that six of the carbons of the C_{15} skeleton were each connected to one hydrogen atom only. The downfield part of the ^{1}H NMR spectrum of **13** showed a 4H AA′XX′ system at δ 8.11 (*d*: H-2′, H-6′, J = 8.9 Hz) and δ 6.94 (*d*: H-3′, H-5′) and two *meta*-coupled doublets at δ 6.47 and δ 6.28 (*d*: H-8, H-6, J = 1.8 Hz). This NMR pattern together with UV-visible spectroscopical data and the observation of a fragment ion of 287 amu in the MS spectrum (Table 2) showed the aglycone of **13** to be 3,5,7,4′-tetrahydroxyflavone (= kaempferol). Based on the chemical shifts obtained by the HSC spectrum (Tables 1 and 3) and the ^{1}H NMR coupling pattern the sugar part of **13** was identified as a glucopyranosyl moiety. The coupling constant of the anomeric signal (J = 7.2 Hz) showed a β-configuration. In the mass spectrum the difference between the molecular ion and the ion of the aglycone was 204 amu, indicating an acetylated hexose unit. In the SEFT spectrum, the most downfield resonance was assigned to a carbonyl function because of its quaternary nature (lacking in the HSC spectrum) and downfield shift value. Together with the observation of a 3H singlet at δ 1.92 in the ^{1}H NMR spectrum, the acyl part was thus identified to be acetic acid.

Shift effects investigated by UV (Table 2) gave evidence for the linkage between the aglycone and the sugar in the

Table 1. ^{1}H NMR data on flavonols from Norway spruce in CD_3OD

	Chemical shifts (ppm)*†														
^{1}H	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
6	6.31 *d*	6.31 *d*	6.30 *d*	6.28 *d*	6.27 *d*	6.28 *d*	6.27 *d*	6.26 *d*	6.27	6.28 *d*	6.27 *d*	6.28	6.28 *d*	6.24 *d*	6.26 *d*
8	6.51 *d*	6.49 *d*	6.48 *d*	6.47 *d*	6.45 *d*	6.47 *d*	6.45 *d*	6.45 *d*	6.45	6.48 *d*	6.45 *d*	6.48 *d*	6.47 *d*	6.44 *d*	6.45 *d*
2′	8.23 *d*	7.33 *s*	7.39 *s*	7.76 *d*	7.80 *d*	7.66 *d*	7.62 *d*	8.17 *d*	8.15 *d*	8.04 *d*	7.62 *d*	8.15 *d*	8.11 *d*	7.61 *s*	7.98 *d*
3′	7.30 *d*	—	—	—	—	—	—	6.97 *d*	6.98 *d*	—	—	6.98 *d*	6.94 *d*	—	—
5′	7.30 *d*	—	—	6.96 *d*	6.96 *d*	—	—	6.97 *d*	6.98 *d*	7.00 *d*	—	6.98 *d*	6.94 *d*	—	6.98 *d*
6′	8.23 *d*	7.33 *s*	7.39 *s*	7.72 *dd*	7.66 *d*	7.39 *d*	7.38 *d*	8.17 *d*	8.15 *d*	7.72 *d*	7.38 *d*	8.15 *d*	8.11 *d*	7.61 *s*	7.69 *dd*
1″	5.38 *d*	5.18 *s*	5.35 *s*	5.18 *d*	5.34 *d*	5.30 *d*	5.47 *d*	5.20 *d*	5.20 *d*	5.32 *d*	5.47 *d*	5.34 *d*	5.24 *d*	5.31 *d*	5.29 *d*
6A″		3.90 *dd*	3.82 *dd*	3.90 *d*	3.81 *d*	3.94 *dd*	3.84 *dd*		3.90 *d*	3.91 *d*	3.84 *dd*	3.78 *dd*	4.26 *dd*		
6B″		3.48 *dd*	3.71 *dd*		3.67 *d*		3.67 *d*	3.71 *dd*	3.62 *dd*		3.67 *dd*	3.62 *dd*	4.15 *dd*		
1‴	5.13 *d*	4.61 *d*	—	4.61 *d*	—	4.63 *d*		—	4.60 *d*	4.62 *d*	—	—	—	—	—
6‴		1.21 *d*	—	1.22 *d*	—	1.20 *d*		—	1.22 *d*	1.19 *d*	—	—	—	—	—
CH_3	—	—	—	—	—	—	—	—	—	—	—	—	1.92 *s*	1.91 *s*	1.91 *s*
OCH_3	—	—	—	—	—	4.03 *s*	4.02 *s*	—	—	4.04 *s*	—	4.02 *s*	—	4.03 *s*	4.04 *s*

* For the coupling constants of the sugar units and the chemical shifts of the sugar protons, see compound **2** in the Experimental section.
† The H-6/H-8 couplings and the *ortho*- and *meta*-couplings measured for the protons on the flavonol B-rings are in the range 1.7–2.1, 8.4–8.9, and 1.9–2.2 Hz, respectively.

Table 2. Spot colours, spectroscopic and chromatographic data on flavonols isolated from Norway spruce

	MS (*m/z*)		UV (nm) with chemical shift reagents														TLC			
	$M+H^+$	$A+H^+$	MeOH		NaOMe		$AlCl_3$		$AlCl_3$/HCl		NaOAc		NaOAc/H_3BO_3		R_f (×100)		Spot colours‡ under UV			
Compound			II	I	II	I	New	II	I	II	I	II	I	II	I	A	B	UV	NH_3	NA
1	611	287	266	342*	272	367	—	244	363	238	359	272	368*	265	345*	59	26	D	D	—
2	627	319	259	361	269	392†	—	271	424	272	407	272	388	258	385	46	15	D	Br	O
3	481	319	257	363	269	398†	—	270	435	272	406	272	383	258	386	24	27	D	Br	O
4	611	303	257	359	271	410	329	273	435	268	401	272	389	261	379	54	25	D	Br	O/Y
5	465	303	258	365	271	410	329	267	435	270	405	272	388	260	379	34	44	D	Br	O/Y
6	641	333	253	362	265	427	321	268	435	270	403	265	421	—	386	45	25	D	Br	O/G
7	495	333	254	361	271	419	327	272	442	270	403	273	389	260	383	24	33	D	Br	O
8	449	287	265	349	273	400	325	272	398	273	398	273	387	266	354	42	57	D	Br	G
9	595	287	265	350	273	400	325	272	396	273	395	272	375	265	352	65	40	D	O	G
10	—		254	355	270	415	330	267	400	267	400	273	386	254	358	63	31	D	G/Y	G
11	449	287	266	350	273	401	325	273	396	273	395	271	371	264	351	41	62	D	O/D	G
12	479	317	253	355	270	415	328	267	401	267	400	273	385	256	358	36	54	D	G/Y	G
13	491	287	265	349	273	401	326	273	396	273	393	272	372	266	351	51	78	D	D	G
14	551	347	253	358	267	425	327	270	405	272	403	274	380	252	361	37	64	D	G/Y	G/Y
15	521	317	254	355	271	413	327	267	401	268	398	273	381	254	357	42	72	D	Br	G

* Long shoulder.

† Decreasing intensity, I–H: UV absorption maxima bands.

‡ Colours: D, dark; Br, brown; O, orange; G, green; Y, yellow.

Table 3. ^{13}C NMR data on flavonols from Norway spruce in CD_3OD

	Chemical shifts (ppm)									
	1	**2**	**3**	**5**	**8**	**9**	**10**	**11***	**12**	**13**
2	x	158.8[a]	x[a]	158.70[a]	158.64[a]	x[a]	158.91[a]	156.37	x[a]	158.57[a]
3	136	x	x	135.90	135.53	135.78	135.70	133.30	135.57	135.28
4	200.26	x	179.75	179.71	179.46	179.3	179.50	177.60	179.58	179.27
5	x	163.31	163.36	163.26	163.1	161.83[a]	x	161.36	161.9	163.00
6	x	100.23	100.19	100.20	100.46	x	100.61	98.84	100.61	100.27
7	x	166.38	166.44	166.38	167.5	167.0	163.28	164.33	x	167
8	x	95.10	x	95.04	95.13	x	95.43	93.78	95.25	95.04
9	158.91[a]	159.80[a]	159.31[a]	159.25[a]	158.80[a]	158.9[a]	159.03[a]	156.53[a]	158.78[a]	159.25[a]
10	106.18	105.91	105.93	105.90	105.2	x	105.67	104.12	105.75	105.28
1′	126.00	122.5	122.21	123.48	122.70	123.08	123.28	121.04	124.72	122.72
2′	132.33	110.63	110.25	116.26	132.34	132.64	114.78	131.03	114.63	132.26
3′	117.43	146.65	146.74	150.12	116.10	116.44	151.19	115.23	151.17	115.98
4′	x	136.2	138.36	146.15	161.83	x	148.62	160.06	148.72	161.60
5′	117.43	146.65	146.74	117.85	116.10	116.44	116.40	115.23	116.30	115.98
6′	132.33	110.63	110.25	123.33	132.34	132.64	124.25	131.03	124.09	132.26
1″	100.25	105.12	104.73	104.64	105.13	105.03	104.78	100.97	103.98	104.38
2″	76.00	75.97	76.00	76.01	73.01	76.06	76.21	74.34	76.22	75.67[b]
3″	78.31	78.51	78.49[b]	78.38[b]	75.04	78.46	78.48	76.52	78.39[b]	77.88
4″	71.61	71.65	71.35	71.47	70.00	71.73	71.90	69.99	71.78	71.30
5″	x	77.55	78.69[b]	78.63[b]	77.10	77.50	77.66	77.63	78.86[b]	75.50[b]
6″	62.89	67.3	62.72	62.82	61.94	68.87	68.80	60.94	62.83	64.27
1‴	x	102.74	—	—		102.73	102.83	—	—	—
2‴	75.14	72.43[b]	—	—		72.39[b]	72.38[b]	—	—	—
3‴	78.61	72.47[b]	—	—		72.58[b]	72.54[b]	—	—	—
4‴	72.15	74.25	—	—		74.19	74.12	—	—	—
5‴	x	70.02	—	—		70.03	70.09	—	—	—
6‴	67.01	18.16	—	—		18.22	18.19	—	—	—
COOH	—	—	—	—			—	—	—	172.51
CH_3			—	—			—	—	—	20.51
OCH_3	—	—	—	—		—	57.02	—	57.05	—

*d-DMSO.

x, signal is missing; [a,b]signals with the same superscript may be interchanged; signals given with one decimal place are weak.

3-position [10]. These UV spectra were almost identical to those obtained for **11**, which confirmed the connection site. The acetyl group was determined to be situated in the 6″ position on the sugar, based on the NMR spectra where the chemical shifts of H-6A″, H-6B″ and C-6″ were shifted 0.5, 0.6 and 3.33 ppm downfield, respectively, and that for C-5″ was shifted 2.13 ppm upfield, compared to the corresponding signals of **11** (Tables 1 and 3). Hence, **13** was determined to be kaempferol 3-*O*-(6″-acetyl)-*β*-glucopyranoside. A similar pigment has previously been isolated from *Senecio aureus* (Compositae) although the binding site of the acetyl group was not determined in that case [11].

The aromatic part of the ^1H spectrum of **14** consisted of only three resonances with the relative areas 2:1:1. The shift values of the two single protons together with their typically *meta*-coupling ($J = 1.9$ Hz), identified them as H-8 and H-6. The upfield singlet at δ 7.61 consisted of two protons consistent with a B-ring possessing oxygen functions in the 3′,4′ and 5′-positions. A singlet at δ 4.03 integrated as six protons and corresponded to two isochronous methoxy groups. Addition of $AlCl_3$ for UV measurement did not result in the typical shift found when the B-ring bears two *o*-hydroxyls. The mass of the aglycone was found to be 347 amu (Table 2), and all these data were consistent with the flavonol aglycone myricetin 3′,5′-dimethyl ether (syringetin). The proton shift of H-2′,6′ was 0.2–0.3 ppm downfield from the analogous signal of myricetin derivatives (Table 3). The mass and ^1H spectra of the glycoside moiety of **14** were similar to those of **13**. The UV spectra of **13** and **14** were different due to the different substitution pattern on the B-ring. Otherwise, the spectral behaviour upon addition of shift reagents was similar for **13** and **14**. Thus, **14** was determined to be the novel compound syringetin 3-*O*-(6″-acetyl)-*β*-glucopyranoside.

The aromatic part of the ^1H NMR spectrum of **15** showed a 3H ABX system at δ 7.98 (*d*: H-2′, $J = 2.1$ Hz), δ 7.69 (*dd*: H-6′, $J = 8.5$, 2.1 Hz), and δ 6.98 (*d*: H-5′, $J = 8.5$ Hz), and a 2H AB system at δ 6.45 and 6.26 (*d*: H-8, H-6, $J = 2.0$ Hz), which together with the singlet resonance at δ 4.04 (3H) and the UV data confirmed the aglycone to be isorhamnetin. The spectral and chromatographic analysis of the glycosidic moiety of **15** was con-

sistent with those observed for **13** and **14**. Thus, **15** was found to be isorhamnetin 3-*O*-(6″-acetyl)-β-glucopyranoside. This compound has previously been found in *Pinus contorta* and *P. sylvestris* [12–13].

The aglycone part of the ^{1}H NMR spectrum of **7** (Table 3) revealed two AB coupling systems; *d*: H-8, H-6, J = 2.1 Hz and *d*: H-2′, H-6′, J = 2.0 Hz. A singlet resonance at δ4.03 integrated for three protons confirmed the presence of an aromatic methoxyl group. The splitting pattern of the B-ring proton signals showed asymmetry in this part of the molecule. The location of the methoxyl group in the 3′ rather than at the 4′ position was determined by the bathochromic shift of Band I (81 nm) in the UV spectrum after addition of $AlCl_3$ to **7** (Table 2). The mass, 333 amu (Table 2), of the aglycone moiety of **7** was in accordance with that of myricetin methyl ether (laricitrin). Compound **7** showed upon MS-analysis, both an ionic fragment of 180 amu and an ion due to a loss of 162 amu from the molecular ion [M + H], indicating a [$C_6H_{12}O_6$–H_2O] moiety [7]. This hexose was determined to be a glucopyranosyl unit bound to the 3 position of the aglycone by comparison of proton shift NMR values, coupling constants and UV data with those for **11** (Tables 2 and 3). Thus, compound **7** was found to be laricitrin 3-*O*-β-glucopyranoside, which has previously been detected in the genus *Larix* [15].

Compound **6** was shown to be laricitrin 3-*O*-(6″-α-rhamnopyranosyl)-β-glucopyranoside. The spectral data (^{1}H NMR, MS and UV) of the aglycone parts of **6** and **7** were quite similar (Tables 1 and 2). However, the molecular ion of **6** (641 amu), and the ^{1}H NMR spectrum (Table 1) showed the glycosidic part to be rutinose. Laricitrin 3-*O*-(6″-α-rhamnopyranosyl)-β-glucopyranoside has previously only been detected in *Larix gmelinii* [15].

Compounds **3**, **5**, **11** and **12** were determined to be the 3-*O*-β-glucopyranosides of myricetin, quercetin, kaempferol and isorhamnetin respectively. Their identities were based on spectral data (Tables 1–3): the molecular ions from the mass spectra, the coupling patterns of the B-ring protons from the ^{1}H NMR spectra, and the ^{13}C NMR data obtained by the SEFT and HSC experiments. The combination of the HSC, ^{1}H and SEFT experiments were used to assign the sugar signals, and the β configurations were based on observations of large (J = 7–8 Hz) anomeric ^{1}H–^{1}H coupling constants. The heteronuclear shift correlation by long-range coupling (COLOC) (Fig. 2) together with the HSC experiments were used to assign the carbon atoms of the aglycone. After irradiation of the H-2′,6′ signal in the ^{1}H NMR spectrum of **11**, a nuclear Overhauser effect (NOE) was observed at H-1″. Together with the shift correlation between H-1″ and C-3 in the COLOC spectrum of **11**, the linkage between the aglycone and the sugar moiety was assigned to the 3-position. Compound **3**, myricetin 3-glucoside, has not previously been reported to occur in Norway spruce.

Compounds **2**, **4**, **9** and **10** have nearly identical spectral properties to the four flavonol 3-glucosides **3**, **5**, **11** and **12** respectively (Tables 1–3). Their molecular ions in the mass spectra are 146 amu higher than the corresponding molecular ions of **3**, **5**, **11** and **12** as expected from the presence of an extra rhamnosyl group. The identity of the rhamnosyl group was confirmed by NMR data (Tables 1 and 3). Thus, the four flavonols are identified as the 3-*O*-(6″-*O*-α-rhamnopyranosyl)-β-glucopyranosides of myricetin (**2**), quercetin (**4**), kaempferol (**9**) and isorhamnetin (**10**). None of these flavonol 3-rutinosides has previously been found in Norway spruce.

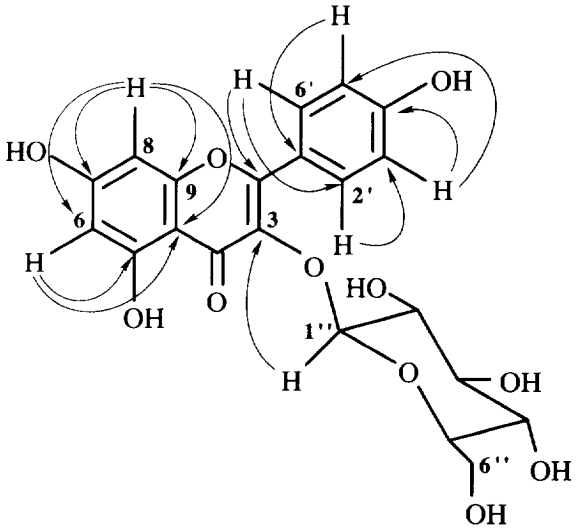

Fig. 2. Observed long-range couplings in the COLOC NMR experiment of **11**.

Based on spectral data (Tables 1–3), the 3,4′-*O*-β-diglucopyranoside and 7-*O*-β-glucopyranoside of kaempferol (**1**, **8**) were also identified in the needles.

Even though no acylated flavonoids have been observed in this species previously, Strack *et al.* have inferred the possible occurrence of an acylated kaempferol 3-glucoside derivative in Norway spruce as an element related to the rapid decrease of the kaempferol 3-*O*-glucoside content during the period May to July [6]. The present finding of the three acetylated flavonols (**13**–**15**) in newly burst shoots may, indeed, indicate metabolic turnover related to the flavonol content in the needles. In this context the finding of the five flavonol rutinosides (**2**, **4**, **6**, **9**, **10**) is also unusual, since no flavonol rutinoside has previously been detected in Norway spruce.

Only one of the compounds earlier reported from Norway spruce needles, isorhamnetin 3-glucoside (**12**), has a methylated flavonol structure. The presence of this compound is now confirmed and the occurrence of the 3-rutinoside (**10**) and the 3-(acetyl) glucoside (**15**) of isorhamnetin are recorded. While syringetin and laricitrin glycosides are rare, some derivatives have been reported to occur in *Larix*, *Cedrus* and *Pinus*. The suggestion that such methylated myricetin derivatives are much more common than until now supposed [9], is supported by our finding of laricitrin 3-rutinoside (**6**) and 3-glucoside (**7**) and of syringetin 3-(Acetyl) glucoside (**14**). This paper reports 10 flavonols (**2**, **3**, **4**, **6**, **7**, **9**, **10**, **13**, **14** and **15**)

which have not been detected in Norway spruce previously. Five of these—**6**, **7**, **13**, **14** and **15**—are either novel or very rare in nature.

EXPERIMENTAL

Plant material. Newly open buds from *Picea abies* were collected at NISK (Fana, Norway) on the 16 June 1994. The shoots were collected at different sites on one tree (Provenance Ukraine, Clone number 5466). The material was immediately frozen to $-10°$ in plastic bags. Extraction with aq. MeOH (80%), purification and isolation by *n*-hexane, ion exchange chromatography and gel filtration with Sephadex LH-20 are according to published procedures [7]. Details regarding the use of centrifugal partition chromatography and preparative HPLC will be published separately.

NMR spectroscopy. The ^1H and ^{13}C NMR spectra were obtained at 400.13 and 100.61 MHz, respectively, on a Bruker AM-400 instrument. The spectra were recorded at 20° using the 5 mm $^1H/^{13}C$ dual probe. The ^{13}C and the residual ^1H signals of the solvents (CD_3OD or DMSO-d_6) were used as secondary references (δ 49.3 and δ 3.4 from TMS in CD_3OD, and δ 39.6 and δ 2.62 from TMS in DMSO-d_6, respectively). The NOEs were measured at 60° by subjecting each line of a multiplet to a 0.5 sec burst of weak irradiation (*ca* 10 Hz). Subtraction of the unperturbed FID (with off-resonance irradiation) from the perturbed FID followed by Fourier transformation and integration, yielded the difference NOEs. The 1D spin echo Fourier transform (SEFT) experiments were performed using the gated decoupler method and optimized for a 1D ^1H–^{13}C coupling constant of 134 Hz. For the direct ^1H–^{13}C chemical shift correlations (HSC) sweep widths of 2401 Hz and 9056 Hz were used for ^1H and ^{13}C, respectively, with 40 t_1 increments. In the long-range heteronuclear shift correlation (COLOC) expts, sweepwidths of 2401 Hz and 18111 Hz were used for ^1H and ^{13}C, respectively, with 96 t_1 increments. The heteronuclear expts were collected with 4K complex data points and optimized for ^1H–^{13}C coupling constant of 143 Hz (HSC) and 5 Hz (COLOC), respectively. Their spectra were processed using a sinebell function shifted 90° and dropped to zero at the last data point in both dimensions.

Mass spectroscopy. The mass spectra were obtained on a Finnigan MAT TSQ 700 triple-stage quadropole mass spectrometer. In the mode of TSP-loop-MS the instrument was equipped with a Finnigan-MAT TSP 2 interface. 0.5 M aq. NH_4OAc was added post-column (0.2 ml min^{-1}) to induce ionization. Ion source temperature 280°, vaporizer 100°, aerosol 320°, filament off and positive mode. Spectra were recorded in the region 150–900 amu. In the mode of D/CI-MS NH_3 was added to induce ionization. Spectra were recorded in the region 100–800 amu.

UV spectroscopy. A Varian CARY 3 UV-visible spectrophotometer was used, and spectra were measured using 10 mm quartz-cuvettes. Shift reagents were prepared and used according to Markham [10].

TLC chromatography. Analytical TLC was carried out on microcrystalline cellulose (F1440/LC254, Schleicher & Schuell) with the solvents 15% HOAc (A) and BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, upper phase) (B). Shift reagents: NH_3(g) and a 1:1 mixture of 2% diphenyl-boric acid–ethanolamine complex in EtOH (Naturstoffreagenz A) and 10% polyethylenglycol 4000 in MeOH (NA).

^1H–^1H coupling constants for the sugars. The following values are given for **2** in CD_3OD: δ 5.18 (*d*: H-1″, J(1″-2″) = 7.8 Hz), 4.61 (*d*: H-1‴, J(1‴-2‴) = 1.6 Hz), 3.90 (*dd*: H-6A″, J(6A″-6B″) = 9.7 Hz), 3.72 (*dd*: H-2‴, J(2‴-3‴) = 3.4 Hz), 3.64 (*dd*: H-3‴, J(3‴-4‴) = 9.5), 3.59 (*dd*: H-2″, J(2″-3″) = 7.9 Hz), 3.52 (*d*: H-5‴, J(5‴-6‴) = 6.3 Hz), 3.48 (*dd*: H-6B″), 3.35 (*d*: H-4‴), 1.21 (*d*: H-6‴).

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