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STRUCTURE OF A NEW DITERPENE ALCOHOL FROM *Pinus sibirica* BUDS

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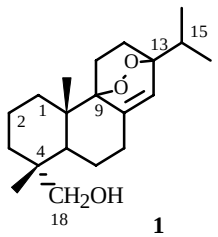
*The new diterpene alcohol (**1**) in addition to the known metabolites isoagatholal (**2**) and β-sitosterol glucoside (as the tetraacetate) (**3**) were isolated from buds of Siberian pine. The structure of the new compound was established as abieto-8(14)-en-18-ol 9α,13α-endoperoxide.*

Key words: *Pinus sibirica,* diterpenoids, abietane, labdane, sterols.

We previously reported the isolation of low-molecular-weight phenolic compounds from buds of Siberian pine *Pinus sibirica* Du Tour [1]. In continuation of these investigations, a study of neutral terpenoids from the $CHCl_3$-soluble portion of the extract was begun.

The $CHCl_3$-soluble portion of the ethanol extract of Siberian pine buds, which was obtained in 11% yield of the absolutely dry substance, contained 25.1% neutral substances, 10.5% strong, and 64.3% weak acids. The acids were isolated by successive treatment of the $CHCl_3$ extract with saturated aqueous $NaHCO_3$ and aqueous NaOH (1%) solutions. The neutral components were repeatedly chromatographed over SiO_2 using solvents with increasing polarity. This isolated a new diterpene alcohol of the abietane series (**1**) and an unknown compound, isoagatholal (**2**), a labdane diterpenoid. The polar fraction was acetylated under mild conditions. The tetraacetate of *β*-sitosterol glucoside (**3**) was isolated from the reaction products. The structure of the new compound was established and the known metabolites were identified using spectral methods, including 2D NMR spectroscopy and mass spectrometry.

Compound **1** contained 20 C atoms according to ^{13}C NMR spectral data (using the APT method). Of these, four were methyls; eight, methylenes; three, methines; and five, C atoms with no bound protons (Table 1). Of the methyls, two were quaternary [δ 17.53 and 17.20 ppm and 1.14 and 0.19 ppm (3H each, s) in the ^{13}C and ^1H NMR, respectively] and two were tertiary within an isopropyl group [δ 18.14 and 18.68 ppm and 0.972 and 0.966 ppm (3H each, d, J = 6.9 Hz) in the corresponding NMR spectra]. The isopropyl proton signal (δ 1.85 ppm, sextet, J = 7.0 Hz) was found from a cross-peak of this signal with those of the corresponding methyls in the 2D ^1H—^1H COSY homocorrelation spectrum.



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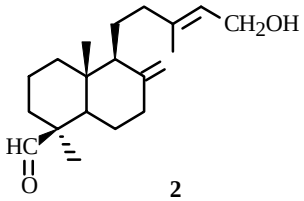

2

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TABLE 1. ^{13}C NMR Chemical Shifts of **1**

C atom	C type	δ, ppm	C atom	C type	δ, ppm
1	CH_2	34.18	11	CH_2	18.72
2	CH_2	17.68	12	CH_2	25.33
3	CH_2	35.75	13	C	79.75
4	C	38.85	14	CH	126.69
5	CH	38.45	15	CH	32.27
6	CH_2	23.49	16	CH_3	18.14
7	CH_2	27.35	17	CH_3	18.68
8	C	144.27	18	CH_2OH	71.73
9	C	82.33	19	CH_3	17.53
10	C	38.74	20	CH_3	17.20

Two signals at weak field in the ^{13}C NMR spectrum were assigned to C atoms of a trisubstituted double bond [δ 144.27 (C) and 126.70 (CH) ppm]. The olefinic proton of this double bond appeared as a triplet with J = 2.3 Hz at δ 6.12 ppm in the PMR spectrum.

Two of the three C atoms bound to O in **1** are quaternary (δ 82.33 and 79.75 ppm) whereas one belongs to the primary hydroxyl (δ 71.73 ppm). Signals for the protons of this group appear in the PMR spectrum as two 1H doublets in an AB system (J = 11.0 Hz) at δ 3.37 and 3.10 ppm.

The molecular weight of **1** (M^+ 320) agrees with the ^{13}C and ^1H NMR spectra and corresponds to the empirical formula $C_{20}H_{32}O_3$. In addition to a peak for the molecular ion in the mass spectrum, a characteristic fragment ion for [M - 32] (*m*/*z* 288), corresponding to loss of molecular oxygen, is also present at high mass numbers. These data and the presence in the ^{13}C NMR spectrum of two quaternary C atoms at weak field together indicate that the molecule contains an endoperoxide group. The location and relative configuration of this group were established by comparison of the spectral data with that in the literature.

Two diastereomeric diterpene acids that were identified as abieto-8(14)-en-18-oic acid 9α,13α-endoperoxide and its (9β,13β)-isomer were isolated previously from *Elodea Canadensis* [2]. Other researchers isolated one isomer of this pair, abieto-8(14)-en-18-oic acid 9α,13α-endoperoxide (as the methyl ester), from deconed shoots of Siberian pine [3]. Using spectral data presented in these studies and comparing them with data for other types of diterpenoids [4], we proposed that **1** has the abietane C skeleton with an endoperoxide group on C-9 and C-13 and a primary alcohol on C-4. By comparing the ^{13}C chemical shifts of the two isomers with the configuration of the endoperoxide, it was possible to establish the relative stereochemistry of this group in the compound isolated by us as 9α,13α.

The stereochemistry of ring A was determined using the chemical shifts for C atoms of this ring. The literature data showed that the signal of an equatorial hydroxymethyl (on C-4) is located at weaker field than that of an axial one. A methyl in a position geminal to any of the hydroxymethyls is shielded. The signal of an axial methyl lies at stronger field than that for an equatorial one. The location of the promary alcohol in **1** was determined using the chemical shifts of the hydroxymethyl δ 71.73 ppm), the quaternary methyl (C-19, δ 17.53 ppm), and C-5 (δ 38.45 ppm), which is sensitive to a change of configuration at C-4. It was equatorial, i.e., 18-CH_2OH.

Thus, the structure of **1** was established as abieto-8(14)-en-18-ol 9α,13α-endoperoxide. Compounds **2** and **3** were isolated from Siberian pine buds for the first time.

EXPERIMENTAL

Melting points were determined on a Kofler block; rotation angles, on a Polamat A polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 instrument in $CDCl_3$ at 26°C with HMDS internal standard. Mass spectra (EI) were measured in an Agilent 5973N GC-MS.

Silica gel (Lachema L 40/100) was used for column chromatography; L 5/40, for TLC and flash chromatography. Compounds were developed on chromatograms by spraying plates with vanillin solution (0.5%) in ortho-phosphoric acid (50%) with subsequent heating to 105°C.

Siberian pine buds (290 g calculated for absolutely dry substance) were collected in May 2002 near the village of Glubokaya in Slyudyan Region of Irkutsk District and were preserved in ethanol at the collection site. The ethanol extract was evaporated. The solid was treated successively with $CHCl_3$ and C_4H_9OH [1]. The $CHCl_3$ extracts (31.6 g) were dissolved in diethylether and treated successively with saturated $NaHCO_3$ solution and NaOH solution (1%). Neutral components (5.73 g) were worked up by flash chromatography over SiO_2 using petroleum ether (PE):diethylether (DE) (5% → 100% diethylether), acetone, and methanol. Elution by PE:DE (6:4) produced a fraction rich in diterpenoids that was then rechromatographed over SiO_2 using hexane:acetone with the fraction of the latter increasing to 20%. Compound **1** (10 mg) was isolated using 4% acetone; **2** (6.8 mg), 5%.

Compound **1** [abieto-8(14)-en-18-ol 9α,13α-endoperoxide], amorph., $[\alpha]_{546}^{23}$ -59.8° (*c* 0.4, $CHCl_3$). Mass spectrum (*m*/*z*): 320 $[M]^+$, 288 $[M - 32]^+$.

Table 1 lists the ^{13}C NMR spectrum.

Compound **2** (isoagatholal), oil, $[\alpha]_{546}^{25}$ +24° (*c* 0.1, $CDCl_3$) (lit. $[\alpha]_D^{20}$ +19.3° [5]).

PMR spectrum (δ, ppm, J/Hz): 0.56 (s, 3H, 20-CH_3), 1.01 (s, 3H, 18-CH_3), 1.66 (s, 3H, 14-CH_3), 4.14 (d, 2H, J = 6.9, 17-CH_2OH), 4.55 and 4.88 (both s, 1H each, 15-H_2), 5.37 (t, 1H, J = 6.8, 13-H), 9.73 (d, 1H, J = 1.4, 19-H).

The ^{13}C NMR spectrum agrees with that published [4].

Neutral components (640 mg) that were soluble in MeOH were dissolved in pyridine (4 mL) and treated with acetic anhydride (2 mL). The reaction was carried out at room temperature for 1 d. After the usual workup, the reaction products were chromatographed over SiO_2 using hexane:acetone (2% → 25% acetone) to afford a fraction of sterol glycoside acetates. Crystals formed in this fraction. They were recrystallized to afford **3** (4 mg).

Compound **3** (β-sitosterol glucoside tetraacetate), white crystals, mp 160-162°C (hexane:acetone) {lit. mp 168-169°C (MeOH) [6]}.

The ^{13}C NMR spectrum agrees with that published [6].

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