NEW FLAVONOL GLYCOSIDES FROM THE NEEDLES OF Larix sibirica

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Flavonoids methylated in ring B, which are widely distributed in the vegetable kingdom, are found extremely rarely in the Pinaceae family: only 3'-methyldihydroquercetin in the bark of the fir [1] and an isorhamnetin glycoside in the needles of the larch [2, 3].

From the needles of the Siberian larch we have isolated two new compounds of glycosidic nature the aglycones of which are 3'-O-methylmyricetin (I) and 3',5'-di-O-methylmyricetin (II). This is the first time that the 3'-methyl ether of myricetin has been isolated, and we have called it laricitrin. Concerning 3',5'-di-O-methylmyricetin (syringetin) it is known that it is present in plants of the genus Lathyrus, but there is no information on its isolation and properties [4].

Both glycosides were obtained from a methanolic extract of the neeldes of Larix sibirica by preparative chromatography on a polyamide sorbent using aqueous and nonaqueous eluting systems.

Characteristic bathochromic shifts of the absorption maxima in the UV spectra with ionizing and complex-forming additives showed the presence of free hydroxy groups in positions 7, 5, 5', and 4' in compound (I) and 7, 5, 4' in compound (II). The PMR spectra of both compounds clearly show the presence of protons in positions 6 and 8 of ring A (Table 1). According to PMR spectroscopy, compound (I) contains one methoxy group (singlet at 3.82 ppm, 3H) and (II) contains two (singlet at 3.82 ppm, 6H). The splittings of the signals of the protons of the ring B in these compounds are different. Thus, in compound (I) the H-2' and H-6' protons are represented by doublets (6.98 ppm and 7.41 ppm) with a constant of meta interaction J = 2 Hz, which shows the unsymmetrical substitution of ring B with the methoxy group most probably in position 3'. Correspondingly, in compound (II) the H-2' and H-6' protons are split by the singlet at 7.10 ppm, which is possible only if the two methyl groups are in the 3',5' positions, preserving the symmetry of the myricetin ring B (see Table 1).

TABLE 1. PMR Spectra* of the TMS Ethers of Flavonoids from the Needles of Larix sibirica Ledb.

Compound	Ring A		Ring B		Anomeric protons		
	1H–6	1H–8	1H–2	1H–6'	OCH_3	1H'	1H''
Laricitrin 3-glucoside (I)	6,08 (d. 2)	6,41 (d. 2)	7,41 (d. 2)	6,98 (d. 2)	3,82 (s.)	5,83 (d.7)	—
Laricitrin	6,10 (d.2)	6,40 (d.2)	7,40 (d. 2)	6,92 (d.2)	3,82 (s.)	—	—
Syringetin 3-rutinoside (II)	6,08 (d.2)	6,41 (d.2)	—	7,10 (s.)	3,82 (s.)	5,88 (d.6)	4,21 (s.)
Syringetin	6,07 (d.2)	6,40 (d.2)	—	7,10 (s.)	3,82 (s.)	—	—

* The nature of the signals and the value of J (Hz), are shown in parentheses (s – singlet; d – doublet).

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The quantitative acid hydrolysis of the glycans gave a ratio of aglycone to carbohydrate residue of 1:1 for (I) and 1:2 for (II). The results of the gas–liquid chromatography of the carbohydrate substituents in the form of the acetates of the aldononitriles [5] showed the presence of glucose (compound I) and of glucose and rhamnose (compound II). The increase in the value of the bathochromic shift of band I in the UV spectra on the addition of $AlCl_3$ + HCl to the aglycone in comparison with the shift for the corresponding glycosides [for (I) about 27 nm and for (II) about 33 nm] showed the attachment of the carbohydrate residue in position 3. This is in harmony with the characteristic value of the chemical shift (CS) of the anomeric proton of the glucose: at 5.83 ppm in (I) and at 5.88 ppm in (II), and β-addition agrees with the value of J = 6-7 Hz for the doublet of the anomeric proton [6].

The results of a calculation of the molecular rotation [7] for compound (I) showed that the glucose substituent has the β-pyranose form. The biose obtained by the oxidation of compound (II) was split on acid hydrolysis into glucose and rhamnose, and its chromatographic behavior was identical with that of rutinose. The absorption of 4 moles of periodate on periodate oxidation shows the presence of a 1→6 bond between the glucose and the rhamnose, which is also confirmed by the results of a comparison of the CSs of the anomeric protons of the glucose and the rhamnose in the PMR spectrum of rutinose [6].

The demethylation of laricitrin and of syringetin led to the same final product – myricetin. Thus, the structure 4',5,5',7-tetrahydroxy-3'-methoxyflavonol 3-O-β-D-glucopyranoside (laricitrin 3-glucoside) has been established for compound (I) and 4',5,7-trihydroxy-3',5'-dimethoxyflavonol 3-O-[O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside] (syringetin 3-rutinoside) for compound (II).

The isolation of these compounds, and also of syringic acid [8] is somewhat unexpected for coniferous plants. Among the extractive phenols of the genus Larix known up to the present time there are methylated derivatives only of the guaiacyl type (lignans, ferulic acid and its esters, vanillin and vanillic acid, and isorhamnetin). The lignin of broad-leaved trees [9], like that of other genera of the Pinaceae family, likewise does not contain syringic structural units.

The appearance in larch needles of phenolic compounds with structural units of the type of 5-hydroxyferulic and sinapic acids may be connected with the presence in them of an O-methyltransferase possessing a meta-specific methylating activity. Such an enzyme has been isolated at the present time from pine seed shoots [10].

EXPERIMENTAL

The UV spectra were taken in a 1-cm cell in methanol on a SF-4A spectrophotometer (concentrations $3.0\text{-}5.0 \cdot 10^{-5}$ M), the IR spectra on a UR-10 spectrophotometer (tablets with KBr), and the PMR spectra of trimethylsilyl ethers of the flavonoids on a BS 487B radiospectrometer (CCl_4, with HMDS as internal standard). The chemical shifts were determined (ppm) on the δ scale, the melting points on a Kofler block, and the angles of rotation on a "Carl Zeiss Jena" polarimeter. In all cases, the elementary compositions corresponded to the calculated figures.

Treatment of the Extract. Freshly collected larch needles (moisture content 46%) (3.42 kg) were extracted with methanol by steeping at room temperature. After partial evaporation in vacuum at 60°C, chlorophyll precipitated. The decanted aqueous methanolic phase was treated successively with petroleum ether, diethyl ether, and butanol. The phenolic compounds were present only in the latter two fractions.

Column chromatography of the butanol fraction (260 g) on polyamide powder (780 g) with elution by water separated the carbohydrates, the glycosides of phenolic acids, and a mixture of a C-glycoside [11] and compound (II) (fraction 1). Further elution with water–methanol (7:3) isolated a mixture of flavonoid glycosides (fraction 2). The rechromatography on polyamide (70 g) of fraction 1 (1.75 g) with elution by water gave compound (II) (0.2623 g). Fraction 2 was separated on polyamide (800 g) in the chloroform–methanol (9:1) system. The 3-glucosides of kaempferol, isorhamnetin, and myricetin were isolated [3], and also a mixture of two compounds difficult to separate: quercetin 3-glucoside and compound (I). In order to isolate the (I) (0.4924 g), this mixture was chromatographed three times under similar conditions.

Compound (I) had mp 175-178°C (prisms) and 253-255°C (rhombic plates, aqueous methanol), $[\alpha]_D^{20}$ −48.0° (c 3.03; pyridine). UV spectrum: λ_{max} (methanol) 255, 360 nm (log ε 4.22, 4.25); (+ sodium acetate) 274, 380 nm; (+ sodium acetate + H_3BO_3) 260, 387 nm; (+ $AlCl_3$) 275, 445 nm; (+ $AlCl_3$ + HCl) 275, 403 nm. IR spectrum, cm^{-1}: 1523, 1573, 1612 (aromatic nucleus), 1663 (CO). Aglycone of (I) (laricitrin) – mp 318-320°C (aqueous methanol). λ_{max} 254, 376 nm (log ε 4.39; 4.49). IR spectrum, cm^{-1}: 1518, 1565, 1602 (aromatic nucleus), 1655 (CO); mol. wt. 332 (mass spectrometrically).

Compound (II) had mp 236-238°C (methanol), $[\alpha]_D^{20}$ −55.0° (c 2.44; pyridine). UV spectrum: λ_{max} (methanol) 255, 362 nm (log ε 4.27, 4.29); (+ sodium acetate) 272, 375 nm; (+ sodium acetate + H_3BO_3) 260, 362 nm; (+ $AlCl_3$) 272, 410 nm; (+ $AlCl_3$ + HCl) 277, 402 nm. IR spectrum, cm^{-1}: 1570, 1605 (aromatic nucleus), 1652 (CO). Aglycone of (II) (syringetin) – mp 283-285°C (aqueous methanol). λ_{max} 253, 376 nm (log ε 4.28, 4.33). IR spectrum, cm^{-1}: 1515, 1550, 1605 (aromatic nucleus), 1660 (CO). Mol. wt. 346 (mass spectrometry).

Acid Hydrolysis. A mixture of 0.01 g of the glycoside and 1 ml of methanol was heated with 2 ml of 10% HCl in the boiling water bath for 2 h. The amount of aglycone was found gravimetrically. The yield of aglycone from compound (I) was 61.2% (63% calculated for a monoside) and from (II) 44.66% (48% calculated for a bioside). The aqueous solution was neutralized on AV-17 anion-exchange resin, and evaporated, and the carbohydrate composition was analyzed.

Cleavage of the Biose. Compound (II) (0.01 g) was oxidized with 0.6 ml of 30% H_2O_2 in the presence of 0.1 N NH_4OH (1 ml) at room temperature for 4 h. After appropriate working up [12], the nature of the biose was determined by paper chromatography.

Periodate Oxidation [13]. Compound (II) (0.01 g) was oxidized with a 0.015 M solution of sodium periodate (10 ml) at room temperature for 4 h. After each 0.5 h, the absorption of the reaction mixture at 223 nm was measured in comparison with the absorption of standard solutions of (II) and of pure sodium periodate at corresponding concentrations. The oxidation of 0.01 g of (II) consumed $6.45 \cdot 10^{-5}$ mole of sodium periodate, which corresponds to the consumption of 4.2 moles of periodate per mole of bioside.

GLC Analysis of the Carbohydrates. The acetates of the aldononitrile derivatives of glucose in the case of compound (I) and of a mixture of glucose and rhamnose in the case of compound (II) were analyzed under conditions described in the literature [3].

Demethylation [14]. A mixture of 0.003 g of laricitrin (or 0.003 g of syringetin) and 0.006 g of pyridine hydrochloride was heated in a salt bath at 180°C for 2 h. Then 1.5 ml of 2 N HCl was added to the mixture and it was extracted with ether. The ethereal extract was chromatographed on paper [acetic acid–formic acid–water (10:2:3)] and on a layer of polyamide [chloroform–methanol (1:1)] in comparison with an authentic sample of myricetin.

CONCLUSIONS

The needles of Larix sibirica Ledb. have yielded two new flavonoid glycosides, for which the structures of 4',5,5',7-tetrahydroxy-3'-methoxyflavonol 3-O-β-D-glucopyranoside (laricitrin 3-glucoside) and 4',5,7-trihydroxy-3',5'-dimethoxyflavonol 3-O-[O-β-D-glucopyranosyl-(1→6)-α-L-rhamnoside] (syringetin 3-rutinoside) have been established.

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