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SYLPIN – A NEW C-METHYLATED FLAVONOID FROM *Pinus sylvestris*

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Among natural flavonoids, C-methylated derivatives form a fairly small group [1]. In the family Pinaceae they are characteristic for species of *Pinus* and are found specifically in the wood. From the wood of some species of *Pinus* have been isolated cryptostrobin (5,7-dihydroxy-6-methylflavanone), strobopinin (5,7-dihydroxy-8-methylflavanone), strobochrysen (5,7-dihydroxy-6-methylflavone), strobobanksin (3,5,7-trihydroxy-6-methylflavone), and from the bark pinoquercetin (6-methylquercetin) and pinomyricetin (6-methylmyricetin) [2-7].

The C-methyl group in these compounds is present in position 6 or 8. Similar methylation of the flavone molecule has been reported for eucalyptus compounds [8, 9].

From needles of a representative of the genus *Pinus* – Scotch pine (*Pinus sylvestris* L.) – we have isolated a new C-methylated flavonoid which we have called sylpin. This is the first case of the detection of C-methylated derivatives in conifer needles. Sylpin (I) was isolated from an ethereal solution of a methanolic extract of the needles by chromatography on polyamide in the chloroform–methanol (95 : 5) system.

The maxima in the UV spectrum (271 and 338 nm) permit sylpin to be assigned to flavones or flavonols with a substituted 3-OH group. According to elementary analysis and mass and NMR spectra, sylpin contains one C-methyl group, one methoxy group, and three hydroxy groups. The presence of three hydroxy groups was confirmed by the preparation of a triacetate. The nature of the substitution of the aromatic nucleus can be judged from the PMR spectrum (Fig. 1), which contains the signals of five aromatic protons: two two-proton doublets at 7.96 ppm and 6.96 ppm with J = 9 Hz (4′-substituted side ring) and a singlet at 6.52 ppm belonging either to H-3 or to one of the protons of the trisubstituted ring A (H-6, H-7, or H-8).

One of these three hydroxyls is in position 5, as is shown by the presence of a singlet at 13.0 ppm in the PMR spectrum. On the basis of the UV and mass spectra (ion C with m/e 121) it may be considered that another hydroxy group is in position 4′. The third hydroxy group may occupy position 6, 7, or 8. The last case is excluded by the negative gossypetone test. The location of an OH group in position 7 does not agree with the UV spectrum (sodium acetate does not lead to a bathochromic shift of the short-wave band). At the same time, the presence of a 6-OH group is shown by characteristic fragmentation in the mass spectrum: an intense $(M-1)^+$ ion is formed by the splitting off of the radical (in this case – H) from the 6-OR group [10]. Furthermore, the UV spectrum in the presence of $AlCl_3$/HCl is also characteristic for 5-hydroxyflavones in which position 6 has the hydroxyl substituent [11].

The remaining methyl and methoxy groups can be located in positions 3 and 8 or 7 and 8. The variant of 3- and 7-substitution is excluded by the Gibbs reaction, which shows that one of the groups is present in position 8.

We made an unambiguous choice of one of the two variants listed on the basis of analysis of the results of the demethylation of sylpin. The fact that demethylation forms flavonol (UV spectrum) corresponds to an initial position of the CH_3O group at C-3 and, consequently, of the methyl group at C-8.

Thus, sylpin has the structure of 4′,5,6-trihydroxy-3-methoxy-8-methylflavone (I). The triacetate (II) and the demethylation product – 3,4′,5,6-tetrahydroxy-8-methylflavone (III) – that we obtained have not been described in the literature, either.

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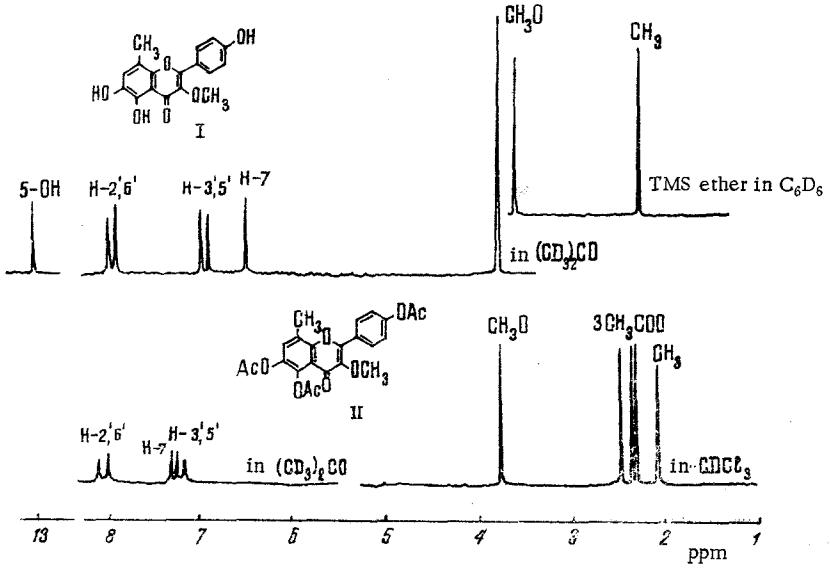

Fig. 1. PMR spectra of sylpin (I) and of its triacetate (II).

EXPERIMENTAL

The spectral characteristics were obtained on the following instruments: Hitachi EPS-3T (UV); Varian HA-100D at 100 MHz with tetramethylsilane as internal standard (PMR); Varian CH-8 at 70 eV (mass spectrum). The melting points were determined on a Kofler block. Elementary analyses were performed on a Hewlett-Packard 185B automatic CHN analyzer.

Isolation. The raw material consists of needles of the Scotch pine collected in July in the Irkutsk oblast. The needles (14.5 kg; moisture content 51%) were comminuted to 1.5-2 cm and were extracted three times with methanol in a ratio of 1 : 8. The combined aqueous methanolic extract was evaporated in a vacuum circulation evaporation at 50-60°C. The liquid phase obtained was decanted from the chlorophyll that had deposited (800 g) and traces of chlorophyll (50 g) were eliminated by treatment with petroleum ether. After this, the extract was treated with diethyl ether. The residue after the evaporation of the ether (32 g) was chromatographed on polyamide sorbent (300 g) in the chloroform–methanol system. At a composition of the mixture of 95 : 5, a compound of flavonoid nature was eluted from the column. After three recrystallizations from methanol, 50 mg of sylpin (I) was obtained.

4',5,6-Trihydroxy-3-methoxy-8-methylflavone (I). Yellow acicular crystals with mp 301-303°C, composition $C_{17}H_{14}O_6$, R_f (TLC, Silufol) 0.7 [benzene–acetone (1 : 1)] and 0.3 [chloroform–methanol (9 : 1)].

Substance (I) is insoluble in chloroform, readily soluble in acetone, and sparingly soluble in ethanol and methanol. With $FeCl_3$ it forms a green coloration, and it does not react with p-benzoquinone (gossypetone test) or with 2,6-dibromobenzoquinone chlorimide (the Gibbs reagent).

Maxima in the UV spectra (nm): MeOH 213, 271, 300, 338; NaOMe 223, 276, 328, 398; NaOAc 271, 300, 368; NaOAc + H_3BO_3 271, 300, 339; $AlCl_3$ 271, 307, 353; $AlCl_3$ + HCl 274, 306, 356.

Mass spectrum at 150°C [m/e (intensity, %)]: M^+ 314 (100), M – H 313 (75), 296 (17), 285 (19), 271 (45), A + H 167 (10), 138 (7), C 121 (18). PMR spectrum (see Fig. 1), ppm: 1) in $(CD_3)_2CO$: 13.0 (1H), 7.96 (2H), 696 (2H), 6.52 (1H), 3.84 (3H); 2) TMS ether in CCl_4: 7.96 (2H), 6.84 (2H), 6.38 (1H), 3.83 (3H), 2.0 (3H); 3) TMS ether in C_6D_6: 3.63 (3H), 2.37 (3H).

Acetylation of (I). A mixture of 5 mg of substance (I), 0.1 ml of pyridine, and 0.5 ml of acetic acid was heated at the boil for 1 h. On the addition of ice water, a precipitate deposited, and this was washed with water and was recrystallized three times from ethanol to give 6 mg of 4',5,6-triacetoxy-3-methoxy-8-methylflavone (II) in the form of a white crystalline compound with mp 209-211°C; composition $C_{23}H_{20}O_9$, R_f 0.3 [TLC; Silufol; benzene–acetone (9 : 2)].

Mass spectrum at 20°C: M^+ 440 (6%), 398 (61), 356 (68), 314 (74), 313 (100), 296 (37), 285 (23), 271 (25), 167 (11), 138 (9), 121 (25).

PMR spectrum (see Fig. 1), ppm: 1) in $CDCl_3$: 8.08 (2H), 7.4 (1H), 7.14 (2H), 3.8 (3H), 2.51 (3H), 2.38

(3H), 2.34 (3H), 2.1 (3H); 2) in $(CD_3)_2CO$: 8.14 (2H), 7.38 (1H), 7.28 (2H).

Demethylation of (I). A mixture of 8 mg of substance (I) and 20 mg of pyridine hydrochloride was heated at 185-190°C for 1.5 h. After cooling, the reaction mixture was treated with ether, the ethereal extract was evaporated, and the residue was chromatographed on polyamide. A flavonoid compound having the bright green fluorescence in UV light characteristic for flavonols was eluted in the chloroform–methanol (4:1) system. The 3,4',5,6-tetrahydroxy-8-methylflavone (III) formed yellow acicular crystals with mp 287-290°C (methanol); composition $C_{16}H_{12}O_6$, R_f 0.25 [TLC; Silufol; chloroform–methanol (9:1)].

Maxima of the UV spectra: MeOH 271, 300, 368, NaOMe 278, 325, 420; NaOAc 272, 300, 375; NaOAc + H_3BO_3 271, 300, 368; $AlCl_3$ 273, 301, 360, 430; $AlCl_3$ + HCl 272, 300, 360, 435.

Mass spectrum at 100°C: M^+ 300 (100%), M – H 299 (21), M – CO 272 (10), M – HCO 271 (12), A + H 167 (5), M^{2+} 150 (9). C 121 (22).

SUMMARY

The new C-methylated flavonoid sylpin has been isolated from the needles of the Scotch pine, and the structure of 4',5,6-trihydroxy-3-methoxy-8-methylflavone has been established for it. During the study of the structure of sylpin two new compounds – 4',5,6-triacetoxy-3-methoxy-8-methylflavone and 3,4',5,6-tetrahydroxy-8-methylflavone – were obtained.

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