GLYCOSIDES OF Fraxinus mandschurica AND F. potamophila

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Continuing a study of the plants mentioned, by chromatographing a methanolic extract on columns of polyamide and silica gel with elution of the former with water and with ethanol and of the latter with organic solvents we have isolated from this extract three substances of a glycosidic nature (I-III).

Glycoside I, $C_{15}H_{10}O_9$, mp 214-215°C (from methanol), $[\alpha]_D^{25} -95°$ (c 0.61; dioxane), R_f 0.47, mol. wt. 340. UV spectrum of (I): λ_{max} 228, 292, 348 nm (log ε 4.1, 3.77, 3.85) – the chromophore of a 6,7-dihydroxycoumarin. IR spectrum, λ_{max} (cm^{-1}): 1700-1710 (coumarin carbonyl), 1630, 1570, 1520 (aromatic nucleus), 3200-3550 (broad band of hydroxy groups). Bands at 880 and 835 cm^{-1} and also at 1040, 1055, 1080, 900, and 770 cm^{-1} show the presence in (I) of a β-glycosidic bond and the pyranose form of the sugar residue. This was confirmed by its enzymatic cleavage and by the calculation of the value of $[M]_D \cdot Kp$ according to Klyne [1].

In the NMR spectra of the glycoside there are the signals of the protons of a totally substituted coumarin nucleus: doublets at 6.10 and 7.53 ppm, J = 9.7 Hz (H-3, H-4), a singlet at 7.05 ppm (2H) – (H-5 and H-8), and also singlets at 4.1 and 4.05 ppm (3H each) (methoxy groups attached to an aromatic nucleus), a multiplet in the 3.9-4.3 ppm region (6H) (the protons of a hexose), a doublet at 5.50 ppm, J = 7Hz (1H) (β-anomeric proton of a sugar residue), and a multiplet at 6.3-6.7 ppm (5H) (the hydroxy groups of a sugar and the phenolic hydroxy group of a coumarin).

The acid hydrolysis of substance (I) in solution yielded D-glucose, detected by paper chromatography, and an aglycone $C_9H_6O_4$ with a yield of 49%, mp 270-271°C, R_f 0.82, M^+ 178.

The UV spectrum of the aglycone taken in an alkaline medium showed a shift of the long-wave maximum by 80 nm and a slight increase in extinction (Δ log ε 0.04), which is characteristic for 6,7-dihydroxycoumarins [2]. On the basis of its composition, melting point, and IR spectrum, the aglycone was identified as 6,7-dihydroxycoumarin (esculetin). Consequently, the initial coumarin glycoside is 7-O-β-D-glucopyranosylesculetin (cychoriin). This is the first time that the size of the oxide ring and the configuration of the sugar residue have been determined.

Glycoside (II), $C_{16}H_{18}O_{10}$, mp 203°C (from methanol), $[\alpha]_D^{25} -85.5°$ (c 0.9; methanol); R_f 0.44, mol. wt. 370. UV spectrum: λ_{max} 350 nm (log ε 3.95). The IR spectrum shows the absorption bands characteristic of the coumarin nucleus, and also bands at 1050, 1070, 1090 cm^{-1} and 830, 850, and 860 cm^{-1} showing the pyranose form of the sugar residue and a β-glycosidic bond. The latter conclusion was confirmed by the results of enzymatic cleavage and by the calculation of the value $[M]_D \cdot Kp$ according to Klyne.

When the UV spectrum was taken in an alkaline medium, a bathochromic shift of the long-wave maximum ($\Delta \lambda_{max}$ 50 nm) and an increase in its intensity (Δ log ε 0.36) were observed, which shows the presence of a free hydroxyl in position 7 of the compound. In the NMR spectrum of (II) there are doublets at 7.45 and 6.05 ppm, J = 9.5 Hz (H-4 and H-3), a singlet at 3.61 ppm (6H) (protons of a hexose), a doublet at 5.52 ppm with J = 7 Hz (the β-anomeric proton of glucose), and a multiplet in the 6.6-6.8 ppm region (6H) (the protons of hydroxy groups and of H-6 or H-8). The hydrolysis of the glycoside (II) with acid yielded D-glucose and an aglycone $C_{10}H_8O_5$ with mp 226-227°C, M^+ 208. The above facts permit the conclusion that the substance obtained is a monoside of a hydroxymethoxycoumarin with a free phenolic hydroxyl at C_7.

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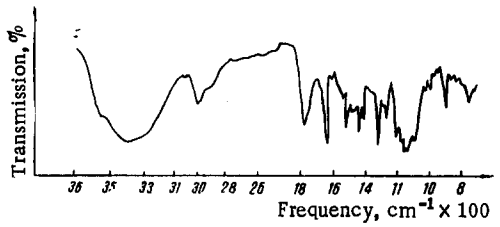

Fig. 1. IR spectrum of fraxinoglucoside.

The UV spectrum of (II) differs from the spectra of derivatives of 5,6,7-trihydroxycoumarin, and the NMR spectrum shows the absence of long-range spin–spin coupling between H-4 and H-8. Consequently, it may be concluded that the substance isolated belongs to the group of 6,7,8-trisubstituted hydroxycoumarins and the signal in the 6.7 ppm region relates to the H-5 proton.

The value of its chemical shift is in close agreement with the calculated value for 8-glucosyloxy-7-hydroxy-6-methoxycoumarin. The composition and constants of the substance correspond to the known coumarin fraxin.

Glycoside (III), $C_{17}H_{20}O_{10}$, mp 134-136°C (from pyridine), $[\alpha]_D^{27}$ −35° (c 0.4; methanol), R_f 0.63, mol. wt. 384, is, judging from its composition and physicochemical properties, a new coumarin glycoside, and we have called it fraxinoside. It dissolves in acetone, ethyl acetate, formamide, dimethylformamide, dioxane, pyridine, butanol, ethanol, and water and is insoluble in petroleum ether, carbon tetrachloride, and benzene.

UV spectrum of (III): λ_{max} 330 nm (log ε 3.98). Its IR spectrum shows absorption bands at (cm^{-1}) 1615, 1560, 1490 (aromatic nucleus), 1750 (α-pyrone carbonyl), 3550-3200 (hydroxy groups), and 2900-2970 (methoxy groups).

The absorption at 830, 1030, 1080, and 1095 cm^{-1} (Fig. 1) shows the presence in the glycoside (III) of a β-glycosidic bond of a sugar residue present in the pyranose form. When the UV spectrum of (III) was recorded in an alkaline medium, no bathochromic shift of the long-wave maximum was observed. Consequently, there is no free phenolic hydroxyl in (III).

The NMR spectrum of (III) has doublets at 7.67 and 6.10 ppm, J = 9.7 Hz (H-4, H-3), singlets at 3.97 and 3.58 ppm (3H each) (two methoxy groups), multiplets at 3.9 and 4.25 ppm (6H) (glucose protons), a doublet at 5.60 ppm, J = 6 Hz (the β-anomeric proton of glucose) and a rise in the 5.3-7.5 ppm region (4H) (the protons of the hydroxy groups of a sugar residue). These facts show that the substance is a monoglycoside, and the position of the H-8 singlet (6.50 ppm) agrees well with its calculated value for 6-glucosyloxy-5,7-dimethoxycoumarin (6.56 ppm).

The acid treatment of fraxinoside gave a hydrolyzate in which paper chromatography showed the presence of D-glucose and an aglycone $C_{11}H_{10}O_5$ with mp 171-172°C, R_f 0.90, M^+ 222 (the yield of the latter amounted to 56% of the glycoside).

The UV spectrum of the aglycone showed sharp absorption maxima at 233, 315, and 340 nm (log ε 4.17, 4.24, and 3.96), which are characteristic for derivatives of 5,6,7-trihydroxycoumarin, and in an alkaline medium a bathochromic shift of the long-wave maximum by 64 nm was found, which shows the presence of a free hydroxy group in position 6.

From its melting point, a mixed melting point, and its IR spectrum, the aglycone was identified as 6-hydroxy-5,7-dimethoxycoumarin (fraxinol). Consequently, the glucoside has the structure of 6-O-β-D-glucopyranosyloxy-5,7-dimethoxycoumarin.

EXPERIMENTAL

The NMR spectra were recorded on a Jeol 4H-100/100 instrument at 60 MHz in pyridine-d (0-14 ppm region, chemical shifts given on the δ scale from the signal of HMDS taken as 0), the UV spectra on a Hitachi spectrophotometer, the IR spectra on a UR-10 instrument (KBr), and the mass spectra on an MKh-1303 spectrometer.

The glycosides were chromatographed on type "M" ["slow"] paper in the BAW (4:1:5) system, (the spots being revealed with diazotized sulfanilic acid after the chromatograms had been sprayed with a 10% solution of sodium carbonate), and the sugars in the pyridine–acetic acid–water (6:3:1) system (the spots being revealed with o-toluidine and aniline phthalate).

Cychoriin. The preparation of the extracts from the leaves of F. mandschurica and the bark of F. potamophila and of their fractions has been described in a preceding paper [2].

Fraction (IV) (leaves of F. mandschurica) was deposited on a column of polyamide sorbent (h = 100, d = 4.5 cm). Elution with water yielded 0.15 g of a crystalline substance, $C_{15}H_{16}O_9$, with mp 214-215°C (from methanol), $[\alpha]_D^{25°} - 95°$ (c 0.5; dioxane).

Fraxin. Fraction (II) (from the bark of F. potamophila) (100 g) was passed through a column of acidic alumina (h = 25, d = 10 cm) and the glycosides were eluted with a 40% solution of methanol in chloroform (by volume), the eluates being evaporated. This gave 15 g of a viscous residue, which was deposited on a column of KSK silica gel (h = 150, d = 2.9 cm). The column was treated with chloroform containing increasing concentrations of methanol. A 7% solution of methanol in chloroform (volume of the eluate 90 ml) gave 0.133 g of a substance $C_{16}H_{18}O_{10}$ with mp 203°C, $[\alpha]_D^{25} - 85.5°$ (c 0.9; methanol).

Fraxinoside. Washing the column with a 10% solution of methanol in chloroform gave an eluate which was evaporated to dryness, and the residue was rechromatographed on the same adsorbent (h = 150, d = 2.1 cm). Elution was performed with chloroform containing 7% of methanol. When the eluate was concentrated, a crystalline substance deposited: $C_{17}H_{20}O_{11}$, mp 134-136°C (from pyridine), $[\alpha]_D^{27} - 35°$ (c 0.4; methanol).

Acid Hydrolysis of the Glycosides. Each of the substances (0.050 g) was dissolved in 3 ml of methanol, 10 ml of 15% sulfuric acid solution was added, and the mixture was heated in the boiling water bath for 2 h. When the hydrolyzates were concentrated, crystals of the aglycones deposited, and these were filtered off, washed, and recrystallized from aqueous methanol. The filtrates were neutralized with a suspension of barium carbonate. The sugars were identified chromatographically in the presence of "marker" sugars. The enzymatic hydrolysis of the substances was performed with β-glucosidase (ratio 1 : 1) at 37°C for 24 h.

SUMMARY

From the leaves of Fraxinus mandschurica Rupr. and the roots of F. potamophila Herd we have isolated and identified cychoriin, fraxin, and a new coumarin glucoside, $C_{17}H_{20}O_{14}$, mp 134-136°C (from pyridine), $[\alpha]_D^{27} - 35°$ (c 0.4; methanol), mol. wt. 384, which we have called fraxinoside. On the basis of UV, IR, and NMR spectroscopy it has been established that fraxinoside is 6-O-β-D-glucopyranosyloxy-5,7-dimethoxycoumarin.

LITERATURE CITED

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