POLYPHENOLS OF Onobrychis vassiltschenkoi AND THEIR POLAROGRAPHIC DETERMINATION

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UDC 547.972

Species of the genus Onobrychis Adans, growing in the Caucasus [1], have great economic value [2, 3]. The amino acids [4], carbohydrates [5], and polyphenolic compounds [6, 7] of some species have been studied. We have investigated the herb Onobrychis vassiltschenkoi Grossh., family Fabaceae [8]. A preliminary chromatographic study showed the presence of 19 substances of polyphenolic nature in it, nine of which were flavonoids.

The polyphenols were isolated by fractional extraction, fractional crystallization, and chromatography on a column of polyamide sorbent. The substances were eluted from the column successively with water and with mixtures of water and ethanol. Arbutin was isolated with hot water. Rutin, hyperoside, and astragalin were eluted successively with 15%, 30%, and 45% ethanol. The subsequent treatment of the column with 60% ethanol gave a mixture of free kaempferol and free quercetin, which were separated by preparative paper chromatography in the HCOOH–ethyl acetate–H_2O (10 : 2 : 3) system. The identities of the compounds isolated were established by UV and IR spectroscopy, acid and enzymatic hydrolysis, and alkaline degradation.

The total amount of flavonoids in the raw material was determined quantitatively by oscillopolarography. For this purpose we studied the polarographic activity of hyperoside and astragalin, which are present in the raw material in the largest amounts. The reduction potentials for hyperoside, astragalin, quercetin, and kaempferol are similar at various pH values of the solution, changing from −1.4 to −1.8 V with a rise in the pH from 3 to 10. The magnitude of the current peak does not change linearly as a function of the pH, and therefore the quantitative determination was performed at pH 4-5 at which the peak is well-defined and in the range of concentrations from 1 to 8 μg/ml the dependence of the current on the concentration is linear. The size of the current peak in the polarography of a mixture of hyperoside and astragalin is close to the sum of the currents for the pure substances. To determine the total flavonoids, the raw material was extracted with 96% ethanol with heating. The extract was transferred quantitatively to a 100-ml measuring flask and was made up to the mark with ethanol, and two dilutions were prepared with 0.1 and 0.2 ml of the initial extract in 0.1 N ammonium acetate buffer mixture to a volume of 25 ml. The concentration of the combined flavonoids in the raw material was determined from the formula

$$C\% = \frac{c \cdot V_1 \cdot V_3 \cdot 100}{V_2 \cdot a},$$

where c is the concentration from the calibration graph, g/ml; V_1 is the volume of the second dilution, ml; V_2 is the volume of the extract taken for dilution, ml; V_3 is the volume of the first dilution, ml; and a is the weight of the sample g.

The amount of total flavonoids in Onobrychis vassiltschenkoi was found to be 3.5%, calculated as hyperoside.

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GLYCOSIDES OF Dorema hyrcanum

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UDC 547.918

In a study of Dorema hyrcanum growing in the region of the town of Kara-Kala, Turkmen SSR, we found two phenolic glycosides. For their isolation, the dried roots were extracted with methanol, and the concentrated extracts were diluted with water and were extracted with ether and then with butanol. When the butanolic fraction was chromatographed on silica gel (0.25 mm) and eluted in the benzene–propanol (10 : 1 and 1 : 1) systems, two substances were isolated.

Substance (I), $C_{15}H_{20}O_9$, mp 200-202°C (water–methanol), $[\alpha]_D^{20}$ −95° (c 1.12; ethanol), R_f 0.6 [BAW (4 : 1 : 5) system, Silufol], giving on acidic hydrolytic cleavage D-glucose and an aglycone $C_9H_{10}O_4$, M^+ 182. Acetylation formed a pentaacetate with mp 140-145°C. On the basis of the physicochemical constants of the substance itself and its derivatives and from its spectral characteristics, the glycoside isolated was identified as pleoside [1].

Substance (II), $C_{21}H_{30}O_{14}$, mp 218-220°C (methanol–water), $[\alpha]_D^{20}$ −11.1° (c 1.11; water), R_f 0.3 [BAW (4 : 1 : 5) system, Silufol], proved to be a new bioside. We have called it hyrcanoside. It is readily soluble in water. Its IR spectrum shows the following maxima: λ_{max} 220-227 nm (shoulder), 285 nm (log ε 2.02, 2.15), which demonstrates the presence in its molecule of the same chromophore as in pleoside. The IR spectrum has bands at 3450 cm^{-1} (hydroxy groups), 1630, 1590, 1510 cm^{-1} (aromatic nucleus), and 1050 and 860 cm^{-1} (β-glycosidic bond).

The acetylation of (II) yielded an octaacetate with mp 158-160°C, which confirms its bioside nature. The acid hydrolysis of (II) yielded an aglycone identical with that from pleoside, and D-glucose was detected in the hydrolyzate by chromatography.

The NMR spectrum of the glycoside showed the signals of the following protons: singlet (3H) at 2.83 ppm – the protons of methylacetophenol; singlet, (3H) 3.58 ppm – methoxy group in an aromatic nucleus; doublets at 6.22 and 6.71 ppm (1H) – meta protons of an aromatic nucleus. In the 4.0-4.5 ppm region there are the signals of protons of two sugar residues (12H). A doublet at 5.48 ppm (J = 7 Hz) shows that one of the anomeric centers has the β configuration. The signal of the second anomeric proton is masked by other signals, but according to a calculation by Klyne's method, the second residue has the α configuration.

The NMR spectrum of the octaacetate has the signals of seven acetyl groups attached to sugar residues – singlet at 1.90 (21H) – and of one attached to the aromatic nucleus – singlet at 2.10 ppm (3H). The signals of the aromatic proton in the acetate of (II) are shifted downfield, as in the acetate of (I), by 0.27 and 0.36 ppm. The nature of the NMR spectrum of the acetate shows the possibility of a 1 → 6 bond between the sugars [2]. This hypothesis was confirmed by the results of the Hakomori methylation of the bioside followed by the hydrolysis of the polymethyl ether, which gave 2,3,4-tri-O-methyl- and 2,3,4,6-tetra-O-methyl-D-glucose, which were identified by chromatography on "Silufol" plates in the chloroform–methanol (25 : 1) system with markers. Thus, it has been established that hyrcanoside has the structure of 2-[O-α-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyloxyl-6-hydroxy-4-methoxyacetophenone.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 101-102, January-February, 1976. Original article submitted May 15, 1975.