FLAVONOIDS OF Bidens tripartita. III

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UDC 547.972:582.998

As reported previously [1], from the herbage of bur beggarticks (Bidens tripartita L.), family Compositae Giseke, we have isolated a flavonoid glycoside (substance 4) with the composition $C_{21}H_{22}O_{11}$, mp 236-239°C (from ethanol), $[\alpha]_D^{20}$ −88° [c 0.05; methanol−dimethylformamide (1 : 1)], assigned to flavanone derivatives on the basis of qualitative reactions.

Acid and enzymatic hydrolysis of the glycoside led to the formation of D-glucose, while the aglycone part of the molecule formed in this process three products which have been assigned by means of qualitative reactions on paper [2] to derivatives of flavanone, chalcone, and aurone. It must be noted that only the aurone derivative was present in appreciable amounts in the hydrolyzate, and the other two products were found in the form of traces.

An attempt to isolate the flavanone aglycone in the individual state using for this purpose column chromatography on a polyamide sorbent with mixtures of chloroform and ethanol of different concentrations as eluents was unsuccessful. In the eluates containing it we invariably found the products of its isomerization. The aurone aglycone isolated under these conditions was, from the results of paper chromatography and its physicochemical properties, identified as 3',4',6,7-tetrahydroxyaurone (maritimetin).

All that has been said above gave us grounds for assuming that the aglycone of the compound isolated was 3',4',7,8-tetrahydroxyflavanone (isookanin). This was confirmed by the oxidation of substance 4 to an aurone glycoside after the hydrolysis of which an aglycone identified as marimetin was obtained.

The alkaline degradation of substance 4 led to protocatechuic acid, which was identified by paper chromatography with an authentic sample. Ring A should form pyrogallol on cleavage. Its absence from the reaction mixture is explained by its extreme ease of oxidation by atmospheric oxygen in an alkaline medium [4].

The amount of sugar residues was determined spectrophotometrically. Since it was impossible to obtain isookanin in the individual state, as standard for the determination of E_{sp} of the aglycone we used naringenin. The ratio of the intensities was 61%, which characterizes the compound isolated as a monoside.

The position of attachment of the glucose to the aglycone was established by comparing the UV spectra of the aurone glycoside obtained by the oxidation of substance 4 and maritimetin. The absence of a bathochromic shift of the maximum of band I on the addition of sodium acetate in the glycoside, in contrast to the aglycone, showed the attachment of the carbohydrate residue at C-6 of maritimetin, which, according to flavanone numbering, corresponds to C-7 of isookanin.

The IR spectrum of the glycoside had three strong absorption bands in the 1100-1010 cm^{-1} region, which shows the pyranose form of the sugar [6]. This was also confirmed by the rate of acid hydrolysis, considerably less than that of furanosides [7]. The splitting off of the glucose on hydrolysis with emulsin showed the presence of a β-glycosidic bond between the sugar and the aglycone [8], as was confirmed by the negative value of the specific rotation and the presence in the IR spectrum of the compound of an absorption band at 887 cm^{-1} [6].

Consequently, substance 4 can be characterized as isookanin 7-O-β-D-glucopyranoside. To confirm the results obtained, and also to establish its absolute configuration, we obtained the PMR spectrum of its trimethylsilyl ether (Fig. 1) and the optical rotatory dispersion spectrum.

Khar'kov State Pharmaceutical Institute. Khar'kov Scientific-Research Institute of Pharmaceutical Chemistry. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 144-147, March-April, 1975. Original article submitted December 29, 1973.

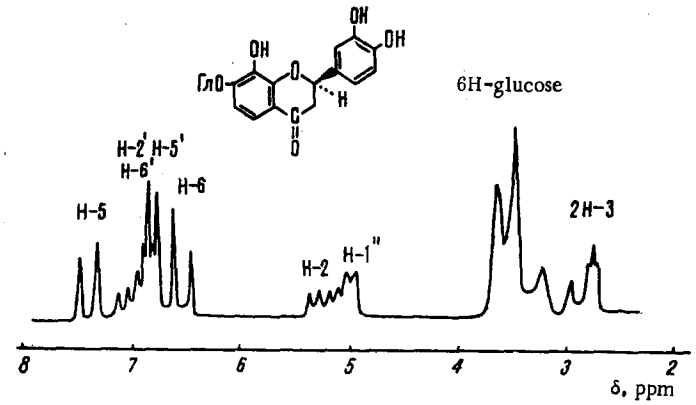

Fig. 1. PMR spectrum of (R-2)flavanomarein.

In the region of aromatic protons, an integral calculation showed the presence of five aromatic protons. Two doublets with J = 10 Hz at 7.41 and 6.54 ppm are due to two protons in positions 5 and 6, respectively. A complex group of signals at 7.19-6.71 ppm is due to three protons of the side chain in positions 2', 5', and 6'. The distribution of the intensity of this group of signals shows the 3', 4'-substitution of ring B.

A doublet at 5.00 ppm with J = 6.5 Hz is due to the axial–axial coupling of the protons at C-1" and C-2" of the sugar, which shows the presence of a β-glycosidic bond between the glucose and the aglycone. In the 3.80-3.02 ppm region there is a group of signals belonging to the other protons of the carbohydrate residue. An integral calculation showed the presence in it of six protons, which confirms the monoside nature of substance 4.

The two protons in position 3, besides interacting with one another (J = 17 Hz) interact with the proton in position 2 with different coupling constants. As a result of the overlapping of the quartets, in the spectrum at 2.86 ppm only two doublets with J = 12 Hz (axial–axial interaction) and J = 5 Hz (axial–equatorial interaction) can be seen in the spectrum. Because of their low intensity, the extreme signals do not appear.

The splitting constants show an axial–axial and axial–equatorial interaction of the protons at C-3 and C-2. Consequently, the 2-aryl group is equatorial, which enables the compound to be ascribed the R configuration at C-2. The R configuration is also confirmed by the presence in the optical rotatory dispersion spectrum of a negative Cotton effect caused by the band of the $n \rightarrow \pi^*$ transition of the carbonyl group at 330 nm.

A quartet at 5.23 ppm is due to the proton in the C-2 position interacting with the axial (J = 12 Hz) and equatorial (J = 5 Hz) protons at C-3.

Thus, the facts given enabled substance 4 to be characterized as (R-2)isookanin 7-O-β-D-glucopyranoside [(R-2)flavanomerin].

EXPERIMENTAL METHOD

Oxidation of Substance 4 to an Aurone Glycoside. A solution of 50 mg of substance 4 in 10 ml of water was treated with two drops of a saturated methanolic solution of ammonia (obtained by passing gaseous ammonia through methanol for 2-3 h), and the mixture was left in the air at room temperature for an hour. Then it was deposited on a small column of Kapron [nylon-6] (d 1 cm, h 10 cm) and was eluted with 100 ml of water; the aurone glycoside was desorbed with methanol, the eluates were evaporated in vacuum to dryness, and the dry residue was crystallized from aqueous ethanol.

Acid Hydrolysis of Substance 4. A solution of 150 mg of substance 4 in 20 ml of 2% aqueous hydrochloric acid was heated in a flask with an air condenser at 100°C for 2 h. The aglycone was extracted from the hydrolyzate with ethyl acetate (4 × 20 ml), the extracts were combined, washed with 20 ml of water, dried with anhydrous sodium sulfate, and evaporated in vacuum to dryness. The dry residue was dissolved in 2 ml of methanol, the solution was mixed with 5 g of Kapron and dried in the air at room temperature, and the dry powder obtained was deposited in the form of a suspension in chloroform on a column of polyamide (d 2 cm, h 28 cm). It was eluted with mixtures of chloroform and ethanol of various concentrations. Maritimetin was desorbed by 15% of ethanol in chloroform.

The aqueous residue after the separation of the aglycone was neutralized by AV-17 anion-exchange resin (OH form), and was evaporated in vacuum to a syrupy consistency, and this residue was dissolved in 2 ml of 96% ethanol and the solution was left to crystallize. This gave a sugar with mp 139-142°C, identical with D-glucose (phenylosazone with mp 200-202°C). The acid hydrolysis of maritimein was performed similarly.

Enzymatic Hydrolysis of Substance 4. To 5 mg of emulsin suspended in 1 ml of water was added 1 ml of a 5% aqueous solution of substance 4, and the reaction mixture was left in a thermostat at 38°C for 12 h. Then the enzyme was precipitated with 50 ml of hot ethanol, the precipitate was separated off, and the filtrate was evaporated to 0.3 ml and was analyzed for the presence of aglycone and carbohydrate residue.

Alkaline Decomposition of Substance 4. A mixture of 3 mg of substance 4 and 30 mg of caustic soda was fused for 1-2 min in a hard glass test tube. After cooling, the reaction mixture was dissolved in 15 ml of water, the solution was made weakly acid with 30% sulfuric acid, and the decomposition products were extracted with diethyl ether (5 × 20 ml). The ethereal extract was washed with 20 ml of water, dried with anhydrous sodium sulfate, and evaporated in vacuum to dryness. The dry residue was dissolved in 0.3 ml of ethanol and was investigated by paper chromatography in 2% acetic acid. Protocatechuic acid was detected.

Determination of the Amount of Sugar Residues. An accurately weighed sample of substance 4 (1 mg) was dissolved in 10 ml of methanol. In a 10-ml pycnometer, 1 ml of this solution was made up with methanol to the mark, and the intensity of the solution obtained was measured in a spectrophotometer at 283 nm. A solution of naringenin was prepared similarly, and its intensity was measured at 278 nm. E_{sp} was determined from the formula $E_{sp} = E/C$, where E is the intensity and C is the concentration of the solution (in %). Then E_{sp} for the glycoside was divided by E_{sp} for the aglycone, and the result obtained was multiplied by 100. The ratio of the intensities of the maxima of the glycoside and the aglycone amounted to 61%, which corresponds to a monoside.

Preparation of the TMS Ether of Substance 4. A published procedure [9] was used. The PMR spectrum of substance 4 was taken on a Hitachi Perkin-Elmer R-20A instrument at a working frequency of 60 MHz. The measurements were performed at 34.5°C with carbon tetrachloride as solvent and tetramethylsilane as internal standard.

SUMMARY

It has been established that substance 4, isolated previously from Bidens tripartita, is (R-2)isookanin 7-O-β-D-glucopyranoside [(R-2)flavanomarein]. The compound isolated is a new one for the genus Bidens L.

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