PINNATIFIDIN – A NEW FLAVONOL GLYCOSIDE FROM Crataegus pinnatifida

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The flowers of Crataegus pinnatifida Bge (Chinese hawthorn) have been shown by paper chromatography to contain nine substances of flavonoid nature, of which we have isolated eight components.

The present paper gives the results of the determination of the structure of pinnatifidin – a new glycoside of the flavonol group with the composition $C_{22}H_{22}O_{12} \cdot \frac{1}{2}H_2O$, mp 240-244°C, isolated by chromatographing a purified ethanolic extract of the flowers on polyamide sorbent.

It can be seen from the NMR spectrum of pinnatifidin that substituents are present in positions 3, 4', 5, 7, and 8 (Fig. 1). One of the substituents is a methoxy group, as is shown by a singlet at 3.74 ppm with an intensity of three proton units; a doublet at 5.74 ppm and the signals of six protons in the 3.3-3.6-ppm region relate to the carbohydrate component [1]. UV spectroscopy showed the presence of free hydroxy groups in positions 4', 5, and 7.

The hydrolysis of pinnatifidin with dilute sulfuric acid gave glucose, identified by paper chromatography [2]. On the basis of a comparison of its constants and NMR, UV, IR, and mass spectra, the aglycone of the substance was identified as 8-methoxykaempferol [3]. Consequently, the glucose in pinnatifidin is present in position 3, which agrees with the UV spectra of the glycoside and its aglycone and the magnitude of the chemical shift of the protons of the anomeric center. The spin–spin coupling constant (8 Hz) shows the β configuration of the anomeric center [1]. The glucose has a pyranose ring, since exhaustive methylation with subsequent methanolysis led to methyl 2,3,4,6-tetra-O-methyl-D-glucoside [4].

Thus, pinnatifidin has the structure of 3-β-D-glucopyranosyloxy-4',5,7-trihydroxy-8-methoxyflavone.

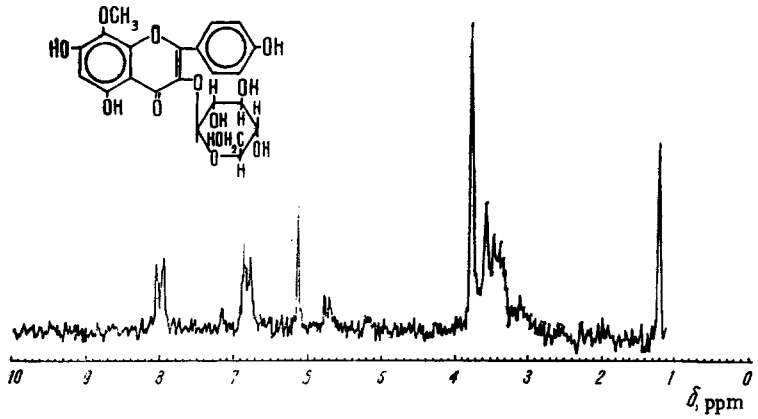

Fig. 1. NMR spectrum of silylated pinnatifidin.

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EXPERIMENTAL

For analysis the substances were dried over phosphorus pentoxide in vacuum at 100°C for 15 h. The NMR spectra were taken on a Varian HA-100D instrument, the UV spectra on a Hitachi spectrophotometer, and the IR spectra on a UR-10 instrument (mulls in paraffin oil). The specific rotations were determined on a type SM polarimeter and the melting points on a Kofler block. The chromatograms were made on Whatman No. 3 paper.

Isolation of Pinnatifidin. The dried flowers (4.88 kg) were extracted with methanol in a ratio of 1 : 5 four times. The extract was evaporated in vacuum, and the residue was treated with hot water (1 liter) and purified with chloroform. The flavonoids were extracted from the purified extract successively with diethyl ether and water-saturated butan-1-ol. The butanolic extract was evaporated to dryness and the residue was dissolved in methanol and was reprecipitated with a fivefold amount of acetone. The combined flavonoids obtained were chromatographed on a column of polyamide sorbent (d 8, h 35 cm). The substances were eluted with water. The fractions enriched in pinnatifidin were combined and evaporated to dryness, and the residue was rechromatographed on a column of polyamide (d 4, h 20 cm). The glycoside under investigation was isolated by means of ethanol–chloroform (7 : 93). After the solvent had been evaporated off, the substance was recrystallized from methanol. Mp 240-244°C, $[\alpha]_D^{20}$ −23.9° (c 0.34; formamide), R_f 0.68 (15% solution of acetic acid), 0.68 [butan-1-ol–acetic acid–water (4 : 1 : 5)]. UV spectra: $\lambda_{max}^{CH_3OH}$ 224, 273, 327, 356 nm; $\lambda_{max}^{CH_3COONa}$ 283, 390; $\lambda_{max}^{CH_3COONa + H_2BO_3}$ 274, 326, 354; $\lambda_{max}^{ZrO(NO_3)_2}$ 285, 316, 356, 416; $\lambda_{max}^{CH_3ONa}$ 283, 335, 410 nm.

Found %: C 54.52; H 4.86. $C_{22}H_{22}O_{12} \cdot \frac{1}{2}H_2O$. Calculated %: C 54.20; H 4.72.

Silylation of Pinnatifidin. A solution of 0.05 g of the substance in 3 ml of pyridine was treated with 0.5 ml of hexamethyldisilazane and 0.5 ml of chlorotrimethylsilane. The pyridine and the excess of the reagents were evaporated off in vacuum. The residue was extracted with 10 ml of carbon tetrachloride, and the solution was filtered, and evaporated to a volume of 1 ml, and its NMR spectrum was recorded: doublet at 8.0 ppm (2H), J = 9 Hz corresponding to H-2',6'; doublet at 6.82 ppm (2H), J = 9 Hz, corresponding to H-3',5'; singlet at 6.12 ppm (1H), corresponding to H-6; doublet at 5.74 ppm (1H), J = 8 Hz, corresponding to H-1 of β-glucose; singlet at 3.74 ppm (3H) due to a $-OCH_3$ group in position 8; signals in the 3.3-3.6 ppm region (6H) – glucose protons.

Hydrolysis of Pinnatifidin. A mixture of 0.1 g of the substance and 2% sulfuric acid was heated in the water bath for 20 min. The precipitate of aglycone was filtered off. After neutralization, the mother solution was evaporated to a volume of 0.5 ml and was chromatographed on paper in the benzene–butan-1-ol–pyridine–water (1 : 5 : 3 : 3) system. Glucose was detected.

After recrystallization from methanol, the aglycone of pinnatifidin had mp 269-271°C, R_f 0.37 (60% solution of acetic acid) and 0.81 [butan-1-ol–acetic acid–water (4 : 1 : 5)]. NMR spectrum in deuteroacetone: doublet at 8.12 ppm (2H), J = 9 Hz, corresponding to H-2', 6'; doublet at 6.96 ppm (2H), J = 9 Hz, assigned to H-3',5'; singlet at 6.25 ppm (1H) showing H-6; and singlet at 3.89 ppm (3H) due to $-OCH_3$ in position 8. UV spectra: $\lambda_{max}^{CH_3OH}$ 273, 327, 376 nm; $\lambda_{max}^{CH_3COONa}$ 257, 281, 318, 390; $\lambda_{max}^{CH_3COONa + H_2BO_3}$ 273, 376; $\lambda_{max}^{ZrO(NO_3)_2}$ 258, 286, 376, 476; $\lambda_{max}^{CH_3ONa}$ 284, 335, 424 nm.

Found %: C 59.98; H 4.05 · $C_{16}H_{12}O_7$. Calculated %: C 60.76; H 3.80. Mol. wt. 316 (mass spectroscopically).

Methylation of Pinnatifidin. With stirring, 0.2 g of sodium hydride was added to a solution of 0.1 g of the substance in 5 ml of dimethyl sulfoxide, and methylation was performed with 2 ml of methyl iodide at room temperature for 12 h. Then the mixture was dissolved in 30 ml of chloroform and the solution was treated with water (2 × 25 ml), sodium thiosulfate solution (25 ml), and water again (25 ml). The chloroform solution of the methylation product was dried with anhydrous sodium sulfate, and the solvent was evaporated off. The residue was subjected to methanolysis with 4 ml of methanol saturated with hydrogen chloride in a sealed tube at 100°C for 3 h. After extraction with chloroform, the methanolysis product was identified by gas-liquid chromatography as methyl 2,3,4,6-tetra-O-methyl-D-glucoside.

SUMMARY

A new flavonoid glycoside, 3-β-D-glucopyranosyloxy-4',5,7-trihydroxy-8-methoxyflavone has been isolated from the flowers of Crataegus pinnatifida.

LITERATURE CITED

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