A NEW FLAVONOID ISOLATED FROM THE LEAVES OF CYNARA SCOLYMUS L.

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As reported previously [1], two flavonoid glycosides have been isolated from the leaves of Cynara scolymus L. (artichoke): luteolin 7-β-glycoside and luteolin 7-β-rutinoside. The present paper gives the results of a study of the structure of a new flavonoid compound (I) which we have called cynarotriside.

This flavonoid was obtained by separating an aqueous extract of artichoke by means of column chromatography. The substance crystallized from aqueous acetone in the form of yellow needle-like crystals exhibiting the specific reactions for flavonoids.

Acid hydrolysis of the flavonoid yielded an aglycone identical in its physicochemical properties with luteolin (VII) (table).

Properties of Cynarotriside and Its Reaction Products

Properties	Cynarotriside	Substance II	Substance III	Luteolin
Empirical formula	$C_{33}H_{40}O_{20}$	$C_{21}H_{20}O_{11}$	$C_{27}H_{30}O_{15}$	$C_{15}H_{10}O_6$
$[\alpha]_D^{22}$, degrees	—	−100	−35	—
mp, °C	274—276	255—258	192—196	326—330
Color reactions				
3% $FeCl_3$ solution	green	green	green	green
Mg + HCl	pink	pink	pink	pink
Ammoniacal solution of $AgNO_3$	negative	positive	positive	positive
R_f value in the following systems:				
butan-1-ol-acetic acid-water (4:1:5)	0.63	0.48	0.32	0.80
ethanol-acetic acid-water (25:10: 65)	0.52	0.26	0.38	0.09
25% acetic acid	0.49	0.24	0.35	0.14
UV spectrum in the region bands (I) and (II), λ_{max} mμ	I II	I II	I II	I II
2×10^{-5} M solution in ethanol	335 255 266*	350 255 267*	350 256 266*	350 255 266*
The same + CH_3COONa	335 266	360 258	375 260	382 269
The same + H_3BO_4 + CH_3COONa	335 266	380 260	375 262	375 261
The same + CH_3CH_2ONa	387 268	400 268	400 270	410 267

*Shoulder

On paper chromatography, D-glucose and L-rhamnose (VI) were found. However, the results of the quantitative hydrolysis of cynarotriside showed the presence of three carbohydrate residues. Stepwise acid hydrolysis gave a substance (II), and the hydrolyzate was found to contain D-glucose (V) and L-rhamnose (VI).

Compound (II) proved to be a monoside the aglycone of which is luteolin and the saccharide component D-glucose (V). To establish the position of the attachment of the D-glucose to theaglycone, the monoside (II) was exhaustively methylated with subsequent hydrolysis with a solution of sulfuric acid. The product obtained was identical in its properties with 5, 3', 4'-trimethoxy-7-hydroxyflavone [2]. The presence of the D-glucose in position 7 is also shown by the bathochromic displacement of the second band ($\Delta\lambda$ +14 mμ) in the UV region of the spectrum when fused sodium acetate is added [3] (see table). The nature of the oxide ring and the configuration of the glucosidic bond of the D-glucose at C_7 were determined by comparing the molecular rotations of the glucoside investigated and the corresponding phenyl glucoside, making allowance for the difference in their molecular weights.

Compound	$[M]_D^{22}$, degrees	K_f	$[M]_D^{22} \cdot K_f$
Phenyl α-D-glucopyranoside	+402	1	+402
Phenyl β-D-glucopyranoside	—182	1	—182
Substance (II)	—448	0.57	—255

Note:

$$K_f = \frac{\text{Mol. wt. (phenyl glucoside)}}{\text{Mol. wt. (substance II)}}$$

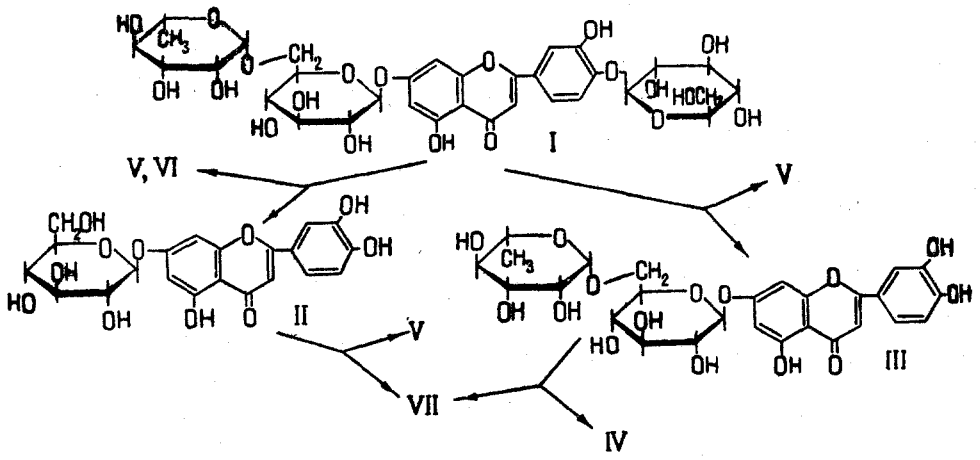
The enzymatic hydrolysis of substance (II) with β-amylase confirmed the presence of a β-glucosidic bond between the sugar and the aglycone. The D-glucose was found to be in the pyranose form by means of the IR absorption spectra, using the method of differential analysis. The bands characteristic for luteolin were eliminated from the IR spectrum of substance (II). The bands at 1096, 914, and 773 cm^{-1} are due to the asymmetrical and symmetrical vibrations of a pyranose ring. The results obtained enable us to state that substance (II) is luteolin 7-β-D-glucopyranoside.

The enzymatic hydrolysis of the flavonoid (I) with β-amylase led to the formation of compound (III) and the sugar D-glucose (V). On complete acid hydrolysis, substance (III) was split into luteolin (VII), D-glucose (V), and L-rhamnose (VI), while stepwise hydrolysis gave the monoside (II) and L-rhamnose (VI). Enzymatic hydrolysis with the stereospecific enzyme rhamnodiastase gave luteolin (VII). The sugar component proved to be identical with rutinose (IV), which we isolated from rutin. These results show that substance (III) is luteolin 7-O-β-D-glucopyranosyl-(6→1)-β-L-rhamnopyranoside.

The position of the second molecule of D-glucose was shown by means of the UV spectrum of cynarotriside obtained by the use of ionizing and complex-forming additives. Absorption maxima were found characteristic for luteolin with substituents in positions 7 and 4' [3]. The presence of a maximum at 335 mμ shows a substituent in position 4', while 7-, 5-, and 3'-substituted luteolins have a maximum at 350 mμ. The absence of a displacement of the maximum of band I on the addition of a mixture of boric acid and sodium acetate also shows the presence of a substituent on the phenolic hydroxyl of the side ring. The substituent in position 4', as was shown by enzymatic hydrolysis with β-amylase, is D-glucose.

The nature of the oxide ring of D-glucose in position 4' was determined, as for the monoside (II), by means of its IR spectrum of cynarotriside (I).

The investigations carried out have shown that cynarotriside is luteolin 7-[O-β-D-glucopyranosyl-<6→1>-O-β-L-rhamnopyranoside]-4'-O-β-D-glucopyranoside.



Experimental

For analysis, the substances were dried in vacuum over P_2O_5 at 110°C for 4 hr. The melting points were determined on a Kofler block.

Isolation of cynarotriside. 10 kg of freshly-ground leaves of Cynara scolymus L. — artichoke (variety "Maikopskii 041") — was extracted with boiling water (three 30-*l* portions). In 1-kg portions the aqueous extract was evaporated under vacuum and mixed with 500 g of polyamide sorbent powder. After drying in the air, the mass was transferred to a column containing 800 g of polyamide sorbent. The column was first washed with water (until the appearance of a

colorless eluate), and then the flavonoid compounds were eluted with 70% ethanol. The alcoholic extract was evaporated under vacuum to dryness, the residue was dissolved in 150 ml of 70% ethanol, and the solution was mixed with 75 g of polyamide sorbent. The dried mass was transferred to a column of polyamide sorbent (height of bed 70 cm, diameter 6 cm) and was eluted first with chloroform and then with a mixture of chloroform and alcohol, the concentration of alcohol being increased in steps of 5% when evaporation of the eluate gave an insignificant amount of residue. 500-ml fractions were collected and were investigated by means of chromatography on "Goznak" paper. The R_f values given are the means of five determinations. Fractions 30-42, containing the cynarotriside and other flavonoid compounds, were combined and evaporated under vacuum to dryness.

The residue was dissolved in 50 ml of 70% ethanol, mixed with 30 g of polyamide sorbent, and chromatographed on a polyamide column, being eluted with water and a mixture of water and alcohol with the concentration of alcohol being increased in 2% steps. Fractions of 300 ml were collected and were chromatographed on paper in the systems shown in the table. Fractions 15-23 containing the flavonoid (I) were concentrated to a resinous residue, which was dissolved in 40 g of ethanol and mixed with 30 g of cellulose powder. The dried mass was transferred to a column of dry cellulose powder (70 cm high and 2.5 cm in diameter) and was eluted with ethanol-acetic acid-water (25:10:65). The separation of the flavonoids was observed in UV light (dark zones).

The alcohol was evaporated off from the aqueous alcoholic fractions under vacuum. The aqueous solution was passed through a polyamide column, which was washed with water to neutrality. Flavonoid (I) was eluted with 70% ethanol, the solvent was distilled off, and the residue was dissolved in 40 ml of a mixture of water and acetone (1:3). After 3 days, yellow needle-like crystals of cynarotriside deposited. Yield 0.12 g.

Acid hydrolysis of (I). A mixture of 100 mg of the substance with 10 ml of 20% sulfuric acid was heated in a water bath for 4 hr. The yellow precipitate which deposited after cooling was separated off and crystallized from aqueous acetone (1:3). The yield of aglycone was 33.3%.

Acetylation of the aglycone gave a tetraacetyl derivative with mp 225-227°C. The products of the alkaline fusion of the aglycone were identified by paper chromatography as phloroglucinol and protocatechuic acid.

After the separation of the aglycone, the mother liquor was passed through a column containing the anion-exchanger AV-17 in the OH form. The eluate was evaporated until a syrup was formed and was chromatographed on paper in the butan-1-ol-acetic acid-water (4:1:5) system. D-Glucose and L-rhamnose were detected (reagent aniline phthalate).

Stepwise acid hydrolysis. A mixture of 40 mg of flavonoid B and 8 ml of 10% sulfuric acid was heated in a water bath for 2 hr. The yellow precipitate which deposited on cooling was crystallized from aqueous methanol. Yellow needle-like crystals of substance (II) were obtained (see table).

The acid hydrolysis of substance (II), carried out under the conditions described, gave the aglycone luteolin, and D-glucose was detected by paper chromatography.

Enzymatic hydrolysis of substance (II). A mixture of 20 mg of substance A and 10 ml of water was acidified with acetic acid to pH 5.6 and 30 mg of an enzyme preparation of the fungus Asperigillus oryzae was added. The mixture was kept in a thermostat at 37-38°C for 18 hr. Paper chromatography of the hydrolysis products showed the presence in them of luteolin and D-glucose.

Production of 5, 3', 4'-trimethoxy-7-hydroxyflavone. A mixture of 0.1 g of substance (II), 0.2 ml of dimethyl sulfate, 2 g of anhydrous potassium carbonate, and 30 ml of anhydrous acetone was heated under reflux on a water bath at 58°C for 18 hr. The pH of the medium was maintained by the addition of dimethyl sulfate or potassium carbonate. Then the acetone was eliminated under vacuum and the residue was heated first with diethyl ether and then with chloroform. The extracts were combined and evaporated under vacuum. The residue (0.09 g) was suspended in 10 ml of 20% sulfuric acid and the suspension was heated in a water bath for 4 hr. After cooling, the solution was treated with diethyl ether (seven 10-ml portions), the ether was distilled off, and the residue was crystallized from 5 ml of a mixture of methanol, acetone, and water (2:2:1), giving prisms with mp 284-285°C.

Found, %: C 63.90; H 4.73. Calculated for $C_{18}H_{16}O_6 \cdot 0.5H_2O$ (mol. wt. 337), %: C 64.09; H 4.74.

Enzymatic hydrolysis of cynarotriside. A mixture of 40 mg of the flavonoid with 20 ml of water (pH 5.6) was treated with 50 mg of an enzyme preparation from Aspergillus oryzae. The mixture was kept in a thermostat at 38-39°C for 8 hr. Then the hydrolyzate was filtered, the residue on the filter was washed with aqueous acetone, and the mother liquor was mixed with an equal volume of acetone. The precipitate which deposited was filtered off, and the solution was concentrated under vacuum. On standing in the cold, a yellow precipitate of substance (III) formed, which crystallized from aqueous acetone in the form of small yellow plate. After the separation of substance (III), the mother liquor was chromatographed on paper; D-glucose was detected. The acid hydrolysis of substance (III) under similar conditions gave luteolin, D-glucose, and L-rhamnose.

Enzymatic hydrolysis of (III). A mixture of 20 mg of substance (III) and 10 ml of water (pH 5.6) was treated with 20 mg of an enzyme preparation of rhamnodiastase. The mixture was placed in a thermostat at 35-37°C for 20 hr. Then the hydrolyzate was treated as in the hydrolysis of cynarotriside. The yellow needle-like crystals isolated were identified as luteolin from the melting point of a mixture and the results of two-dimensional paper chromatography. The sugar component of the hydrolyzate was identified as rhamnose by paper chromatography.

The stepwise acid hydrolysis of substance (III) gave the monoside (II). Paper chromatography showed the presence of L-rhamnose.

The UV spectra of the compounds obtained were determined on a SF-4 spectrophotometer. The IR spectra of the substances, taken on a UR-10 spectrophotometer in a KBr tablet, were discussed by I. P. Kovalev.

Summary

The fresh leaves of the artichoke Cynara scolymus L. have yielded a new flavonoid glycoside, cynarotriside, having the structure luteolin 7-[O-β-D-glucopyranosyl-<6→1>-O-β-L-rhamnopyranoside]-4'-O-β-D-glucopyranoside.

REFERENCES

1. L. I. Dranik, V. T. Chernobai, and D. G. Kolesnikov, Med. prom. SSSR, no. 5, 23, 1964.
2. S. Hattori and H. Matsuda, J. Amer. Chem. Soc., 76, 5792, 1954.
3. L. Jurd, "Spectral properties of flavonoid compounds," The Chemistry of Flavonoid Compounds, ed. T. A. Geissman, The Macmillan Co., New York, 1962.

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