CRATENACIN—A NEW FLAVONE GLYCOSIDE FROM CRATAEGUS CURVISEPALA

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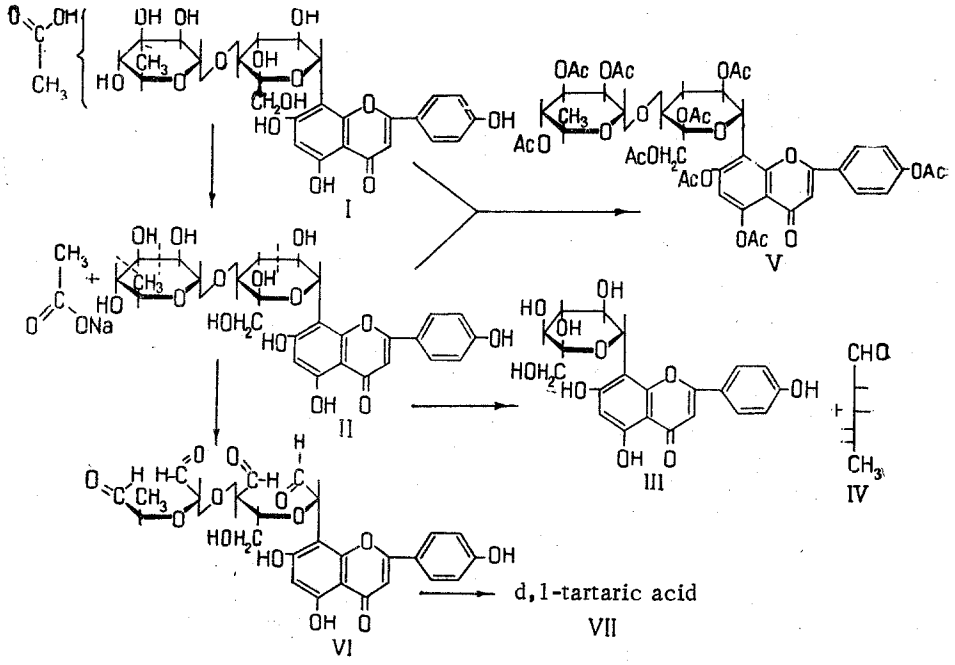
The first of the flavonoids with a C-glycosidic bond, vitexin, was isolated by Perkin from Vitex littoralis in 1900 [1]. In 1955, Fiedler [2] isolated two flavonoids of this series from Crataegus oxyacantha L. (English hawthorn) one of which proved to be vitexin and the other vitexin 4'-rhamnoside. In 1964, Horowitz and Gentilli [3] definitively established the formula of vitexin.

This paper gives the results of a chemical investigation of a new flavonoid with a C-glycosidic bond isolated from the leaves of Crataegus curvisepala, Lindm., family Rosaceae, which we have called cratenacin. In previous communications, it was given the designation Zh [4, 5].

Cratenacin (I) has the composition $C_{29}H_{32}O_{15}$, mp 254° C, $[\alpha]_D^{20}$ −29° (in methanol, λ 589 mμ, SPU-3 spectropolarimeter), λ_{max} 270 mμ (log ε 4.178) and 335 mμ (log ε 4.160). It is readily saponified by a dilute alcoholic solution of alkali with the formation of substance (II). Paper chromatography of the alkaline hydrolyzate of cratenacin in the ethanol–ammonia (9:1) system showed the presence of acetic acid (R_f 0.48) in addition to compound (II). The presence of an acetyl group in the molecule of cratenacin is also confirmed by the identity of the acetates of cratenacin and substance (II). Both compounds melt at 148°–151° C and they give no depression of the melting point.

The products of the acid hydrolysis of (II) contained a substance (III) the UV spectrum of which, on the addition of sodium acetate, underwent a bathochromic displacement of 33 mμ in the long-wave region and one of 9 mμ in the short-wave region. These shifts indicate that hydroxyl groups are present in positions 7 and 4'. The addition of aluminum chloride gave a bathochromic shift of 9 mμ, which indicates the presence of a hydroxyl group in position 5. The IR spectrum of substance (III) has absorption bands at 920, 890, and 756 cm^{-1}, which are characteristic for a β-D-glucopyranose ring [6]. The size of the ring and its β anomerism have been proposed previously by Gentilli on the basis of results obtained by means of the NMR spectrum [3]. The acetyl derivative of substance (III) contains seven acetyl groups, four of which are present in a lateral ring.

The stability of (III) to hydrolysis with concentrated solutions of inorganic acids shows that the side ring is connected with the flavonoid nucleus through a carbon–carbon bond. The physicochemical properties of substance (III) mentioned above correspond to the properties of vitexin, in which the sugar component is connected with the nucleus of 5, 7, 4'-trihydroxyflavone by means of a carbon–carbon bond [3].



From a hydrolyzate of substance (II) we isolated, in addition to vitexin, a crystalline sugar (IV) which was identified by paper chromatography and by its mixed melting point with an authentic sample of rhamnose (mp 90° C).

From the data of IR spectroscopy (absorption bands at 920, 844, and 755 cm^{-1}), vitexin is present in the form of a glycoside with α-L-rhamnose. In its physicochemical properties substance (II) is identical with vitexin 4'-rhamnoside, isolated by Fiedler from Crataegus oxyacantha L. [2].

As a result of the chemical reactions and spectroscopic properties of cratenacin, vitexin rhamnoside, and vitexin on the addition of sodium ethoxide and sodium acetate, it has been established that the rhamnose is present not in the nucleus of the flavonoid, as Fiedler [2] stated, but is attached to the glucose molecule in position 4.

To establish the position of attachment of the sugars in vitexin rhamnoside, the method of oxidative degradation of disaccharides with periodic acid and subsequent reduction of the products formed, as modified by us, was used [7]. We did not reduce the oxidation products but oxidized them with nitric acid. Periodic acid, which oxidizes vicinal diol groups, leads to rupture between the 2nd and 3rd carbon atoms of glucose with the formation of an aldehyde (VI) which reduces Fehling's solution and simultaneously gives the cyanidin reaction. On further oxidation and hydrolysis of the aldehyde with nitric acid, part of the molecule, consisting of the 3rd, 4th, 5th, and 6th carbon atoms of glucose, is converted into tartaric acid. The appearance of tartaric acid in the oxidative degradation of disaccharides possessing another type of linkage is unlikely.

Thus, cratenacin is 5, 7, 4'-trihydroxyflavone 8C-[β-D-glucopyranosyl-(1→4)-monoacetyl-O-α-L-rhamnopyranoside].

Experimental

Saponification of cratenacin. 1 g cratenacin was dissolved in 20 ml of water, 20 ml of 1% caustic potash solution was added, and after 2 min the solution was neutralized with sulfuric acid, after which it was extracted several times with diethyl ether. The ethereal extract was concentrated to small bulk in the cold and was chromatographed in the ethanol–ammonia (9:1) system with a reference sample of acetic acid. The chromatogram was shown up with bromothymol blue.

After some time, the alkaline hydrolyzate of cratenacin deposited plate-like crystals of substance (II) with mp 215°C (from aqueous acetone), $[\alpha]_D^{20}$ −35° C (in methanol, λ 589 mμ, SPU-E spectropolarimeter), UV spectrum λ_{max} 270 mμ (log ε 4.155) and 335 mμ (log ε 4.123).

Found, %: C 55.63, 55.81; H 5.09, 5.11. Calculated for $C_{27}H_{30}O_{14}$, %: C 55.05; H 5.19.

Cratenacin acetate. A mixture of 0.1 g of substance (I), 2 ml of pyridine, and 1 ml of acetic anhydride was kept at room temperature for 24 hr. The mixture was poured into 50 ml of cold water. The precipitate which deposited was recrystallized from butan-1-ol and then from a mixture of isopropyl and ethyl alcohols (3:1), mp 148°–151° C.

Found, %: C 55.86, 56.11; H 4.83, 4.93. Calculated for $C_{45}H_{48}O_{23}$, %: C 56.48; H 5.02.

Acetate of substance (II). Substance (II) was acetylated in a similar manner to cratenacin. Crystals with mp 148°–151° C were obtained.

Found, %: C 56.09, 56.39; H 4.76, 4.85. Calculated for $C_{45}H_{48}O_{23}$, %: C 56.48; H 5.05.

Hydrolysis of substance (II). 0.5 g of substance (II) was boiled in 10 ml of 10% sulfuric acid for 2 hr. The plate-like crystals of substance (III) that deposited were recrystallized from a mixture of methyl alcohol and acetone (mp 264° C, $[\alpha]_D^{20}$ −14.0° C (in pyridine), UV spectrum λ_{max} 271 mμ (log ε 4.304) and 335 mμ (log ε 4.155).

Found, %: C 57.81, 57.99; H 4.58, 4.60. Calculated for $C_{21}H_{20}O_{10}$, %: C 58.33; H 4.63.

Acetate of substance (III). A mixture of 0.1 g of substance (III) and 5 ml of acetic anhydride was heated, a drop of concentrated sulfuric acid was added, and the mixture was poured into cold water. The precipitate that deposited was recrystallized from alcohol. The plate-like crystals had mp 249°–258° C.

Found, %: C 56.95, 57.03; H 4.79, 4.81. Calculated for $C_{35}H_{34}O_{17}$, %: C 57.85; H 4.69.

Oxidation of substance (II) with sodium metaperiodate. At room temperature, 0.4 g of substance (II) was oxidized with 0.5 g of sodium metaperiodate for 24 hr. The aldehyde (IV) (mp 185°–195° C) that deposited on the following day was dissolved in 10 ml of 25% nitric acid and evaporated in an evaporating dish. After the elimination of traces of nitric acid, the acids formed were precipitated with calcium chloride, the precipitate was decomposed by boiling in dilute sulfuric acid, and the solution, freed from the precipitate of calcium sulfate, was evaporated to a volume of 1–2 ml. Tartaric acid was isolated in the form of the potassium hydrogen salt. The potassium hydrogen tartrate obtained was converted into the neutral potassium salt and passed through a column of KU-2 cation-exchanger, and the

eluate was concentrated to a volume of 0.5 ml and left to crystallize. After having been dried under high vacuum, the crystals were identical with racemic acid in respect of R_f values in various systems and by a mixed melting point (mp 204°–205° C).

The IR spectra were taken by I. P. Kovalev.

Summary

The structure of a new C-glycoside, cratenacin, has been established; it is 5,7,4'-trihydroxyflavone 8C-[β-D-glucopyranosyl-(1→4)-monoacetyl-O-α-L-rhamnopyranoside].

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