Constituents of wild rose seeds

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Received 27 November 1974

Accepted for publication 10 February 1975

The constituents of the ethereal and methanolic extracts of wild rose seeds were identified by means of gas-chromatographic data and paper-chromatographic evidence. The ethereal portion consisted mainly of drying oil (triglycerides of unsaturated fatty acids), accompanied with further components of lipidic character common to plants. Free saccharides, glucose and fructose, of bound saccharides glucose, arabinose, and traces of xylose were found to be present in the methanolic portion.

Были определены компоненты эфирного и метанольного экстракта, полученного из семян шиповника. Эфирная доля содержит прежде всего высыхающее масло (триглицериды непредельных жирных кислот) вместе с другими веществами липидного характера, которые обычно встречаются в растениях. Метанольная доля содержит в свободном виде глюкозу и фруктозу, а в связанном — глюкозу, арабинозу и следы ксилозы.

Rosa canina L. (wild rose) of *Rosaceae* family belongs to our well known medicinal plants mainly due to the high contents of vitamin C in the fruits [1—3]. Fruits of rose are collected not only from *Rosa canina*, the pure population of which is quite rare, but also from other species and hybrids of *Rosaceae* family.

The pulpous pericarp from which downs and seeds were removed serves for preparing tea. The seeds are so far of no practical use and since there is little known about their constituents, we investigated them minutely. From the ground seeds ethereal and methanolic extracts were prepared. In the first, extracts of substances of lipidic character were found while in the second one free and bound saccharides were identified.

Experimental

The seeds for investigation were obtained from the wholesale VDP in Zvolen. Loss on drying was determined according to [4]. Silica gel was employed both for column [5] and TLC chromatography [6]. The purity of the individual fractions was tested by thin-layer chromatography. For partition chromatography paper Whatman No. 4

and solvent system butanol—pyridine—water 10 : 3 : 3 were used. Transesterifications were performed in sealed tubes in water-free methanol containing 5% of dry hydrogen chloride at 60—70°C for several hours.

Chromatograph Pye series 104 Model 124 equipped with two flame-ionization detectors and a dual system of glass columns (0.4 × 150 cm) was employed for gas chromatography; as packings, 10% diethyleneglycol succinate on Chromosorb W (column *A*), 3% SE-30 on Gas Chrome Z (*B*), 10% butanediol succinate on silanized Chromosorb W (*C*), and 3% OF-1 on Gas Chrome O (*D*) were used. All supports were of 100—120 mesh. The chromatography was carried out either at a constant or at a linearly programmed temperature (2—3°C min^{-1}). The samples were injected as 1% carbon tetrachloride solutions using a Hamilton syringe. Authentic specimens were used to identify individual substances; homologues in a homologous series were identified also by the usual graphical method [7]. For quantitative determination the product of the retention time and the peak height (for homologues) or the triangulation method were employed. All identifications of the obtained chromatographic waves were verified at least on two different columns using the authentic samples.

The infrared spectra were taken with a UR-10 (Zeiss, Jena) spectrophotometer in 0.1-cm cells. The concentration of samples in carbon tetrachloride was approx. 4%.

Preparation of the extract

The wild rose seeds (20 g; 94.0% dry weight) were ground and sifted through a 20 mesh sieve. This material was first extracted in a Soxhlet apparatus with ether for 5 hrs, then the solvent was removed under reduced pressure and the residue dried at 105°C for 3 hrs. The yield amounted to 1.6 g (8% of substances soluble in ether). Afterwards the material was extracted with methanol (9 hrs). By evaporation *in vacuo* of the extract a semifluid substance was obtained (3.3 g; 16.5%).

Analysis of the ethereal portion of the extract

The results of the silica gel (100 g) column chromatography of the extract (2 g) are given in Table 1. The separate fractions were further resolved in a gas chromatograph.

Analysis of the methanolic portion of the extract

Free saccharides were identified by a one-direction descending chromatographic method [8]. The methanolic extract was spotted directly. Chromatograms were twice developed. The chromatographic chamber was saturated for 2 × 16 hrs, development of chromatograms took 2 × 9 hrs with anilinium hydrogen phthalate as a detection reagent [9]. At 105°C the spots of hexoses turned brown, while those of pentoses pinkred.

Bound saccharides were obtained from the methanolic portion (2 g), which was dissolved in methanol (100 ml), precipitated with 10% basic lead(II) acetate (10 ml) and kept at 60°C for 15 minutes. After cooling the precipitate was filtered off, washed, dissolved in 1 N-H_2SO_4 (20 ml), and hydrolyzed in a sealed tube at 90°C for 20 hrs. The solution was then neutralized with 1 N-NaOH and filtered. The filtrate was evaporated and extracted with pyridine at 100°C for 10 min in a steam bath in order to remove the undesirable by-products. After filtration, the solution was evaporated and the desalinized residue was dissolved in aqueous ethanol (1 1; 3 ml). Saccharides present in this solution were identified by paper chromatography under the same conditions as given before.

Results and discussion

By means of gas chromatography it has been found, that the hydrocarbons present in fraction *1* (see Table 1) formed a homologous series of C_{15}—C_{33} *n*-alkanes. Odd-carbon hydrocarbons prevailed in higher members of the series whereas the

Table 1

Chromatographic separation of the ethereal extract

Fraction	Solvent	Amount g	Group	Calculated in % of extract	Calculated in % of seeds
1	*n*-Hexane	0.02	Hydrocarbons	1.0	0.08
2	*n*-Hexane—ether 9 : 1	0.01	Esters	0.5	0.04
	n-Hexane—ether 8 : 2	1.53	Triglycerides	76.5	6.12
4	*n*-Hexane—ether—methanol 7 : 2 : 1	0.15	Alcohols	7.5	0.60
	Residue in the column	0.29		14.5	1.16

quantitative preponderance of odd-carbon hydrocarbons in the lower ones was not so significant. The predominant members of the homologous series were found to be nonacosane (C_{29}, 40%), hentriacontane (C_{31}, 10%), and heptacosane (C_{27}, 7%). In addition to *n*-hydrocarbons, trace amounts of branched hydrocarbons were identified. No unsaturated hydrocarbons were determined using the method proposed by [10].

Simple esters

Fraction *2* was carefully transesterified [5] with methanol and the resulting products (free alcohols and methyl esters of acids) were analyzed by gas chromatography. β-Sitosterol was found to be the main alcoholic and the unsaturated oleic acid the main acid component (~80%); hence it follows that β-sitosteryl oleate is the major component of this fraction. Oleic acid was accompanied by a homologous series of saturated even-carbon C_{10}—C_{20} acids.

Glycerides

Glycerides were the major component (76.5%) of the total ethereal extract. On TLC this fraction gave a single spot with an R_F value corresponding to that of synthetic triglyceride (trimystine). Gas chromatographic analysis of methyl carboxylates obtained by transesterification of triglycerides with methanol showed the main components to be the C_{18} unsaturated acids: oleic (15%), linoleic (62%), and linolenic (18.5%) acids. Saturated acids were found to be present only in minor concentrations: palmitic (2.5%), stearic (2.0%), arachic (1%), and higher acids. The presence of glycerol was proved after reduction of triglycerides with lithium aluminium hydride followed by acetylation; the resulting glycerol triacetate was compared with an authentic specimen using gas chromatography.

Free alcohols

According to TLC fraction *4* (7.5%) was not uniform. In an infrared spectrum the vibration characteristic of a hydroxyl group at 3610—3460 cm^{-1} and a weak absorption of the carboxylic group at 2500—3200 cm^{-1} could be observed. The R_F values in TLC became considerably higher after acetylation of the sample but after a subsequent esterification with diazomethane they did not change any more. The acetylated fraction (150 mg) was separated by TLC into five portions which were separately analyzed by means of gas chromatography. In the first fraction using reference substances β-sitosterol acetate was identified in addition to trace amounts of stigmasterol and campesterol acetates. A short homologous series of higher primary alcohols (C_{20}—C_{28}) was found in the same fraction. In the remaining fractions, the presence of some triterpenic alcohols was assumed.

In the methanolic extract both free and bound saccharides were identified by means of paper chromatography. Results are listed in Table 2. Instead of R_F the R_x values indicating the relative motion towards *l*-rhamnose were calculated.

Table 2

Identification of saccharides by means of paper chromatography

Saccharide	R_x		Colour of spots
	Reference substance	Sample	
	Free saccharides		
Glucose	0.5060	0.5106	Brown
Fructose	0.5869	0.6010	Brown
Xylose	0.7186	0.7197	Pink
	Bound saccharides		
Glucose	0.5120	0.5198	Brown
Arabinose	0.6298	0.6189	Pink
Traces of xylose	0.7186	0.7201	Pink

As seen, glucose as well as xylose appeared both in the group of free and bound saccharides. Fructose was identified only in the group of free saccharides and arabinose in the group of bound saccharides.

Acknowledgements. The authors thank Mr K. Konečný for technical assistance and valuable advices and Dr S. Vašíčková (both from the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague) for measurement and interpretation of infrared spectra.

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Translated by Z. Votický