

SUBSTANCES DETECTED ON THE SURFACE OF THE ROOTS  
OF VARIOUS SPECIES OF THE GENUS *Onobrychis adans*  
AND IN THE SOIL SURROUNDING THEM

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Undamaged fresh roots (1.2 kg) of *Onobrychis kachetica* which were brown were placed in a flask, covered with 5 liters of a mixture of chloroform and ethyl acetate (1:1), and shaken for 1 min. The roots then assumed a light-cream color. The washing solution, which had acquired a dark-orange color was filtered and concentrated. This gave 37.4 g of residue (6.74% calculated on the air-dry roots). By photochromatography in six different solvent systems, more than 30 different compounds were isolated which showed a yellow or blue color with a 3% solution of  $\text{AlCl}_3$ , a yellow, pale-blue, or deep-blue color with 10%  $\text{NaOH}$ , a pink or blue color with 3%  $\text{FeCl}_3$ , blue or green with 5% phosphotungstic acid, yellow with vanillin in concentrated  $\text{H}_2\text{SO}_4$ , red or orange with diazotized sulfanilic acid, and red with a 1% solution of phloroglucinol in concentrated  $\text{H}_2\text{SO}_4$ .

To detect these substances on the surfaces of the roots, undamaged fresh roots were carefully cleaned with a brush, and the detached parts of the sample that had been removed from the surface of the roots were dissolved in a mixture of chloroform and ethyl acetate (1:1). Paper chromatography showed the presence in this extract of the whole complex of substances found by washing the roots.

Similar results were obtained in an investigation of other species of sainfoin: *O. meshcetica*, *O. radiata*, *O. iberica*, *O. petrea*, *O. angustifolia*, *O. sosnowskji*, *O. kemularia*, *O. oxytropoides*, *O. kluchorica*, *O. daghestanica*, *O. inermis*, *O. arenaria*, *O. hamata*, *O. trancaucasica*, *O. cyri*, *O. meschetica* and *O. radiata*.

To detect the presence of these substances in the roots, 100 g of each material was previously washed, first with a mixture of chloroform and ethyl acetate (1:1) and then with ethanol until the reaction for this group of substances was negative. After this, the roots were comminuted and were extracted with chloroform-ethyl acetate (1:1). The extract (yield 0.1-0.2%) was found to contain the whole complex of substances present on the surface of the roots.

Then experiments were performed to determine these substances in the soil around the roots of the plants mentioned. Soil was taken from around the roots at a radius of 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, and 45-50 cm. The soil samples were shaken several times with chloroform-ethyl acetate-methanol (5:5:1), and the extract was filtered, concentrated, and chromatographed. The soil taken at a radius of 1-5 cm contained all the substances found on the surface of the roots. In the 5-10- and 10-15-cm zones three of the substances secreted by the roots were absent, and in the 15-20- and 20-25-cm samples another five to nine substances were absent. At a radius of 25-30 cm, only in some case were two or three substances found. Not one of these substances was found at a radius greater than 30 cm.

An extract from the soil around the roots of *O. kachetica* (5.0 g) was separated on a column of Sephadex LH20. Eight individual substances were obtained which were assigned on preliminary evidence - specific reactions and UV and IR spectral characteristics - to aromatic aldehydes, flavonoids, coumarins, lignans, and hydroxystilbenes. One of the substances was assigned by Professor G. K. Nikonov to the esters of dihydroxycinnamic acid. We identified another substance, with the composition  $\text{C}_8\text{H}_8\text{O}_3$ , mp 81-82°C, as vanillin. A substance with the composition  $\text{C}_7\text{H}_6\text{O}_2$  proved to be p-hydroxybenzaldehyde.

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Among the substances found in the soil and on the surface of the roots of the species mentioned, the main ones are substances of flavonoid nature. It is assumed that these substances when present in the soil are secreted by the roots of the plants mentioned.