METHOD FOR THE QUANTITATIVE DETERMINATION OF OSAJIN IN MACLURA AURANTIACA

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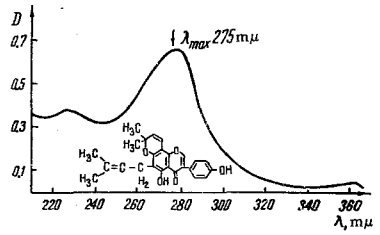
It is known that flavonoids are widely distributed in plants and have valuable pharmacological properties. The comparatively little studied group of isoflavones is of great interest, and this applies particularly to osajin, present in the fruit of Maclura aurantiaca Nutt., family Moraceae (osage orange) [1], which inhibits the growth of transplanted animal tumors by 80% [2] and possesses other valuable properties [3].

Literature information on the content of osajin in osage orange is lacking. We have determined it by a chromatospectrophotometric method which does not require large amounts of raw material and is distinguished by its high accuracy.

A consideration of the UV spectrum of osajin (figure) shows three maxima, in the 224-, 275-, and 362-mμ regions. The first and third of these maxima are not suitable for analytical purposes since they are of feeble intensity. Furthermore, the use of the short-wave maximum is unsuitable for purely technical reasons. The most suitable is the maximum in the 275-mμ region, which has the highest intensity.

The specific absorption, $E^{1\%}_{1cm}$, at this wavelength is fairly high, 1128, which makes it possible to use it for analytical purposes. We have determined the specific absorption of solutions of various concentrations (from 0.01 to 0.1 μg). At this wavelength the absorption obeys the Bouguer-Lambert-Beer law.



UV spectrum of osajin in ethanol (c 0.01 mg/ml).

Pure osajin, and also ethanolic extracts, were chromatographed on FN-3 paper. The best separation of osajin from the accompanying polyphenols [4] took place in a mixture of ethyl acetate, formic acid, and water on paper impregnated with ethylene glycol. Methanol was used as the solvent. A determination of the optical densities of extracts obtained under various conditions showed that the optimum conditions for elution are boiling for 30 min. In this case only about 96.74% (±0.4%) of the adsorbed osajin is eluted. Consequently, in the formula used for our calculations we introduced a correction factor of 1.034, and the specific absorption was taken as 1090.

The best solvent for the extraction of osajin from the plant raw material is ethanol; the most rapid equilibrium between the content of osajin in the raw material and in the extract was found with boiling for 30 min.

A combination of the optimum conditions for extraction, chromatography, and elution has enabled us to propose the method described below. To determine the accuracy of the method, we have carried out experiments with the addition of definite amounts of osajin to a weighed sample of raw material. These amounts were found in all experiments with an accuracy of ±2.29%. Six parallel experiments on one of the samples also confirmed the accuracy

of the proposed method (see table).

Content of Osajin in the Fruit of Osage Orange
(% of the Absolutely Dry Weight)

Weight of raw material	Osajin found, %	Deviation from the arithmetic mean	Square deviation from the arithmetic mean
0.25	6.30	0	0
0.25	6.40	+0.1	0.01
0.25	6.40	+0.1	0.01
0.25	6.25	−0.05	0.0025
0.25	6.30	0	0
0.25	6.25	−0.05	0.0025

Mean square deviation from the arithmetic mean	±0.0022
Confidence interval	0.0001
Maximum error of the result	0.0006
Relative error of the result	0.99 %
Interval value	6,3±0.0001

We have studied various organs of the osage orange by this method. The amount of osajin present in the fruit (6.4%) is greater than in the leaves (0.23%); it is completely absent from the bark of the stem.

EXPERIMENTAL

An accurately weighed 0.25-g sample of the air-dried material which had been comminuted and passed through a sieve (with apertures having a diameter of 0.2 mm) was placed in a 25-ml flask fitted with a reflux condenser, 10 ml of ethanol was added, and the mixture was heated in a boiling water bath for 30 min. The flask and its contents were then cooled, and the extract was brought to its original weight with pure solvent and shaken. The filtered extract, 5 ml, was mixed with 5 ml of pure solvent, and 0.02 ml of the extract was deposited by a micropipette on the starting line of a sheet of FN-3 chromatographic paper previously impregnated with a 20% solution of ethylene glycol in water and dried in air. Chromatography was carried out by the ascending method in an ethyl acetate–formic acid–water (5 : 2 : 3) system at 20° C for 24 hr. The chromatograms were brought to the air-dried state, the sections of the paper corresponding to the spots of osajin in the R_f 0.63 region (dark spots in UV light) were cut out, reduced to pieces (1–2 mm), and transferred into a 25-ml flask fitted with a reflux condenser. Then 5 ml of methanol was added to the flask and it was heated in a water bath for 30 min. After cooling, the flask was weighed and pure solvent was added to restore the original weight. The resulting solution was placed in the cell of a spectrophotometer (layer thickness of the liquid 1 cm), and the optical density of the solution was measured at a wavelength of 275 mμ.

The content of osajin (X) was calculated from the formula

$$X = \frac{D \cdot V_1 \cdot V_2 \cdot 10}{1090 \cdot a \cdot V_3 (100 - c)},$$

where D is the optical density of the eluate from the chromatographic paper;

V_1 is the volume of the extract from the raw material, after dilution with ethanol, ml;

V_2 is the volume of the solvent used for elution, ml;

V_3 is the volume of the extract deposited on the chromatogram, ml;

a is the weight of the raw material, g; and

c is the moisture content, %.

CONCLUSIONS

A chromatospectrophotometric method for the quantitative determination of osajin in various organs of Maclura aurantiaca Nutt. has been proposed. It has been established that the best type of raw material is the fruit of the plant, which contains about 6.4% of osajin.

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