## EXPERIMENTAL

Extraction of the Rotenoid Glycosides. Seeds of the *Amorpha fruticosa* crushed in a roller mill (50 kg) were extracted with chloroform–ethanol (1:1) by the steeping method. Three changes of solvent were made. The first contact of the phase lasted 5.5 h, the second 4 h, and the third 2.5 h. The extracts were combined (a total of 210 liters of extract) and were evaporated in a vacuum-evaporating apparatus to 25 liters and left for a day.

Elimination of Weakly Polar and Nonpolar Impurities from the Deposit. After a day, the precipitate that had deposited was separated off on a suction filter, suspended three times in chloroform (3 × 8 liters), the solvent being removed by suction each time, and the product was well dried in a vacuum-drying chest.

Crystallization and Recrystallization of Frutitsin. With stirring, 850 g of technical frutitsin was dissolved in 128 liters of boiling water and the resulting solution was filtered through a heated pressure filter and was left for crystallization at room temperature. After 12-16 h, the precipitate was separated off on a suction filter and the moist product was recrystallized from 47 liters of ethanol. The yield of frutitsin was 500 g, or 1% on the weight of the raw material.

## SUMMARY

1. The conditions for the isolation and purification of frutitsin by various solvents and mixtures have been studied.

2. A method for isolating frutitsin which increases the yield of the preparation is proposed.

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# QUANTITATIVE DETERMINATION OF THE AGLYCONE OF THE TOTAL PATRINOSIDES IN THE ROOTS OF *Patrinia intermedia*

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UDC 543.062+547.918:633.88

A method has been developed for the quantitative determination of the aglycone of the total patrinosides present in the roots of *Patrinia intermedia*. The method comprises the extraction of the patrinosides from the raw material, their hydrolysis to oleanolic acid, and the potentiometric titration of the aglycone obtained.

The roots of *Patrinia intermedia* Roem. et Schult. contain a whole set of triterpene glycosides the main ones of which are patrinoside B, C and D. From 9 to 11 individual glycosides are found in different populations of the plant [1]. The structures of only two of the glucosides — patrinosides D and C — have been established, but it is known that they all have a common aglycone — oleanolic acid [2].

A densitometric method has been developed for evaluating introduced forms of *P. intermedia* for their content of the main patrinosides, B, C, and D [3]. The low accuracy of the method, the presence of other triterpene glycosides in the raw material, and also the necessity for using standard samples of the glycosides being determined do not permit its use for the

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TABLE 1. Influence of the Solvents, the Conditions, and the Time of Extraction on the Completeness of Extraction of Patrinosides from the Raw Material

| Solvent | Conditions and time of extraction | Oleanolic acid content, % |
|---|---|---|
| 1. Water | Steeping for | |
| | 24 h | 5.24 |
| | 48 h | 5.82 |
| | 72 h | 6.76 |
| 2. Methanol | Soxhlet apparatus | |
| | 7 h | 6.45 |
| 3. 50% ethanol | Boiling under reflux for | |
| | 3 h | 6.77 |
| | 4 h | 6.84 |
| | 5 h | 7.20 |
| | 6 h | 7.20 |

quantitative evaluation of a raw material. We have developed a method for the quantitative determination of oleanolic acid — the common aglycone of the total patrinosides. The method comprises three stages: extraction of the patrinosides from the raw material, their hydrolysis to oleanolic acid, and the potentiometric titration of the aglycone obtained.

The patrinosides are readily soluble in water, methanol, and aqueous ethanol. All these solvents were tested in selecting the optimum conditions for the extraction of the patrinosides from the raw material. The methods and times of extraction were also varied (Table 1). It was established that the most complete extraction of the patrinoside from the raw material is achieved by boiling a comminuted sample of the raw material in 50% ethanol for 5 h.

Various methods for hydrolyzing triterpene glycosides have been described in the literature in which the hydrolyzing reagents used have been hydrochloric and sulfuric acids in various concentrations, and also mixtures of these acids with acetic acid in various ratios. Each of the methods of hydrolysis mentioned has its advantages and disadvantages. For convenience of passage from the stage of extracting the patrinosides to their hydrolysis, we used the method of hydrolyzing the triterpene glycosides proposed by Japanese authors [4]. Hydrolysis was carried out by boiling the glycosides with 2 N sulfuric acid in 50% ethanol for 2 h. We monitored the process of hydrolyzing the patrinosides with the aid of thin-layer chromatography. The presence of patrinosides in the reaction mixture was checked in the chloroform—methanol—water (61:32:7) system, and the purity of the oleanolic acid formed in the chloroform—methanol (48:2) system. The time of hydrolysis had to be increased to four hours, since after a shorter time the patrinosides are not completely hydrolyzed. Figure 1 shows a chromatogram of the products of the hydrolysis of an aqueous ethanolic extract of the patrinia in the chloroform—methanol (48:2) system. The substance with the $R_f$ value higher than of oleanolic acid is obviously a product of its partial degradation. Not more than 1% of this by-product is formed.

Pure standard oleanolic acid was treated under the conditions of hydrolysis. On a Silufol plate, 100 and 200 μg of the product of hydrolytic treatment and 1 and 2 μg of pure oleanolic acid were deposited. A correspondence was detected between the intensity of the coloration of the spot of the oleanolic acid in 1 μg and of the by-product when 200 μg of the mixture was deposited taking into account the fact these substances have similar sensitivities.

It is known that, in addition to triterpene glycosides, the roots of *Patrinia intermedia* contain accompanying glycosides, the aglycone of which is not a triterpene compound [5]. The substance with an $R_f$ value lower than that of oleanolic acid is apparently this aglycone. This substance, when isolated preparatively, is not titrated under the conditions of the method.

Since oleanolic acid is insoluble in water, the potentiometric titration of the product obtained was carried out in ethanol using as titrant a 0.05 N solution of caustic potash in ethanol. The metrological characteristics of the results of the analysis of three samples of *P. intermedia* roots in sextuplicate are given below. The error of an individual determination amounts to ±5%.

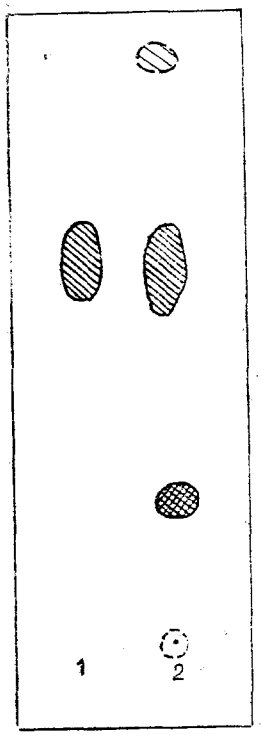


Fig. 1. Chromatogram of the products of the hydrolysis of the patrinosides: 1) oleanolic acid marker; 2) product of the hydrolysis of the patrinosides [solvent system: chloroform–methanol (48:2)].

| $f$ | $\bar{X}$ | $S$ | $P$, % | $t(P, f)$ | $\Delta X$ | $E$, % |
|---|---|---|---|---|---|---|
| 5 | 5,71 | ±0,099 | 95 | 2,571 | ±0,25 | ±4,44 |
| 5 | 5,58 | ±0,109 | 95 | 2,571 | ±0,28 | ±5,02 |
| 5 | 5,29 | ±0,102 | 95 | 2,571 | ±0,26 | ±4,97 |

The absence of systematic losses of the substance being determined was shown by addition experiments. To an aqueous ethanolic extract of patrinia with a known oleanolic acid content before hydrolysis were added accurately weighed amounts of pure oleanolic acid. It can be seen from the result given below that the relative error is within the limits of accuracy of the procedure developed (oleanolic acid content 5.58%):

| Weight of raw material | Weight of oleanolic acid, g | Total initial amount of oleanoic acid, g | Found, g | Relative error, % |
|---|---|---|---|---|
| 1.0010 | 0.0161 | 0.0719 | 0.0711 | −1.10 |
| 0.5000 | 0.0216 | 0.0495 | 0.0505 | +2.02 |
| 1.0000 | 0.0221 | 0.0779 | 0.0759 | −2.57 |

Determination Procedure. A 2-g sample (accurately weighed) of the comminuted roots of *Patrinia intermedia* with a particle size of 0.25 mm in a filter-paper thimble was covered with 100 ml of 50% ethanol and this was boiled under reflux on the water bath for 5 h. Of the extract obtained, 50 ml was treated with 30 ml of ethanol and 4.4 ml of concentrated sulfuric acid. This mixture was boiled under reflux on the water bath for 4 h. Then it was diluted twofold with water. The precipitate that had deposited was filtered off, washed with water until the reaction to universal indicator paper was neutral, and was dried. The dried precipitate was dissolved in hot ethanol and was titrated potentiometrically with a 0.05 N solution of caustic potash in ethanol. The percentage content of oleanolic acid was calculated from the formula

$$X = \frac{0.02285\ K(Y-Y_1)\ 100 \cdot 100}{P(100-B)\ 50} = \frac{0.0457\ K(Y-Y_1)\ 100}{P(100-B)},$$

where 0.02285 is the amount of oleanolic acid corresponding to 1 ml of 0.05 N caustic potash solution, g;

K is the correction for the titer of the 0.05 N KOH solution;

Y is the amount of titrant consumed in the titration of the sample, ml;

$Y_1$ is the amount of titrant consumed in the titration of a blank sample, ml;

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

B is the loss in weight of the raw material on drying, %.


## SUMMARY

1. A method has been developed for the quantitative determination of the aglycone of the combined patrinosides in the roots of *Patrinia intermedia*. The error of a single determination is ±5%.

2. The method can be used for the quantitative evaluation of a raw material.


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# THE STRUCTURE OF THELENOTOSIDES A AND B FROM THE HOLOTHURIAN *Thelenota ananas*


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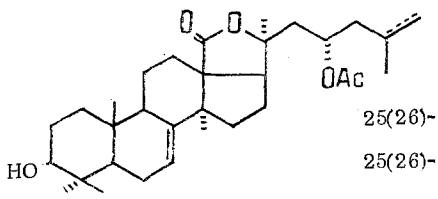
Two new triterpene tetraosides — thelenotosides A and B — have been obtained from the holothurian *Thelenota ananas*. Their complete structures have been determined as 23(S)-acetoxy-3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-quinovopyranosyl-(1→2)-β-D-xylopyranosyloxy]holost-7-ene and 23(S)-acetoxy-3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranosyloxy]holost-7-ene, respectively.


Continuing an investigation of the glycosides of holothurians of the family *Stichopodidae* [1, 2], we have studied the composition of the glycosidic fraction of the industrial Pacific Ocean holothurian *Thelenota ananas*. Two new physiologically active glycosides — thelenotosides A and B — have been obtained.

The native genins of the glycosides of *T. ananas* have a 7(8)- and not an 8(9)- double bond in the holostane nucleus, as was considered previously [3, 4] and are 23(S)-acetoxyholost-7-en-3β-ol (I) and 23(S)-acetoxyholosta-7,25-dien-3β-ol (II), respectively [5, 6].




25(26)-Dyhydro — I

25(26)-Dehydro — II

In order to determine the complete structures of the thelenotosides, we separated the total glycosides from *T. ananas* into a series of chromatographically individual fractions. Analysis of the $^{13}C$ NMR spectra of each of them showed that they were two-component mixtures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