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XANTHONES FROM *Halenia corniculata*. 2. QUANTITATIVE DETERMINATION OF TOTAL γ-PYRONE CONTENT IN THE AERIAL PART

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A method for quantitative determination of total γ-pyrone content in the aerial part of Halenia corniculata *was developed. Their total content in various samples of the aerial part was investigated.*

Key words: *Halenia corniculata,* γ-pyrone compounds, chromato-spectroscopy, quantitative determination.

We previously established that the principal active compounds from *Halenia corniculata* (L.) Cornaz (Gentianaceae) are xanthones and flavones [1].

The development of objective methods for quantitative determination of the content of biologically active compounds in starting material and medicinal preparations is an important step in the development of medicinal preparations.

Therefore, our task was to develop a method for quantitative determination of the total γ-pyrone content in the aerial part of *Halenia corniculata*.

The present article also includes results from a study of the total γ-pyrone content in various samples of the aerial part of this plant.

We used a chromato-spectrophotometric method for quantitative determination of the γ-pyrone content. The standard was 1-hydroxy-2,3,4,5-tetramethoxyxanthone (**1**), the dominant component of the total γ-pyrones. Its absorption maximum in the UV spectrum (260 ± 1) is close to those of the second dominant xanthone, 1-hydroxy-2,3,5-trimethoxyxanthone (257 ± 3), and luteolin (253 ± 1) and to that of the alcohol extract of the hydrolyzed raw material (254 ± 1) (Fig. 1). The analytical wavelength was 254 nm, which is situated between the peaks in the UV spectra of the xanthones, flavone, and the alcohol extract. It has been found that the optical density as a function of concentration for **1** is linear in the range $1.2 \cdot 10^{-6}$-$12.0 \cdot 10^{-6}$ g/mL.

The optimal conditions for the extraction became clear during development of the quantitative determination method: extractant, 60% ethanol; extraction temperature, 90°C; particle size of raw material, 0.5-1 mm; raw-material/extractant ratio, 1/60; time and repetition of extraction: I, 60 min; II, 60 min; III, 45 min.

The extract was purified of accompanying substances by chromatographic separation over a column of polyamide sorbent. Ballast substances (blue band in UV light) were eluted by water; γ-pyrones (absorbing band in UV light), by 95% ethanol. However, it was noted that preliminary elution with water gave effluents containing O-glycosides of γ-pyrones (TLC monitoring). In order to avoid these losses (up to 4%), the plant material was extracted with 60% ethanol containing HCl (5%). The glycoside bonds were completely destroyed in 1 h (TLC).

A correction factor was introduced into the formula for quantitative determination of total γ-pyrones in the aerial part of *Halenia corniculata*. This took into account incomplete desorption from the column of the eluted substances. This coefficient (K_{elu}), equal to 1.093, was determined experimentally for **1** in a series of ten independent determinations.

Table 1 gives the metrological parameters of the quantitative method for determining the total γ-pyrone content in the aerial part of *Halenia corniculata* (calculated for 1-hydroxy-2,3,4,5-tetramethoxyxanthone). The relative uncertainty of a single measurement was ±3.72%.

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TABLE 1. Metrologic Parameters of the Quantitative Method for Determining Total γ-Pyrone Content in the Aerial Part of *Halenia corniculata*

n	f	$\overline{x}$	S^2	S	S_x	P	t (p, f)	±Δx	E
10	9	6.53	0.1156	0.34	0.1075	95	2.26	0.24	3.72

TABLE 2. Validity Check of the Developed Method by the "Added—Found" Method

Added **1**, g · 10^{-3}	Theoretical content of γ-pyrones calculated for **1**, g · 10^{-3}	Found γ-pyrones calculated for **1**, g · 10^{-3}	Relative uncertainty, %
0.40	7.02	6.83	-2.71
0.60	7.22	7.33	+1.52
0.80	7.42	7.23	+2.56

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γ-Pyrones in an aliquot calculated for **1** was $6.62 \cdot 10^{-3}$.

TABLE 3. Quantitative Determination of Total γ-Pyrones in Various Samples of the Aerial Part of *Halenia corniculata*

Site and date of collection	*Total γ–pyrone content, %* (of abs. dried mass)
Region:	
Selenginsk (2001)	9.22
Selenginsk (2002)	7.86
Barguzinsk (2002)	8.58
Selenginsk (2003)	5.80
Nerchensko-Zavodsk	
Chita district (2003	8.53
Zakamensk (2004)	10.69
Okinsk (2004)	9.05

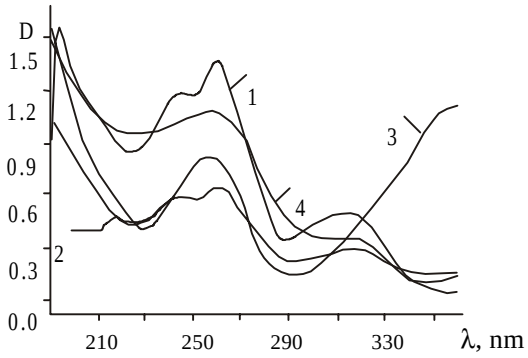

Fig. 1. Absorption spectra of 1-hydroxy-2,3,4,5-tetramethoxy-xanthone (*c* $4 \cdot 10^{-6}$ g/mL, 1), 1-hydroxy-2,3,5-trimethoxyxanthone (*c* $4 \cdot 10^{-6}$ g/mL, 2), luteolin (*c* $4 \cdot 10^{-6}$ g/mL, 3), and the alcohol extract of the aerial part of *Halenia corniculata* after hydrolysis (4).

The relative uncertainty for the "added—found" method was less than 2.71% (Table 2).

The method developed by us was used to analyze various samples of raw material from the aerial part of *Halenia corniculata*. We have found that the total γ-pyrone content lies in the range 5.80-10.69% (Table 3). Thus, we developed a chromato-spectrophotometric method for standardizing the total γ-pyrone content of the aerial part of *Halenia corniculata* and found that the total xanthone and flavone contents should be at least 5.80% (of the abs. dry mass).

EXPERIMENTAL

We used freshly distilled solvents and pure-grade reagents.

Optical density of solutions was measured on an SF-26 spectrophotometer.

Extract of *Halenia corniculata* was separated chromatographically over a polyamide column (TU 6-09-10-822-73) using purified water and 95% ethanol as eluents.

The separation was monitored using TLC (Silufol, hexane:ethylacetate, 7:3).

The aerial part of *Halenia corniculata* was collected during mass flowering in 2001-2004 in Selenginsk, Barguzinsk, Zakamensk, and Okinsk regions of the Republic of Buryatiya and Nerchensko-Zavodsk region of Chita district.

Quantitative Method for Determining γ-Pyrones in the Aerial Part of *Halenia corniculata*. An analytical sample of the raw material was ground until the particles passed through a 1-mm sieve.

Ground raw material (~1 g, accurate weight) was placed in a 100-mL round-bottom flask and treated with ethanol (60%, 60 mL) containing HCl (5%). The flask and contents were connected to a reflux condenser and heated on a water bath for 60 min at 90°C, cooled, and filtered into a 200-mL volumetric flask. The solid in the flask and on the filter was washed with ethanol (60%, 20 mL). The extraction was repeated twice under the same conditions using 90% ethanol for 60 and 45 min, respectively. The effluents were combined. The total volume was adjusted to the mark with 60% ethanol (solution A).

Solution A (1 mL) was placed on a polyamide column. The column was eluted with purified water (50 mL) at 4 mL/min. The aqueous effluent was discarded. Total γ-pyrones were eluted with 95% ethanol (50 mL) with monitoring of their movement using UV light. When the band reached the lower part of the sorbent, the effluent was collected in a 50-mL volumetric flask. The volume of the effluent was adjusted to the mark with 95% ethanol (solution B).

The optical density of solution B was determined on the spectrophotometer at 254 nm in cuvettes with a 1-cm pathlength.

The reference solution was 95% ethanol.

The optical density of a solution (0.0006%) of 1-hydroxy-2,3,4,5-tetramethoxyxanthone in ethanol (95%) was determined in parallel.

The total γ-pyrone content (X) in percent calculated for 1-hydroxy-2,3,4,5-tetramethoxyxanthone and absolute dry raw material was calculated using the formula:

$$X = \frac{D}{D'} \times \frac{100\times50}{1} \times \frac{m'}{m} \times \frac{10\times5}{50\times100\times25} \times K_{ins} \times K_{elu} \times \frac{100}{100 - W} \times 100,$$

where D and D' are the optical densities of the investigated solution and the solution of standard 1-hydroxy-2,3,4,5-tetamethoxyxanthone; m is the raw-material mass (g); m' is the mass of 1-hydroxy-2,3,4,5-tetramethoxyxanthone (g), K_{ins} is the instrument correction for the spectrophotometer and cuvettes (0.997); K_{elu} is the elution coefficient (1.093); and W is the mass lost on drying the raw material (%).

Column Preparation. Polyamide sorbent (1.5 g, TU 6-09-10-822-73) was placed in a 50-mL cylinder, treated with purified water (30 mL), stirred, and poured into the column (d = 1.5 cm, h = 30 cm), the lower part of which contained a cotton filter. The column was filled with an open stopcock. A cotton plug was placed over the upper sorbent layer.

Preparation of a Standard Solution (0.0006%) of 1-Hydroxy-2,3,4,5-tetramethoxyxanthone. Standard 1-hydroxy-2,3,4,5-tetramethoxyxanthone (15 mg, accurate weight) was placed in a 50-mL volumetric flask. Ethanol (95%) was added to the mark (solution A). Solution A (10 mL) was transfered to a 100-mL volumetric flask and adjusted to the mark with the same solvent (solution B). Solution B (5 mL) was transfered to a 25-mL volumetric flask and adjusted to the mark with the same solvent (solution C).

REFERENCE

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