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XANTHONES FROM *Halenia corniculata*. 3. PREPARATION OF STANDARD 1-HYDROXY-2,3,4,5-TETRAMETHOXYXANTHONE

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1-Hydroxy-2,3,4,5-tetramethoxyxanthone, which is used as a standard for quantitative determination of the total content of γ-pyrones, was isolated from the aerial part of Halenia corniculata *L. Cornaz., an available raw material in Russia. A method for preparing standard 1-hydroxy-2,3,4,5-tetramethoxyxanthone was developed.*

Key words: xanthones, *Halenia corniculata*, standard sample, 1-hydroxy-2,3,4,5-tetramethoxyxanthone.

We previously proposed a method for quantitative determination of the total content of γ-pyrones in the aerial part of *Halenia corniculata* L. Cornaz. [1]. The standard sample in this method was 1-hydroxy-2,3,4,5-tetramethoxyxanthone (**1**), which is the dominant component of the xanthones in the aerial part of *H. corniculata*.

Several methods for preparing **1** are known: from roots and the aerial part of *Swertia bimaculata* Hf. & T. in 0.003% yield [2]; from roots of *Frasera caroliniensis* Walt., in 0.07% yield [3]; and from roots and the aerial part of *H. asclepidea* (HBK) Don, *H. elliptica* D. Don, and *H. campanulata* in yields of 0.10, 0.12, and 0.13%, respectively [4-6].

Drawbacks of these methods are the unavailability of the raw material, the low yields (0.003-0.13% per mass of absolute dry raw material), and the duration and labor-intensiveness of the process.

The goal of the present work was to develop a method for preparing standard **1** from the aerial part of *H. corniculata* growing in Russia.

Several methods for preparing **1** were studied during the course of the work. Method 1 produced **1** in 0.04% yield. Methods 2-4 under the same conditions but using different eluents for column chromatography also isolated **1** in yields of 0.25, 0.12, and 0.04%, respectively. Method 5 involves simultaneous extraction of raw material and hydrolysis by HCl (2%). The resulting product (0.02% yield) was contaminated with oleanolic acid, which interferes with the separation and preparation of **1** as a pure compound.

Method 2 gave the highest yield of **1** from the aerial part of *H. corniculata* and was used to produce three batches of **1**. The content of **1** as determined by HPLC was 95.31-96.87% (Fig. 1). The compounds were identified using TLC, melting points, and UV and PMR spectroscopies. The physicochemical properties of the final product were analogous to those of chemically pure **1**.

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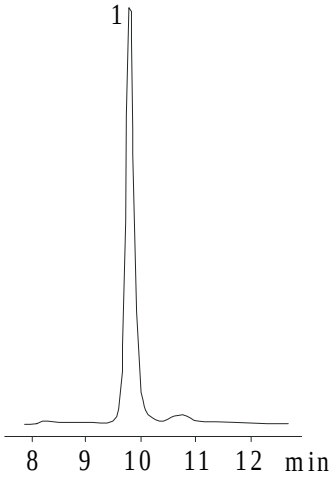

Fig. 1. HPLC of standard **1** prepared by method 2.

EXPERIMENTAL

We used freshly distilled solvents and pure-grade reagents. Melting points were determined on a Kofler microstage. Elemental analyses were obtained on a CHN-analyzer (Carlo Erba, model 1106). IR spectra were recorded on a spectrometer (Vector 22) in KBr disks; UV absorption spectra, in a UV-Vis spectrometer (HP 8453) in ethanol ($c = 10^{-4}$ M). Molecular weights and elemental formulas were determined in a high-resolution mass spectrometer (Finnigan MAT 8200). NMR spectra were obtained on a spectrometer (Bruker AC 200, working frequency 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C) in $CDCl_3$. Signals in the PMR spectra were assigned using COSY H—H correlation spectroscopy. The multiplicity of signals in the ^{13}C NMR spectra were determined using standard methods for recording spectra with J-modulation (JMOD). Xanthones were separated by column chromatography over silica gel (L 100/400 and L 100/250) with elution by $CHCl_3$ (I) and hexane:ethylacetate (7:3, II; 8:2, III; 6:4, IV). The separation of compounds was monitored using TLC on Silufol plates and solvent system II with subsequent development by $AlCl_3$ solution (5%) in ethanol and by visually following the movement of bands on the column in UV light. The purity of the compounds were determined by HPLC using a microcolumn liquid chromatograph (Millichrom A-02). The chromatography conditions were: 2×75 mm column, ProntoSIL-120-5-C-18 AQ sorbent (No. 0322, 5 μm), 35°C, 3.2 MPa, elution rate 150 μL/min, UV detection at 244, 260, and 320 nm, eluent trifluoroacetic acid in CH_3OH (0.1%).

The aerial part of *H. corniculata* was collected in August 2001 during flowering in Kaban and Ivolgin regions (Republic of Buryatia).

Method 1. Air-dried and ground *H. corniculata* (100 g) was extracted with ethanol (95%, 3×1000 mL). The combined alcohol extracts were filtered and concentrated to a volume of 200 mL. The resulting extract was transferred to a separatory funnel and diluted with purified water (100 mL). Xanthone aglycons were extracted with $CHCl_3$ (3×1000 mL). The combined $CHCl_3$ extracts were concentrated to a volume of 100 mL, mixed with silica gel (L 100/250 μm, 40 g), dried, and chromatographed over a silica-gel column (L 100/250 μm, 100 g, 4×50 cm) using $CHCl_3$. Fractions (50 mL) were collected. Fractions 14-18 containing **1** were combined and concentrated to dryness. The solid was recrystallized twice from ethanol.

Method 2. The $CHCl_3$ fraction obtained using Method 1 was placed on a silica-gel column (L 100/250, 100 g, 4×50 cm) and eluted using solvent system II. Fractions (50 mL) were collected. Fractions 9-12 containing **1** were combined and concentrated to dryness. The solid was recrystallized twice from ethanol.

Methods 3 and 4. These were analogous to Method 2 but used solvent systems III and IV.

Method 5. Air-dried and ground *H. corniculata* (100 g) was extracted with HCl (2%) in ethanol (95%) on a boiling-water bath for 3 h (3×1000 mL). The combined alcohol extracts were filtered and concentrated to a volume of 200 mL. The resulting extract was transferred to a separatory funnel and diluted with purified water (100 mL). Xanthone aglycons were extracted with $CHCl_3$ (3×500 mL). The combined $CHCl_3$ extracts were washed with water until the washings were neutral and concentrated to a volume of 100 mL. Subsequent workup was analogous to that of method 1.

1-Hydroxy-2,3,4,5-tetramethoxyxanthone (1). mp 146-147°C (EtOH), R_f 0.29 (II), 0.71 (IV).

IR spectrum (ν, cm^{-1}): 721, 778, 843, 951, 991, 1052, 1502, 1587, 1610 (C=C), 1655 (C=O).

UV spectrum (λ_{max}, MeOH): 244, 260, 275sh, 314, 378 nm.

PMR spectrum (δ, ppm, J/Hz): 3.92 (3H, s, OCH_3), 4.00 (6H, s, 2×OCH_3), 4.12 (3H, s, OCH_3), 7.22 (2H, m, H-6,7), 7.77 (1H, dd, J = 7.5 and J = 2.0, H-8), 12.55 (1H, s, OH).

^{13}C NMR spectrum (δ_C, ppm): 56.42 (CH_3), 61.47 (CH_3), 61.72 (2×CH_3), 95.12 (C-4), 106.79 (C-8a), 115.92 (C-6), 116.49 (C-7), 120.83 (C-5a), 123.56 (C-8), 132.69 (C-4a), 135.42 (C-1a), 145.62 (C-5), 148.66 (C-3), 150.49 (C-2), 154.08 (C-1), 181.58 (C-9).

Mass spectrum (*m*/*z*, %): 347 (13.55), 332 (83.87), 317 (100), 302 (13.27), 289 (10.83), 259 (11.17), 175 (11.68). $C_{17}H_{16}O_7$. Calc. *m*/*z*: 332.08959. Exp. *m*/*z*: 332.08801.

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