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B R I E F C O M M U N I C A T I O N S

BIOLOGICALLY ACTIVE COMPOUNDS FROM *Cacalia hastata* LEAVES. 3. ORGANIC ACIDS

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Chromatographic analysis of *Cacalia hastata* L. leaves detected organic acids that had not previously been investigated. Quantitative determination by a potentiometric titration method [1] of organic acids in leaves collected from the end of May to the start of September 1998-2002 in Pribaikal (Pr), Mukhorshibirsk (Mk), and Zaigraevsk (Zr) regions of Buryatiya Republic showed that their content is maximal during budding to the start of flowering: 9.97-10.24% (1998, Pr), 11.20-11.56 (1999, Pr), 13.30-13.64 (2000, Pr, Mk), 11.97-12.75 (2001, Pr, Mk), 10.98-11.22 (2002, Mk, Zr).

We attempted to isolate and identify the dominant compounds of this class. The literature method [2] afforded total organic acids (27.12 g, 9.04% of the absolute dry weight), which were a light brown hygroscopic powder without a scent and with an acid-bitter taste that was very soluble in water and ethanol. Extraction chromatographic separation of the total organic acids [2] isolated four compounds. The chemical structures and purities of the isolated compounds were established using color reactions [3-6], the Buch test [7], melting points, lack of melting-point depression in mixtures, chromatographic mobilities, and ^{13}C NMR spectroscopy (with signals assigned taking into consideration spectra of pure compounds).

One-dimensional paper chromatography was performed in ascending mode at 5-7°C on FN-16 paper using butanol:formic acid:water (system I, 9:1:3) and ethylether:formic acid:water (system II, 18:5:5). Alcoholic sodium bromphenol blue (0.5%) was used as the developer.

Compound 1, 0.639 g, 0.21%, $C_2H_2O_4$, colorless needle-like crystals, mp 99.9-101.5°C (H_2O), positive H_2SO_4—gallic reaction, R_f 0.57 (I), 0.50 (II), ^{13}C NMR (125.7 MHz, DMSO-d_6, ppm): 161.1 (s, COOH), ethanedicarboxylic acid (oxalic acid).

Compound 2, 1.138 g, 0.38%, $C_4H_6O_6$, colorless prismatic crystals, mp 170.5-171.1°C (H_2O), positive vanillin reaction, R_f 0.38 (I), 0.36 (II), ^{13}C NMR (125.7 MHz, DMSO-d_6, ppm): 72.3 (s, CHOH), 173.3 (s, COOH), 2,3-dihydroxybutanedioic acid (tartaric acid).

Compound 3, 1.016 g, 0.34%, $C_6H_8O_7$, colorless needle-like crystals, mp 151.5-152.2°C (H_2O), positive reaction with diphenylamine, R_f 0.48 (I), 0.45 (II), ^{13}C NMR (125.7 MHz, DMSO-d_6, ppm): 42.8 (s, CH_2), 72.6 (s, COH), 171.4 (s, COOH), 174.6 (s, COOH), 2-hydroxy-1,2,3-propanetricarboxylic acid (citric acid).

Compound 4, 1.689 g, 0.56%, $C_4H_6O_5$, colorless crystals, mp 129.8-103.6°C (H_2O), positive reaction with β-naphthol, R_f 0.68 (I), 0.56 (II), ^{13}C NMR (125.7 MHz, DMSO-d_6, ppm): 39.5 (s, CH_2), 67.2 (s, CHOH), 172.1 (s, COOH), 174.9 (s, COOH), 2-hydroxybutanedioic acid (malic acid).

REFERENCES

1. D. N. Olennikov, L. M. Tankhaeva, and G. G. Nikolaeva, *Vestn. Buryat. Gos. Univ.*, **3**, 163 (2003).
2. S. V. Soldatenkov and T. A. Mazurova, *Biochemical Methods in Plant Physiology* [in Russian], Moscow (1971).
3. A. R. Jones, E. J. Dowling, and W. J. Skraba, *Anal. Chem.*, **25**, 394 (1953).
4. I. I. Grandberg, *Practical Studies and Seminars in Organic Chemistry* [in Russian], Moscow (2001).

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0009-3130/04/4003-0289

5. R. Pohlovdek-Fabini and T. Beyrich, *Organic Analysis. Handbook on Analysis of Organic Compounds* [Russian translation], Leningrad (1981).
6. J. Houben and T. Weyl, *Methoden der Organischen Chemie*, Band II, *Analytische Methoden* [Russian translation], Moscow (1963).
7. M. L. Buch, R. Montgomery, and W. L. Porter, *Anal. Chem.*, **24**, 489 (1952).