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BIOLOGICALLY ACTIVE SUBSTANCES FROM *Cacalia hastata* LEAVES. 5. COUMARINS AND TRITERPENES

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In continuation of research on the chemical composition of *Cacalia hastata* L. leaves, a batch (1.0 kg) was processed in a Soxhlet apparatus successively by C_6H_{14}, $CHCl_3$, and EtOAc. After saponification (10% KOH/EtOH), the $CHCl_3$ extract was diluted in water (1:5). The resulting solid was filtered off and chromatographed over silica gel ($CHCl_3$:EtOH, 100:0→90:10) with TLC monitoring (C_6H_6:MeOH, 4:1; C_6H_{14}:C_6H_6:$CHCl_3$:MeOH, 5:4:2:1) to isolate two compounds **1** and **2**.

The aqueous phase after saponification was acidified and extracted with $CHCl_3$. The extract was chromatographed over silica gel ($CHCl_3$:EtOH, 100:0→95:5) with paper chromatographic (formamide:$CHCl_3$, system I) and TLC ($CHCl_3$:EtOH, 19:1, system II) monitoring. The isolated compounds were rechromatographed in a free layer of silica gel (40/100, system I) and recrystallized from MeOH to afford three compounds **3**-**5**.

Qualitative reactions established that **1** and **2** were triterpenes; **3**-**5**, coumarins. The isolated compounds were identified using UV spectroscopy, chromatographic mobility, melting and mixed melting points, chemical transformations, and optical rotations.

Compound **1**, yield 0.05%, mp 139-140°C, $[\alpha]_D^{20}$ -36° (*c* 0.5, $CHCl_3$) [1], identified as β-sitosterol.

Compound **2**, oleanolic acid [2], yield 0.12%, pale yellow needlelike crystals, mp 308.0-309.8°C, $[\alpha]_D^{20}$ +78° (*c* 0.5, $CHCl_3$), UV spectrum (H_2SO_4, λ_{max}, nm) 310 [1]. Acetylation of **2** produced the acetate with mp 257-258°C.

Compounds **3**-**5** were reduced with hydriodic acid [3] to give a compound with mp 66-68°C, UV spectrum (EtOH, λ_{max}, nm) 272, 328, that was characterized as coumarin [4].

Compound **3**, yield 0.015%, mp 202-203°C (MeOH), TLC R_f 0.55 (system I), 0.52 (system II), UV spectrum (EtOH, λ_{max}, nm) 231, 295, 340, scopoletin [4].

Compound **4**, yield 0.009%, mp 230-232°C (EtOH), TLC R_f 0.18 (system I), 0.041 (system II), UV spectrum (EtOH, λ_{max}, nm) 228, 256, 330, umbelliferone [5]. The structure of **4** was confirmed by direct synthesis from malic acid and resorcinol by a Pechmann reaction [6].

Compound **5**, yield 0.001%, mp 269-270°C (EtOH), TLC R_f 0.02 (system I), 0.15 (system II), UV spectrum (EtOH, λ_{max}, nm) 262, 305, 356, esculetin [5].

Coumarins in *C. hastata* leaves were determined quantitatively using chromatography and spectrophotometry and the specific extinction coefficients E (1 cm, 1%) [7]; triterpenes calculated for oleanolic acid, by spectrophotometry using a halochromic reaction with conc. H_2SO_4 [8]. It was found that the contents of scopoletin, esculetin, and triterpenes in *C. hastata* leaves increased during mass flowering, reaching values of 0.031, 0.002, and 0.92%, respectively, and slightly decreased by autumn (Table 1). The exception was umbelliferone, the concentration of which increased toward the end of vegetation.

Triterpenes in representatives of the genus *Cacalia* L. have been observed in *C. aconitifolia* (oleanolic acid acetate) [2] and *C. kamtschatica* (β-amurin, β-sitosterol, stigmasterol, campesterol) [9, 10]. Coumarins in *C. hastata* have not been previously investigated. All compounds were isolated from *C. hastata* leaves for the first time.

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TABLE 1. Accumulation Dynamics of Coumarins and Triterpenes in *C. hastata* L. Leaves, %

Substance	Start of vegetation (June 1)	Vegetation (June 11)	Vegetation (June 22)	Budding (July 28)	Mass flowering (August 15)	Fruiting (August 28)
Scopoletin	0.010±0.001	0.010±0.002	0.013±0.002	0.028±0.004	0.031±0.005	0.012±0.005
Umbelliferone	0.005±0.001	0.006±0.001	0.015±0.001	0.017±0.001	0.020±0.002	0.028±0.003
Esculetin	-	-	0.001	0.001	0.002	0.001
Triterpenes	0.31±0.03	0.44±0.04	0.74±0.09	0.86±0.08	0.92±0.11	0.82±0.09

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