COUMARINS FROM *Eucalyptus viminalis* LEAVES

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The genus *Eucalyptus* L'Her numbers over 500 species [1]. According to the USSR State Pharmacopoeia, XIth Ed., leaves of *E. viminalis* Labill. are an official plant raw material [2].

We have previously reported research on hydroxycinnamic acids, flavonoids, and tanning agents from leaves of *E. viminalis* [3]. In continuation of that work we have isolated and identified six coumarins from this raw material.

Air-dried leaves of *E. viminalis* (5.0 kg) were extracted with purified water (15.0 L) at 95°C for 2 h and left to stand for 12 h. The extraction was carried out three times with new portions of purified water. The aqueous extracts were combined (40.6 L), evaporated at 85°C in a vacuum-circulator apparatus at 690 mm Hg to a volume of 1.0 L, and left to stand for 4 d. The supernatant liquid was separated. Polysaccharides were precipitated first from the aqueous solution by adding a three-fold amount of EtOH (96%). The precipitate was filtered off. The filtrate was evaporated to an aqueous residue that was fractionated using $CHCl_3$, EtOAc, and BuOH. The completeness of the fraction separations was monitored using PC and TLC.

Next the $CHCl_3$ and EtOAc fractions were studied. Paper chromatography showed that the $CHCl_3$ fraction contained coumarins, chlorophylls, phospholipids, and triterpene compounds; the EtOAc extract, coumarins, flavonoids, and hydroxycinnamic acids. The $CHCl_3$ fraction was treated successively with NH_4OH and $NaHCO_3$ and NaOH solutions (1%), by which the coumarins were transferred to the aqueous phase as salts. The resulting aqueous solutions were acidified with HCl to pH 4–5 and re-extracted with $CHCl_3$ (fractions 1, 2, and 3, respectively). The $CHCl_3$ and EtOAc fractions were evaporated. The dry solid was dissolved in a small amount of EtOH (96%), spotted using a capillary on chromatographic paper impregnated beforehand with formamide–acetone (1:3), and chromatographed in descending mode using $CHCl_3$ and hexane. Chromatograms were visualized using a diazo reagent and alcoholic KOH (10%) to identify at least six coumarin-type compounds that were separated over a column of silica gel (fraction:sorbent, 1:250, $h = 80$, $d = 3$ cm, elution by C_6H_6:$CHCl_3$ with increasing concentration of the latter after 1 L). The separation was monitored using UV light and PC.

Fraction 1 afforded **1** (0.058 g) and **2** (0.043); fraction 2, **3** (0.033); fraction 3, **4** (0.034); EtOAc fraction, **5** (0.048) and **6** (0.065).

Physicochemical and chemical methods (PC, TLC, UV and IR spectroscopy, specific rotation, melting point, acid and enzymatic hydrolysis) were used to identify the isolated compounds [4]. Products from acid hydrolysis of **5** and **6** contained D-glucose according to polarography and chromatography using *n*-BuOH:CH_3CO_2H:H_2O (4:1:2) and $(CH_3)2CO$:*n*-BuOH:H_2O (7:2:1).

Thus, the compounds isolated from leaves of *E. viminalis* were identified as:

1, $C_9H_6O_3$, mp 228–230°C, λ_{max} 231, 258, 327 nm; 7-hydroxycoumarin or umbelliferone;

2, $C_{10}H_8O_4$, mp 202–204°C, λ_{max} 230, 255, 296, 346 nm; 6-methoxy-7-hydroxycoumarin or scopoletin;

3, $C_{19}H_{13}O_7$, mp 254–256°C; 7-methoxy-6-hydroxy-3,7′-dicoumarinic ether or daphnoretin;

4, $C_9H_6O_2$, mp 67–69°C; coumarin;

5, $C_{16}H_{18}O_9$, mp 218–220°C, λ_{max} 231, 330 nm, $[\alpha]_D^{20}$ −8.5° (DMF); 6-methoxy-7-*O*-*β*-D-glucopyranosylcoumarin or scopolin;

6, $C_{15}H_{16}O_8$, mp 218–220°C, λ_{max} 220, 252, 325 nm, $[\alpha]_D^{20}$ −80° (MeOH); 7-*O*-*β*-D-glucopyranosylcoumarin or skimmin.

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Thus, coumarin, umbelliferone, scopoletin, daphnoretin, and their glycosides skimmin and scopolin were isolated for the first time from leaves of *E. viminalis*.

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