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COMPONENTS OF *Capsicum annuum* FRUIT

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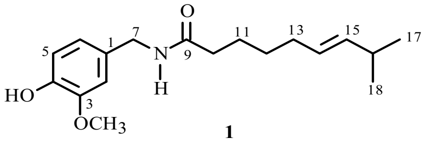
The pepper *Capsicum annuum* L. is one of the most important sources of local irritants of plant origin [1–3]. The therapeutic activity of fruit from this plant is known to be due to alkaloids, capsaicinoids, the main one (up to 70%) of which is capsaicin, *trans*-8-methyl-*N*-vanillyl-6-nonenamide [3, 4]. Other known capsaicinoids are homocapsaicin, dihydrocapsaicin, homodihydrocapsaicin, and nordihydrocapsaicin [3–5]. A tincture, ointments, creams, and other combination drugs are based on *C. annuum*. However, methods for standardizing the raw material and preparations of this plant have not yet been harmonized. We developed earlier a chromatographic-spectrophotometric procedure for quantitative determination of total capsaicinoids in peppers [6].

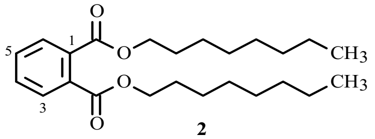
The goal of the present work was to compare the chemical compositions of the raw material and tincture of pepper.

The subjects of the study were commercial samples of pepper tincture (OAO Tverskaya Pharmaceutical Factory) and the medicinal plant raw material, pepper fruit. TLC analysis of the chemical compositions of these samples (PTSKh-AF-A-UV Sorbfil plates, $CHCl_3$:EtOH, 19:1) used a standard capsaicin sample (VFS 42-1753-87).

Pepper fruit (200 g) was extracted with EtOH (90%, 1:12 ratio) at room temperature for 24 h. The resulting extract (1000 mL) was purified of carotinoids by dilution with purified H_2O (1:1 ratio) and passage over a layer of Al_2O_3 that was formed as a suspension in 50% EtOH on a glass filter (sorbent layer thickness 10 cm). The resulting filtrate was evaporated in *vacuo* to 10 mL and placed in a refrigerator. Pepper tincture was treated analogously. In both instances, amorphous solids were obtained, dissolved acetone, mixed with sorbent (silica gel L 40/100 μm), and dried. Then, the silica gel with the compounds was placed on a layer of silica gel formed as a suspension in $CHCl_3$ and eluted in gradient mode by $CHCl_3$ and $CHCl_3$:EtOH mixtures of various proportions. This produced two compounds, the alkaloid capsaicin (**1**) and a phthalic acid derivative, dioctylphthalate (**2**).



1



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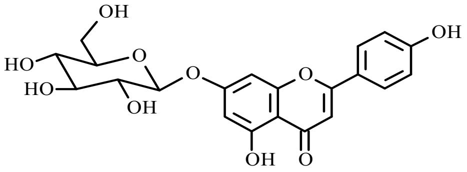

3

In order to isolate compound **3**, the EtOH (90%) extract (1000 mL) of pepper was evaporated to an aqueous residue that was passed through a layer of polyamide formed in H_2O and then eluted with EtOH (20, 40, 70, and 96%). Fractions with **3** (eluates of 40–70% EtOH) were rechromatographed over a column of silica gel L 40/100 with elution by $CHCl_3$:EtOH mixtures of various proportions to obtain **3**, which was identified as cosmosiin (flavonoid).

PMR and ^{13}C NMR spectra were taken on Bruker AM 300 (300 MHz) instruments; mass spectra, in a Kratos MS-30 spectrometer; IR spectra, on a Nicolet IS 40 instrument (Thermo Scientific); UV spectra, using a Specord 40 spectrophotometer (Analytik Jena).

Chromatograms were developed using a basic solution of diazobenzosulfonic acid. The dominant spot had a reddish-raspberry color and R_f~0.6 (at the level of the analogous spot of the capsaicin standard). This indicated that the alkaloid was

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phenolic in nature (capsaicin). Under these conditions, capsaicin was clearly differentiated from all other components. In our opinion, this could provide a basis for determining the authenticity of raw material and preparations containing pepper.

Capsaicin (1). Syrupy light-yellow compound, $C_{18}H_{27}NO_3$. Mass spectrum (70 eV, 200°C, *m/z*, %): 305 (30) $[M]^+$. IR spectrum (cm^{-1}): 1626 (C=O). UV spectrum (EtOH, λ_{max}, nm): 230, 280. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.88 (3H, d, J = 6.0, H-17), 0.96 (3H, d, J = 6.0, H-18), 1.12–1.70 (4H, m, H-11, 12), 1.90–2.10 (2H, m, H-13), 2.20 (2H, t, J = 7.5, H-10), 2.30–2.40 (1H, m, H-16), 3.88 (3H, s, OCH_3), 4.37 (2H, d, J = 5.5, H-7), 5.35 (2H, m, H-14, 15), 5.65 (2H, br.s, NH, OH), 6.75 (1H, dd, J = 7.5, 2.0, H-6), 6.82 (1H, d, J = 2.0, H-2), 6.87 (1H, d, J = 7.5, H-5). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 22.71 (C17), 22.73 (C-18), 25.36 (C-11), 29.52 (C-12), 31.05 (C-16), 32.30 (C-13), 36.86 (C-10), 43.64 (C-7), 56.04 (OCH_3), 110.80 (C-2), 114.46 (C-5), 120.92 (C-6), 126.57 (C-14), 130.52 (C-1), 138.20 (C-15), 145.24 (C-4), 146.80 (C-3), 172.90 (C-9). The spectral properties of **1** agreed with those of capsaicin [7, 8].

Dioctylphthalate (2). Colorless syrupy compound, $C_{24}H_{30}O_4$. IR spectrum (cm^{-1}): 1725 (ester C=O). UV spectrum (EtOH, λ_{max-}, nm): 231, 281. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.90 (6H, d, J = 6.0, $2CH_3$), 1.2–1.75 (24H, m, $12CH_2$), 4.15–4.30 (4H, m, $2CH_2$–O-), 7.50–7.75 (4H, m, aromatic H-3, 4, 5, 6). The combined spectral data enabled **2** to be identified as dioctylphthalate [9].

Cosmosiin (3). Light-yellow crystals, $C_{21}H_{20}O_{10}$. Aglycon $[M]^+$ 270 (100%), mp 225–227°C (aqueous alcohol). UV spectrum (EtOH, λ_{max}, nm): 270, 334; +NaOAc: 269, 378; +NaOMe: 278, 397; +$AlCl_3$: 279, 330, 347, 384; +$AlCl_3$ + HCl: 279, 330, 347, 384. PMR spectrum [300 MHz, $(CD_3)_2CO$, δ, ppm, J/Hz]: 3.3–4.0 (glucose 6H), 5.18 (1H, d, J = 7.2, glucopyranose H-1″), 6.43 (1H, d, J = 2.5, H-6), 6.80 (1H, d, J = 2.5, H-8), 6.90 (1H, s, H-3), 7.04 (2H, d, J = 9, H-3′, 5′), 7.97 (2H, d, J = 9, H-2′, 6′), 12.50 (1H, s, 5-OH). Flavonoid **3** was degraded by acid hydrolysis (10% HCl, 100°C, 2 h) and *β*-glucosidase (Fluka, Hungary) into glucose and the aglycon, which was identified by TLC as apigenin (5,7,4′-trihydroxyflavone). The presence in the NMR spectrum of a singlet for the flavonoid 5-OH group at 12.50 ppm in combination with results from enzymatic hydrolysis and UV spectral data (lack of a bathochromic shift of the short-wavelength absorption band in the presence of NaOAc) enabled the carbohydrate unit to be assigned to the 7-OH group [10]. The glucose was bonded as a *β*-D-glucopyranosyl unit (characteristic doublet for the anomeric proton at 5.12 ppm with J = 7.2 Hz). Based on the physical chemical and spectral properties, **3** was identified as cosmosiin (apigenin 7-*O*-*β*-D-glucopyranoside) [11].

Thus, the study of the component composition of tincture and fruit of pepper isolated and characterized the dominant alkaloid of pepper and dioctylphthalate. The flavonoid cosmosiin was isolated for the first time from pepper fruit. Dioctylphthalate was described for the first time from fruit of the genus *Capsicum* L.

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