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MINOR CONSTITUENTS OF TOTAL CAPSAICINOIDS FROM *Capsicum annuum*

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The annual pepper plant includes over 2,000 species such as chili pepper *Ancho*, Hungarian pepper *Hot wax*, cayenne pepper variety *Dindicut*, *Chile piquin*, *Habanero*, *Red habanero*, *Red savina habanero*, etc. [1]. The main irritant of chili pepper is the alkaloid capsaicin, the content of which varies from 0.007 to 1.9% (calculated per dry substance) [2]. About 20 capsaicinoids have been identified in red peppers [3]. Capsaicin and its analogs were the subjects of reviews [4, 5].

Our goal was to determine the minor components in total capsaicinoids isolated from red chili pepper *Capsicum annuum* L. growing in Uzbekistan (Fergan Valley, Kokand).

Total capsaicinoids were isolated according to the literature method [6] with small changes. The active constituents were extracted by petroleum ether with subsequent column chromatography over C_{18} hydrophobic silochrom sorbent. The yield of total capsaicinoids was 0.65 ± 0.05% per dry weight of starting material.

Pure capsaicinoids were isolated by HPLC over a Zorbax 300SB-C_{18} column (4.6 × 250 mm). The mobile phase was TFA solution (0.1%) and CH_3CN. Gradient elution used CH_3CN at 5% up to 5 min; 5–60%, 5-35 min; 60%, 35–40 min; and a reverse linear gradient from 60 to 5% over 5 min at flow rate 1 mL/min. Detection was performed at 269 nm. The standard was dihydrocapsaicin (Sigma, No. M1022-50).

Capsaicinoids were detected and identified using a Waters ZQ-4000 LC–MS mass spectrometer with direct injection of pure compounds into the mass spectrometer under the following conditions: ESI+ ionization source; drying gas flow rate 450 L/h; drying gas temperature 250°C; skimmer cone potential 30, 35, 50, 70 V; mass range 100–400 *m/z*; positive-ion mode; mobile phase water:MeOH, 1:9; flow rate 0.25 mL/min. Mass spectra were taken at various cone potentials by injecting each sample twice into the instrument. Good results were obtained at 35 and 50 V cone potentials, at which the molecular weights (MWs) of the alkaloids appeared as peaks for the molecular ions. The mass spectra also contained peaks for six singly charged and one doubly charged positive ions. The scheme below uses capsaicin as an example to show the molecular fragmentation and all possible pathways for formation of secondary ions.

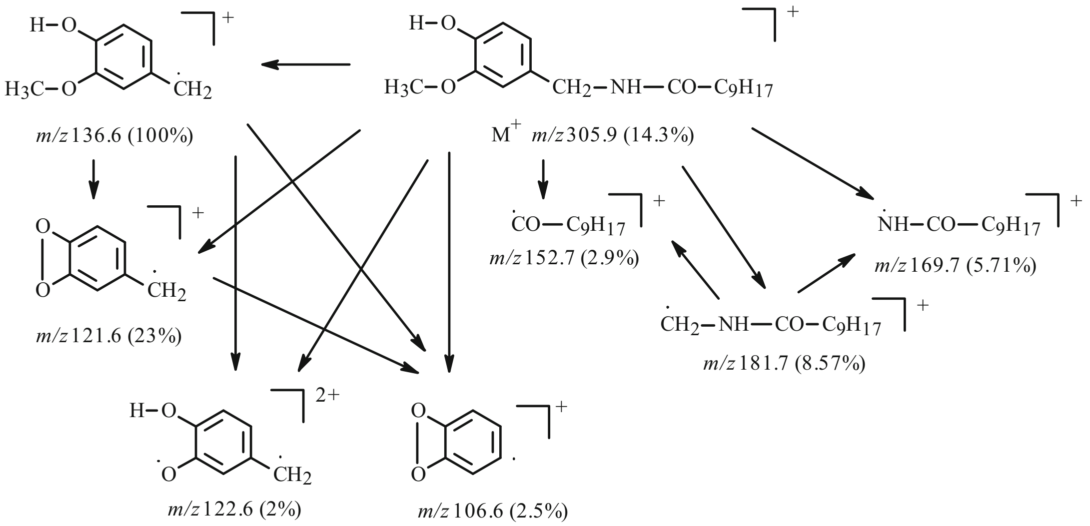

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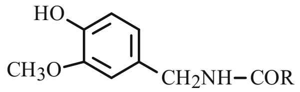
TABLE 1. Content and Elution Time of Pure Capsaicinoids in Extract of *Capsicum annuum* Grown in Uzbekistan



Trivial name	Radical (R)	Content, %	Elution time, min
Capsaicin	*trans*-$(CH_2)_4CH=CHCHMe_2$	45.29	37.025
Dihydrocapsaicin	$(CH_2)_6CHMe_2$	38.35	39.347
Nordihydrocapsaicin	$(CH_2)_5CHMe_2$	1.91	36.479
Homodihydrocapsaicin	$(CH_2)_7CHMe_2$	1.56	42.604
Homocapsaicin	*trans*-$(CH_2)_5CH=CHCHMe_2$	1.31	33.087
Norcapsaicin	*trans*-$(CH_2)_3CH=CHCHMe_2$	1.26	30.931
Nornorcapsaicin	*trans*-$(CH_2)_2CH=CHCHMe_2$	0.267	35.508
Homocapsaicin II	*trans*-$(CH_2)_4CH=CHCH_2CHMe_2$	0.158	40.051
Homodihydrocapsaicin II	$(CH_2)_6CHMeCH_2Me$	0.148	42.604
–	$(CH_2)_9Me$	0.146	34.821
Nordihydrocapsaicin II	$(CH_2)_4CHMeCH_2Me$	0.140	42.604

Peaks for the positive ion 3-methoxy-4-hydroxybenzylidene (*m*/*z* 136.6, 100%); secondary positive ions with *m*/*z* 106.6 (2.5%), 121.6 (23%), and 122.6 (2%); positive molecular ions that were reported for known capsaicinoids [4]; positive ions formed after fragmentation of the molecular ion (–R–CO–, R–CO–NH–, and R–CO–NH–CH_2–) were observed in mass spectra of 11 of the isolated pure compounds.

The structures of the radicals were determined from the MW of the starting capsaicinoid and the retention time in HPLC analysis, which depended on the nature of the radical and its structural features. Table 1 presents the formulas of the radicals and the commonly accepted trivial names of identified capsaicinoids in the order of decreasing overall content in the analyzed sample.

Thus, we determined for the first time the qualitative and quantitative compositions of minor constituents of total capsaicinoids isolated from red chili pepper *C. annuum* growing in Uzbekistan.

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