Journal of Natural Products
Vol. 50, No. 3, pp. 510-522, May-Jun 1987

CONSTITUENTS OF THE ESSENTIAL OIL OF *CALAMINTHA NEPETA*

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Calamintha nepeta (L.) Savi (Syn. *Melissa nepeta* L., *Satureia nepeta* Frtsch) (1) is a plant of the Labiatae family, locally known as calamithra, calamithros, phliscouni, and agrioriganum (2). Isolation of polyphenolic constituents from the plant has been reported (3). The plant is known for its medicinal uses as a stimulant, tonic, antiseptic, and antispasmodic (4,5). In this work we report on the composition of the oil obtained by steam distillation of the leaves and flowers of *C. nepeta* growing in Greece, comparing it with the composition of oils reported in the literature (6).

EXPERIMENTAL

PLANT MATERIAL AND ISOLATION.—The leaves and flowers were collected in August from the island of Leucada in Greece and identified by the Laboratory of Systematic Botany, University of Athens. A specimen has been deposited in the Botanical Museum of the University of Athens.

The dried leaves and flowers (500 g) were pulverized and subjected to steam distillation for 3 h to give 10 g of a pale yellow essential oil. This was dried over anhydrous Na_2SO_4 and stored in a refrigerator.

ANALYSIS.—The oil was analyzed with a Hewlett-Packard 5980A gas chromatograph equipped with a 3m×1.8 mm i.d. glass column, packed with 5% carbowax 20 M (Chromosorb W-aw/DMCS, 80/100 mesh). Injector temperature was 150°, and the FID was heated to 300°. The column temperature was programmed from 75° to 230° at 3°/min. The carrier gas was He at a flow rate of 36 cc/min. The mass spectra were obtained with a Hewlett-Packard 5989 A (70eV) instrument (Data System) coupled to the chromatograph.

The compounds were identified by a comparison of their retention times and mass spectra with those of authentic samples and with data reported in the literature.

RESULTS AND DISCUSSION

The essential oil (2% by weight of dry material) showed the following physical constants: $[\alpha]^{20}D$-19,5°, $n^{20}D$ 1,3252. The identification of constituents resulted from the comparison of the gc and gc/cims analysis (See Table 1).

TABLE 1. Composition of *Calamintha nepeta* Essential Oil

Constituents	Rt (min)	% in Oil	Constituents	Rt (min)	% in Oil
α-pinene	2.	0.25	*cis*-thujanol	12.5	tr
camphene	2.5	tr	*cis*-isopulegone	13.6	tr
β-pinene	2.8	0.3	terpinen-l-ol-4	14.5	0.2
myrcene	3.1	tr	menthol	15.3	tr
limonene	3.5	1.5	pulegone	17.1	39.7
cineol	3.9	0.9	α-terpineol	18.5	tr
3-octanone	4.2	tr	piperitone	20.02	0.75
π-cymene	4.8	0.35	piperitone oxide	21.3	tr
2-ethyl-hexanol	5.9	tr	piperitenone	29.1	2.4
3-octanol	7.2	0.85	piperitenone oxide	30.95	0.45
trans-thujanol	9.3	0.25	caryophyllene oxide	32.05	tr
menthone	10.	24.7	thymol	41.2	tr
iso-menthone	10.8	25.6			

According to the biogenetic studies of the monoterpenes and ketones in the Labiatae family (7), our finding of pulegone as the main compound of the oil and the presence of menthone, isomenthone, and piperitone shows that the biogenesis of the terpenes in our sample of *C. nepeta* followed the pulegone pathway.

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Received 22 May 1985

FLAVONOIDS OF *CUPRESSUS SEMPERVIRENS* AND *CUPRESSUS CASHMERIANA*

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The genus *Cupressus* (Cupressaceae), comprising 12 species, is distributed in the Mediterranean region, tropical Asia, and North America (1). A survey of the literature shows that three biflavonoids—amentoflavone, cupressuflavone, and 4‴-mono-*O*-methyl amentoflavone (podocarpusflavone-A)—have been reported in *Cupressus sempervirens* var. *sempervirens* and var. *stricta* Ait. (2,3), but no flavonoid glycosides are known in any *Cupressus* species. The present paper describes the isolation and characterization of a flavonol glycoside, quercetin-3-*O*-α-L-rhamnopyranoside, along with quercetin, hinokiflavone, isocryptomerin, and reported biflavonoids from the leaf extracts of *Cupressus sempervirens* var. *horizontalis* (Mill.) Gordon. The chemical analysis of the leaf extracts of *Cupressus cashmeriana* Royle Carriere resulted in the isolation of a flavonol diglycoside, quercetin-3-*O*-(6″-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside together with amentoflavone, cupressuflavone, hinokiflavone, 7-*O*-methyl amentoflavone (sequoiaflavone), and isocryptomerin. No flavone aglycones were detected. Sequoiaflavone was found to be present only in this species while others contained podocarpusflavone-A. The presence of a flavonol diglycoside is, thus, reported in the Cupressaceae; this type of compound may serve as a useful taxonomic marker.

EXPERIMENTAL

PLANT MATERIALS.—*C. sempervirens* var. *horizontalis* was collected from Munger, Bihar, and *C. cashmeriana* from the Forest Research Institute, Dehra Dun, UP, India. These were identified by N. Bahadur, Officer-in-Charge, Systematic Botany Branch, F.R.I. Dehra Dun. Voucher specimens are deposited there in the Herbarium.

EXTRACTION AND ISOLATION OF FLAVONOIDS.—MeOH extracts of air-dried and powdered leaves (4 kg in each case) were concentrated in vacuo and treated successively with *n*-hexane, C_6H_6, and $CHCl_3$. The residue was then poured into H_2O and filtered. The filtrate was extracted with *n*-BuOH, concentrated under reduced pressure, and purified by cc (Si gel, $CHCl_3$-MeOH, 4:1) followed by pc (Whatman No. 3, *n*-BuOH-HOAc-H_2O, 4:1:5) to yield quercetin-3-*O*-α-L-rhamnopyranoside (300 mg) from *C. sempervirens* and quercetin-3-*O*-(6″-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (500 mg) from *C. cashmeriana*. These were characterized by chromatographic and hydrolytic studies, ^{1}H-nmr spectral studies of their acetates, and uv spectral shift data of the glycosides as well as the aglycones of permethylated glycosides (4). The latter glycoside was further clarified by ^{13}C-nmr data.

The dark brown precipitate on successive column chromatography (Si gel, C_6H_6/EtOAc) and tlc (Si gel, BDH, C_6H_6-pyridine-formic acid, 36:9:5) yielded quercetin (150 mg), amentoflavone (300 mg), cupressuflavone (200 mg), hinokiflavone (180 mg), podocarpusflavone-A (130 mg), and isocryptomerin (120 mg) from *C. sempervirens* var. *horizontalis* and amentoflavone, cupressuflavone, hinokiflavone, sequoiaflavone (140 mg), and isocryptomerin from *C. cashmeriana*. These were identified by comparison of ^{1}H-nmr spectral data of their acetates, the characteristic fluorescence in uv light of their permethyl ethers, Rf, mp, and mmp with authentic samples (5-7).

Full details of the isolation and identification of the compounds are available on request to the senior author.