VOLATILE CONSTITUENTS OF *LIPPIA NODIFLORA*

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In our investigations of allelopathic plants, those plants which produce compounds capable of inhibiting the growth of other plants, we have examined the creeping perennial herb *Lippia nodiflora*[1] (L.) Greene (family Verbenaceae) which is known for its rampant growth (2). *L. nodiflora* has also been valued as an antipyretic and diuretic in Indian medicine (3). Preliminary experiments in our laboratory showed that extracts of *L. nodiflora* reduced the radical length of lettuce seedlings, suggesting the presence of allelochemicals. Because of the observed rampant growth of *L. nodiflora*, characteristic of allelopathic plants, combined with our observations that *L. nodiflora* extracts inhibited lettuce seedling growth, and the known high incidence of biological activity in the volatile fractions of members of the genus *Lippia*, we have characterized the steam volatile components of *L. nodiflora*.

MATERIALS AND METHODS

L. nodiflora was collected from a planted plot on the irrigated bank of a holding pond, University of California, Davis campus, August 1983. Botanical identification was made by Dr. Lars Anderson, U.C. Davis. An herbarium specimen was filed by K.L. Stevens at the USDA, ARS, WRRC, Berkeley, CA. Plants were air dried for 12 days and then ground in a hammer mill equipped with an ⅛ in. screen and stored in plastic bags at room temperature until used.

A 524 g sample of dried, ground *L. nodiflora* in 500 ml of deionized H_2O was extracted with heptane at 100 mm in a Likens-Nickerson steam distillation continuous extraction apparatus for 15 h. The heptane extract was concentrated to about 0.5 ml by distillation at 100 mm through a Vigreaux column. A portion of this concentrated extract was separated into hydrocarbon and oxygenated components by elution through a Waters Associates, Inc., Milford, MA, Sep-pak C_{18} cartridge with 20 ml of hplc-grade hexane followed by 20 ml of Et_2O. The hexane and Et_2O solutions were concentrated by room temperature distillation of the solvents under reduced (aspirator) pressure.

Gas chromatographic analysis involved a Hewlett Packard 5830 instrument equipped with a 23 m SE 30 glass capillary column and flame ionization detector. The column was programmed from 40 to 300° at 5°/min. Gc-ms data were obtained at 70 eV with a VG Micromass 7070 HS double focusing ms coupled to a DS 2000 data system, and connected to either a 20 m × 0.35 mm i.d. BD-1701 or a 32 m × 0.259 mm i.d. DB-1 J and W fused silica capillary column each with a 0.25 μm film thickness. Compounds were identified by comparison with published mass spectra (4) and by comparison with mass spectra available in our laboratories. Identifications of compounds of similar gc retention times were confirmed by co-injection of standards.

RESULTS

The steam distillate was divided into hydrocarbons and oxygenates. The hydrocarbon fraction was a very complex mixture with no single component dominating. Fourteen components were identified as presented in Table 1; the majority of the hydrocarbons were not identified. The sesquiterpenes calamenene and β-caryophyllene each represented almost 20% of the identified hydrocarbons; the remaining 12 hydrocarbon components each comprised less than 12% of the total.

The oxygenated fraction contained 18 components having concentrations greater than 0.5% in the 2.65 to 30 min retention time range. Seventeen (95%) of these components were identified (Table 1). Small amounts of β-ionone and driminol were detected in this oxygenated fraction at retention times greater than 30 min. No single volatile component dominated the oxygenated fraction. Five components, 1-octen-3-ol, phenethyl alcohol, linalool, *p*-cymen-8-ol, and methylsalicylate, were present in amounts between 10 and 20% of the total; the remaining 12 components each comprised less than 7% of the total.

Monoterpenoids (both hydrocarbons and oxygenated compounds) have previously been implicated in allelopathy (5). Muller *et al.* found camphene, camphor, cineole, dipentene, α-pinene, and β-pinene among the volatile inhibitors produced by *Salvia* species (6). Asplund (7) investigated the inhibitory na-

[1]*Lippia* is properly called *Phyla* (1), but is referred to as *Lippia* in the phytochemical literature.

TABLE 1. Composition of Steam Volatiles from *L. nodiflora*

Compound	% Composition
Monoterpene Hydrocarbons[a]	
β-pinene	8.1
2,6-dimethyloctane	12.3
1-methyl-4-isopropylcyclohexane	7.8
p-cymene[b]	—[c]
β-ocimene	—[c]
terpinolene	1.5
γ-terpinene	6.3
Sesquiterpene Hydrocarbons[a]	
α-copaene	8.4
β-caryophyllene	18.7
α-bergamotene	4.2
β-bisabolene	3.6
δ-cadinene	4.2
4,10-dimethyl-7-isopropylbicyclo-[4.4.0]1,4-decadiene	4.8
calamenene	19.9
Oxygenated Steam Volatiles[d]	
benzaldehyde	6.80
1-octen-3-one	<1.0
1-octen-3-ol	15.29
3-octanol	3.95
benzyl alcohol	2.59
phenylacetaldehyde	2.68
6-methyl-3,5-heptadien-2-one	1.65
2-phenethyl alcohol	16.40
linalool	13.79
p-cymen-8-ol	10.61
methyl salicylate	10.63
α-terpineol	4.86
dihydrobenzofuran	<1.0
carvone	<1.0
thymol	2.74
isothymol (carvacrol)	3.22
eugenol	<1.0

[a]Compounds listed in order of elution on DB1701 column. Reported as % of identified hydrocarbons as estimated from peak heights.

[b]Could not be distinguished from dimethyl, ethylbenzene.

[c]The gc peaks were not well-resolved, and areas could not be calculated.

[d]Compounds listed in order of elution on SE 30 column. Reported as % of total oxygenated compounds of area >0.5% that eluted within 30 min on a 23 m SE 30 capillary column.

ture of camphor, pulegone, borneol, cineole, limonene, α-phellandrene, *p*-cymene, α-pinene, and β-pinene toward radish seed germination and found that although compounds with a ketone group, camphor and pulegone, were more inhibitory than the others, all were inhibitory. Sesquiterpene hydrocarbon inhibitors have also been reported (5). β-Carophyllene, bisabolene, and chamazulene were found inhibitory in *Artemisia absinthium* (8). Other sesquiterpenoids have been implicated in allelopathy (5).

The terpenes β-pinene, *p*-cymene, β-caryophyllene, and bisabolene are all found in the steam volatiles of *L. nodiflora*. In fact, β-caryophyllene comprises almost 20% of the identified hydrocarbons. These terpenes may well be acting as allelochemicals in *L. nodiflora*. They may contribute to its ability to grow rampantly and to inhibit lettuce seedling growth.

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FLAVONOID DIGLYCOSIDES FROM *MYOPORUM TENUIFOLIUM*

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Myoporum tenuifolium G. Forster is widely used in the gardens of eastern Spain. We have been conducting an investigation on foliar flavonoids of this plant. The hydroalcoholic extract was successively extracted with Et_2O, EtOAc and *n*-BuOH. The Et_2O and EtOAc extracts, which especially extract free aglycones and mono-glycosides, respectively, were studied previously, and luteolin, chrysoeriol, apigenin, tricin, luteolin-7-*O*-β-D-glucoside, and tricin-7-*O*-β-D-glucuronide were isolated and identified from them (1,2).

In continuation of this previous work, we have now studied the diglycoside flavonoid compounds present in the *n*-BuOH extract, and luteolin-7-*O*-β-D-rutinoside, chrysoeriol-7-*O*-β-D-rutinoside, apigenin-7-*O*-β-D-rutinoside, eriodictyol-7-*O*-β-D-rutinoside, luteolin-7-*O*-β-D-gentiobioside, and chrysoeriol-7-*O*-β-D-gentiobioside were isolated and identified.

The structures were determined by uv, ms, and ^{1}H-nmr standard procedures (3-6), followed by hydrolysis and chromatographic studies of the resulting aglycones and sugars (7-9).

This is the first report of flavonoid diglycosides from a member of the Myoporaceae. Previously the dihydroflavonol, pinobanksin, was isolated from *Eremophila alternifolia* and *Eremophila ramosissima* (10), 5-hydroxy-3,6,7,3′,4′,5′-hexamethoxyflavone from *Eremophila fraseri* (11), and luteolin, apigenin, chrysoeriol, tricin, luteolin-7-*O*-β-D-glucoside, and tricin-7-*O*-β-D-glucuronide from the Et_2O and EtOAc extracts of *M. tenuifolium* (2).

EXPERIMENTAL

PLANT MATERIAL.—Plant material was collected from Cabo-Roig in southeastern Spain in April, 1982, by the senior author and identified by Dr. F. Alcaraz. A voucher specimen is deposited in the Herbarium of the Faculty of Biology, University of Murcia (accession no. 3732).

EXTRACTION AND ISOLATION OF FLAVONOIDS.—Powdered leaf material (1 kg) was extracted with EtOH-H_2O (7:3). The EtOH was removed under reduced pressure, and the aqueous concentrate extracted with Et_2O, EtOAc, and *n*-BuOH, successively. The diglycosides were isolated from the *n*-BuOH extracts by preparative pc on Whatman No. 3 with H_2O and 30% HOAc and on Whatman No. 1 with *n*-BuOH-EtOH-H_2O (20:5:11). Compounds were purified by tlc on Polyamide DC-6 with H_2O-MeCOEt-*n*-BuOH-HOAc (7:1:1:1).