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Volatile Constituents of the *Liatris* Species, *L. spicata*, *L. elegans* and *L. gracilis*

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The volatile fractions obtained from the leaves of *Liatris spicata*, *L. elegans*, and *L. gracilis* have been examined. In all, ninety-three compounds were identified using combined gas chromatography-mass spectrometry. They include mono- and sesquiterpenoids, norisoprenoids, α, β-unsaturated straight-chain aldehydes and ketones, and fatty acids; the majority of them are common to all three species. A chemical comparison with three previously examined *Carphephorus* species is made and the chemotaxonomic significance of the results is discussed.

Previous studies in this laboratory of various plant materials used as tobacco additives [1–3] have included the volatile fractions obtained from dried leaves of *Carphephorus odoratissimus* (deer tongue), a coumarin-containing plant possessing attractive flavour properties.[4] This study, which was performed on a small scale, resulted in the identification of a large number of components, of which many proved important from a flavour point of view. Subsequent studies primarily directed towards acquiring information on the flavour components of two additional, virtually coumarin-free, *Carphephorus* species have comprised the volatile fractions of *C. corymbosus* and *C. paniculatus*.[5] These, like that of *C. odoratissimus*, were found to be complex and, in all, more than one hundred and thirty constituents were identified.

With this considerable chemical information at hand, which was readily obtained by GLC-MS, it became of obvious interest to evaluate the possible chemotaxonomic significance of these volatile trace components. This was notably so because two of the *Carphephorus* species, *C. odoratissimus* and *C. paniculatus*, had recently been transferred from the genus *Trilisa*, which in turn had previously been generated by division of the genus *Liatris*.[6]

As reported earlier the comparison revealed that the compositions of the volatile fractions derived from these three species were very similar, indicating a close relationship between the plants. However, in view of the limited

information available on volatile trace components present in other plants, definitive conclusions from this isolated study were not considered justified.[5] In order to obtain further data both on the contents of volatiles and on their chemotaxonomic validity, we have now examined three members of the genus *Liatris*, *L. spicata* var. *spicata*, *L. elegans* (Walter) Michaux, and *L. gracilis* Pursh.

RESULTS

In agreement with the findings for the *Carphephorus* species, low-pressure distillation of the acetone extracts of dried leaves of *L. spicata*, *L. elegans*, and *L. gracilis* yielded only small amounts of volatiles having typical "green-leaf" aromas. The distillates were initially partitioned between pentane and water, and the pentane-soluble portions were subsequently divided into neutral and acidic components.

Neutrals. As expected, the neutral volatile fractions derived from the three *Liatris* species proved to be very complex. They were therefore further separated by chromatography on silica gel into two subfractions containing hydrocarbons and oxygenated constituents, respectively, prior to detailed GLC and GLC-MS analyses. Since the present examination was performed on a very small scale, the identifications of the compounds listed in Table 1 are based solely on comparisons of mass spectra and, when possible, retention times with those of authentic samples.

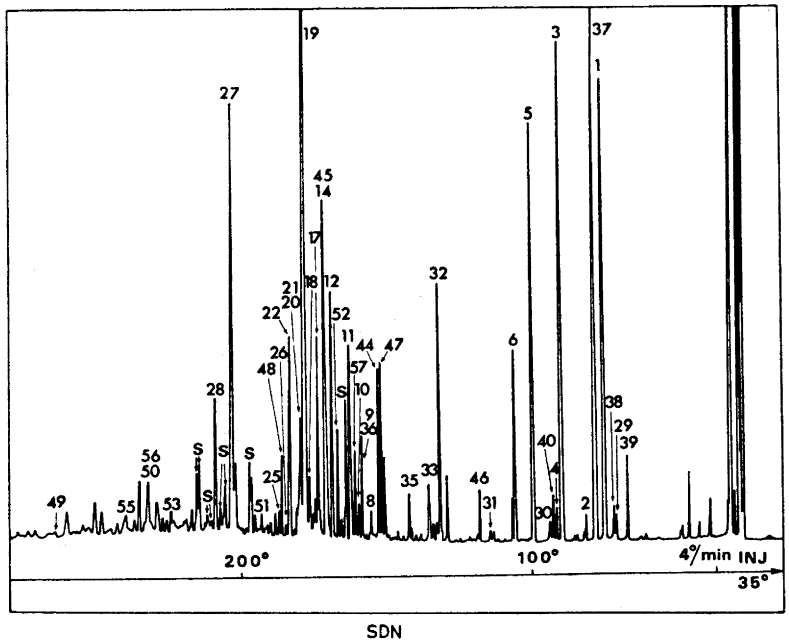
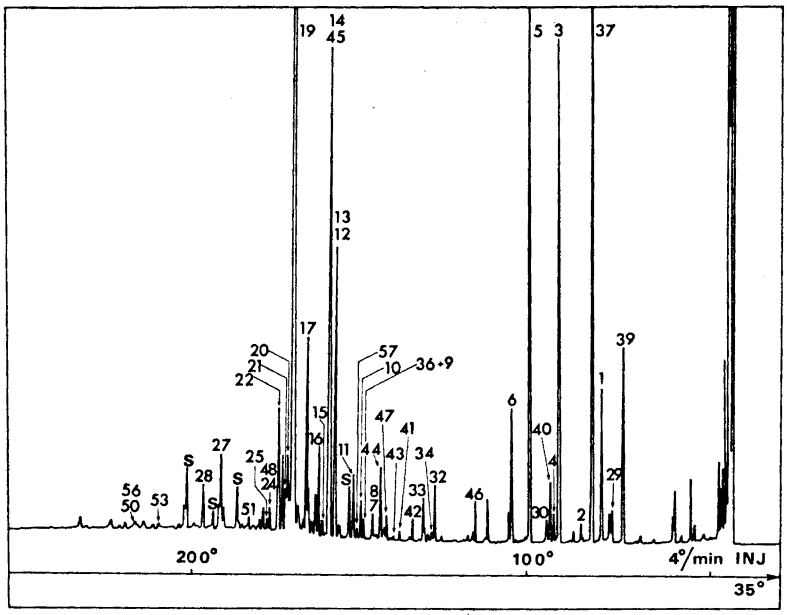
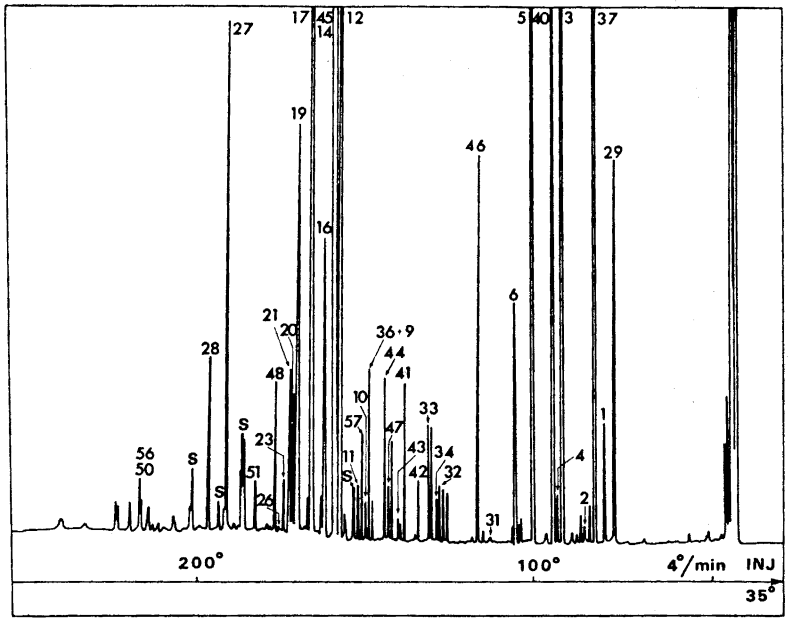


Fig. 1. Gas chromatogram of the neutral fraction from *L. spicata*. Column: HB 2000, 50 m × 0.35 mm.



EDN

Fig. 2. Gas chromatogram of the neutral fraction from *L. elegans*. Column: HB 2000, 50 m × 0.35 mm.



GDN

Fig. 3. Gas chromatogram of the neutral fraction from *L. gracilis*. Column: HB 2000, 50 m × 0.35 mm.

Table 1. Neutral constituents.

Peak No.	Compound	Method of identification			Ref.
		L. spicata	*L. elegans*	*L. gracilis*	
	Hydrocarbons				
	Naphthalene	MS, GLC	MS, GLC	MS, GLC	[a]
1	α-Pinene	MS, GLC	MS, GLC	MS, GLC	26
2	Camphene	MS, GLC	MS, GLC	MS, GLC	26
3	β-Pinene	MS, GLC	MS, GLC	MS, GLC	26
4	Sabinene	MS, GLC	MS, GLC	MS, GLC	26
5	Myrcene	MS, GLC	MS, GLC	MS, GLC	26
6	Limonene	MS, GLC	MS, GLC	MS, GLC	26
	p-Cymene	MS, GLC	MS, GLC	MS, GLC	26
7	α-Cubebene		MS		27
8	δ-Elemene	MS	MS		27
9	α-Ylangene	MS, GLC	MS, GLC	MS, GLC	28
10	α-Copaene	MS, GLC	MS, GLC	MS, GLC	27
11	β-Bourbonene	MS, GLC	MS, GLC	MS, GLC	27
12	β-Elemene	MS, GLC	MS, GLC	MS, GLC	27
13	β-Ylangene		MS, GLC		[a]
14	Caryophyllene	MS, GLC	MS, GLC	MS, GLC	29
15	β-Copaene	MS, GLC	MS, GLC		[a]
16	γ-Elemene		MS	MS	27
17	α-Humulene	MS, GLC	MS, GLC	MS, GLC	27
18	γ-Murolene	MS, GLC			27
19	Germacrene D	MS, GLC	MS, GLC	MS, GLC	[a]
20	α-Muurolene	MS, GLC	MS, GLC	MS, GLC	27
21	β-Bisabolene	MS	MS	MS	27
22	δ-Cadinene	MS, GLC	MS, GLC		27
23	γ-Cadinene			MS, GLC	27
24	4,10-Dimethyl-7-isopropyl-bicyclo[4.4.0]-1,4-decadiene		MS, GLC		27
25	Calamenene	MS, GLC	MS, GLC		[a]
	α-Calacorene	MS	MS		[a]
	Ethers				
26	Thymohydroquinone dimethyl ether	MS, GLC		MS, GLC	[a]
27	β-Caryophyllene epoxide	MS, GLC	MS, GLC	MS, GLC	[a]
28	Humulene epoxide II	MS, GLC	MS, GLC	MS, GLC	[a]
	Aldehydes				
29	Hexanal	MS, GLC	MS, GLC	MS, GLC	[a]
	Heptanal			MS, GLC	[a]
	3-Pentenal	MS		MS	30
30	2-Hexenal	MS, GLC	MS, GLC		[a]
31	2-Heptenal	MS, GLC		MS, GLC	31
32	2,4-Heptadienal	MS	MS	MS	[a]
33	2,4-Heptadienal	MS	MS	MS	[a]
	2,4-Decadienal	MS			32
34	Benzaldehyde		MS, GLC	MS, GLC	[a]
	3-Phenylpropanal	MS, GLC			33
35	α-Campholene aldehyde	MS, GLC			34
36	Myrtenal	MS	MS	MS	35
	Ketones				
	Pentan-2-one	MS	MS	MS	30
	2-Methyl-3,6-heptadione	MS			[a]

Table 1. Continued.

37	Mesityloxide	MS, GLC	MS, GLC	MS, GLC	*a,b*
38	Pent-*trans*-3-en-2-one	MS			36
39	4-Methylpent-4-en-2-one	MS	MS	MS	Tentative
40	Hex-3-en-2-one	MS	MS	MS	Tentative
	Hept-3-en-2-one			MS	37
	Oct-3-en-2-one			MS	37
41	Non-3-en-2-one		MS	MS	37
42	Octa-3,5-dien-2-one		MS	MS	17
43	Octa-3,5-dien-2-one		MS	MS	17
	5-Methylhex-3-en-2-one			MS	37
44	3,5-Dimethylcyclohex-2-enone	MS	MS	MS	Tentative
45	2,2,6-Trimethylcyclohex-5-enone	MS	MS	MS	38
	6-Methylheptanone	MS, GLC	MS, GLC	MS, GLC	*a*
46	6-Methylhept-5-en-2-one	MS, GLC	MS, GLC	MS, GLC	37
47	6-Methylhepta-3-*trans*-5-*trans*-dien-2-one	MS, GLC	MS, GLC	MS, GLC	*a*
48	Geranylacetone	MS, GLC	MS, GLC	MS, GLC	39
49	Farnesylacetone	MS, GLC	MS, GLC		*a*
50	Hexahydrofarnesylacetone	MS, GLC	MS, GLC	MS, GLC	*a*
51	β-Ionone	MS, GLC	MS, GLC	MS, GLC	*a*
52	Verbenone	MS			35
	1-Phenylpropan-2-one			MS	Tentative
	4-Phenyl-but-3-en-2-one	MS			40
	Esters and lactones				
53	Isopropyl myristate	MS, GLC	MS, GLC		*a,b*
54	Benzyl benzoate			MS, GLC	*a*
55	Coumarin	MS, GLC			*a*
56	Dihydroactinidiolide	MS, GLC	MS, GLC	MS, GLC	41
	Alcohols				
57	*trans*-Pinocarveol	MS	MS	MS	27
	2-Phenylethanol	MS			*a*

[a] Mass spectrum of an authentic sample. [b] Probably an artefact formed from the acetone used in the extraction.

A comparison of the gas chromatograms shown in Figs. 1 – 3 reveals immediately that the neutral fractions from the three *Liatris* species are indeed very similar. They contained predominantly mono- and sesquiterpene hydrocarbons, while oxygenated compounds of terpenoid and non-terpenoid origin were present in fairly low concentrations. The main differences between the fractions were encountered as differences in the relative concentrations of certain constituents as indicated below.

The macrocyclic and labile germacrene D, postulated as an important intermediate in the biogenesis of the sesquiterpenoids,[7] was the major neutral constituent of the distillates from *L. spicata* and *L. elegans*. In harmony with this, the majority of the sesquiterpenes encountered in these two species proved to belong to the germacrane class, *e.g.* β- and δ-elemene, δ-cadinene, α- and γ-muurolene, calamenene, α-calacorene, β-bourbonene, α-ylangene, α- and β-copaene were identified in *L. spicata*. Germacrene D was less pre-

ponderant in the volatile neutral fraction from *L. gracilis*. However, many of the sesquiterpene components were still of germacrene type. In fact, β-elemene was a major constituent of this plant in addition to α-humulene and caryophyllene, both of which belong to the humulane class. The latter two compounds were also present, although in comparatively lower concentrations, in *L. spicata* and *L. elegans*.

β-Caryophyllene oxide and humulene epoxide II proved to be constituents of all three species. The mass spectral results indicated the presence of additional epoxides of the caryophyllane and humulane types as well as a bourbonene epoxide. Since, however, no reference material was available, it has not been possible to characterize them fully at the present stage. The same also applies to the sesquiterpene ketones and alcohols denoted by S in Figs. 1 – 3.

The majority of the monoterpenoids identified were common to all three plants, *i.e.* α- and β-pinene, camphene, sabinene, myrcene, limonene, myrtenal, verbenone and *trans*-pinocarveol. Of these, α- and β-pinene and myrcene were major neutral volatiles of *L. spicata*, whereas only the latter two were of a similarly high concentration in *L. elegans* and *L. gracilis*. The remaining monoterpenoids were present in small amounts only. The aromatic thymohydroquinone dimethyl ether, often encountered in members of the *Compositae*, was found in *L. spicata* and *L. gracilis*.

In accordance with the results for the *Carphephorus species*,[4,5] compounds falling within the groups of nor-isoprenoids, saturated aldehydes, α,β-unsaturated and α,β,γ,δ-diunsaturated aldehydes and ketones were present in all three *Liatris* species, although generally in modest quantities only. The nor-isoprenoids encountered, which in all probability are formed *via* degradation of carotenoids and higher isoprenoids,[8–10] were represented by 6-methylheptanone, 6-methylhept-5-en-2-one, 6-methylhepta-3,5-dien-2-one, geranylacetone, farnesylacetone, hexahydrofarnesylacetone, β-ionone, dihydroactinidiolide, 3,5-dimethylcyclohex-2-enone and 2,2,6-trimethylcyclohex-5-enone. Most or all of these have proved to be constituents of other thoroughly examined plant materials such as tea,[11–13] tomato,[10,14] tobacco,[15] and various essential oils.[16] Similarly, the *Liatris* straight-chain oxo derivatives *trans*-2-hexenal, different stereoisomers of 2,4-heptadienal, 2,4-decadienal, and 3,5-octadienone also occur in tea.[11,17,18] Of these compounds, *trans*-2-hexenal has recently been shown to be derived from linolenic acid in Ginkgo leaves [19] and in black tea,[20] and it hence seems likely that the others may arise in a similar fashion. Since many of these nor-compounds also have aroma properties, it is reasonable to assume that an important portion of the "green-leaf" aroma originates from them.

Acids. The acidic fractions, of which that from *L. elegans* was minute, were examined after methylation using diazomethane. They contained predominantly fatty acids; hexanoic and palmitic acids being the major constituents (*cf.* Table 2). Coumarin was present in a fairly high concentration in the acidic fraction from *L. spicata*.

Concluding remarks. It follows from Tables 1 and 2 and the results presented above that the majority of the compounds are common to all three plants, and that the main differences lie in their relative concentrations. Even if the compari-

Table 2. Acidic constituents.

Compound	Method of identification			Ref.
	L. spicata	*L. elegans*	*L. gracilis*	
Pentanoic acid	MS, GLC		MS, GLC	[a]
Hexanoic acid	MS, GLC	MS, GLC	MS, GLC	[a]
Heptanoic acid	MS, GLC		MS, GLC	[a]
Octanoic acid	MS, GLC	MS, GLC	MS, GLC	[a]
Nonanoic acid	MS, GLC	MS, GLC	MS, GLC	[a]
Decanoic acid	MS, GLC		MS, GLC	[a]
Undecanoic acid	MS, GLC			[a]
Dodecanoic acid	MS, GLC	MS, GLC	MS, GLC	[a]
Tridecanoic acid	MS, GLC			[a]
Tetradecanoic acid	MS, GLC	MS, GLC	MS, GLC	[a]
Pentadecanoic acid	MS, GLC	MS, GLC	MS, GLC	[a]
Hexadecanoic acid	MS, GLC	MS, GLC	MS, GLC	[a]
Heptadecanoic acid	MS, GLC	MS, GLC	MS, GLC	[a]
3-Hexenoic acid	MS			36
Oleic acid	MS, GLC		MS, GLC	[a]
Linoleic acid	MS, GLC	MS, GLC	MS, GLC	[a]
Linolenic acid	MS, GLC	MS, GLC	MS, GLC	[a]
Benzoic acid			MS, GLC	[a]
Thymol	MS			[a]
Coumarin	MS, GLC			[a]

[a] Mass spectrum of an authentic sample.

son is extended to the *Carphephorus* species the similarities prevail, which would indicate that *Carphephorus* and *Liatris* are indeed closely related. However, it may also be that the present set of compounds are not suited for distinguishing between the species investigated. This would mean that neither the enzyme systems involved in the biogeneses of the mono- and sesquiterpene hydrocarbons, nor those associated with the production of the nor-compounds are specific to any of the species.[10,17,18] Although admittedly, little is still known about the occurrence of nor-compounds in nature; the present results and those mentioned above for other thoroughly examined plants seem to indicate that these compounds are in fact widely distributed and accordingly of limited value for chemotaxonomic purposes.

The required differentiation and hence evidence in favour of or against the above arguments might be obtained from a comparison of the polar compounds present in these plants. Thus, current studies have demonstrated that various *Liatris* species including *L. spicata* contain large amounts of sesquiterpene lactones of the germacranolide and guaianolide types.[21–23] These compounds possess the elaborate oxygenation pattern probably necessary for differentiation of the species.

However, it should be emphasized that the sesquiterpene lactones were isolated from extracts of the entire above-ground parts of the *Liatris* species. In contrast, the extracts of the leaves of the three *Liatris* species and *C. corymbosus* and *C. paniculatus* on TLC analysis showed major spots corresponding to non-polar constituents, while only minor spots were ascribed to

sesquiterpene lactones. This is consistent with our previous examination of non-volatiles from the leaves of *C. odoratissimus*, which revealed that relatively non-polar triterpenoids and lignans were the major constituents.[24,25] An investigation of the entire above-ground parts of the *Carphephorus* species is therefore highly desirable, since it might provide the complementary chemical data.

EXPERIMENTAL

Experimental apparatus and techniques have been described previously.[5]

Extraction and separation. Crushed dried leaves of *L. spicata*, *L. elegans*, and *L. gracilis* were extracted with acetone in a Soxhlet apparatus for 24 h ultimately affording green gummy concentrates (*cf.* Table 3). These were distilled separately at reduced pressure (90°, 0.1 mmHg), using carbon dioxide as a carrier gas. The distillates, containing residual acetone, were diluted with pentane (1000 ml) and subsequently extracted with water (3 × 200 ml). The pentane-soluble portions from the three distillates were separated into acidic and neutral constituents. No bases were present.

Table 3. Extraction of dry leaves of three *Liatris* species and separation of concentrates.

	Dried leaves g	Acetone extract g	Distillate g	Neutral fraction g	Acidic fraction g
L. spicata	165	25	2.2	0.7	0.5
L. elegans	188	27	1.6	1.0	0.1
L. gracilis	228	45	2.6	0.5	0.5

The neutral fractions from the three plants were initially chromatographed on silica gel (pentane-ether) to give two subfractions containing hydrocarbons and oxygenated constituents, respectively, which were studied by GLC and GLC-MS.

The acidic fractions were reacted with excess ethereal diazomethane overnight and subsequently examined by GLC and GLC-MS.

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