ACTA CHEMICA SCANDINAVICA 26 (1972) 2837–2846

Volatile Constituents of *Carphephorus odoratissimus* (J. F. Gmel) Hebert

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The volatile fraction obtained from an acetone extract of *Carphephorus odoratissimus* (deertongue) is shown to be a complex mixture containing coumarin as the predominant constituent. In all, ninety-six compounds were identified using combined gas chromatography-mass spectrometry as the main tool. These include mono- and sesquiterpenoids, nor-isoprenoids, α,β-unsaturated straight-chain aldehydes and ketones, saturated aldehydes, and fatty acids.

Carphephorus odoratissimus (J. F. Gmel) Hebert (Compositae), also referred to as deertongue, is a coumarin-containing plant native to south-eastern USA. Extracts of the dried leaves are frequently used in perfumery and as an additive to tobacco because of their attractive flavour properties.[1]

Previous investigations on the chemical composition of the leaves have dealt mainly with nonvolatile constituents and have demonstrated the presence of a series of triterpenoids of the α-amyrin, β-amyrin, and lupane types, and certain sterols and lignans.[2,3] Coumarin, dihydrocoumarin, and 2,3-benzofuran have been identified as the predominant volatile constituents.[2] The present study of a volatile fraction, derived from an acetone extract of dried leaves of *C. odoratissimus*, was undertaken with a view to identifying further components which contribute to the flavour.

RESULTS

In agreement with earlier findings, low-pressure distillation of the acetone extract yielded a very small volatile fraction, containing predominantly coumarin and having a heavy, somewhat sweet aroma.[1,2] In order to facilitate the study of the less-abundant flavour components, most of the coumarin was initially removed by partition of the distillate between pentane and water. The pentane-soluble part of the distillate was subsequently separated into fractions containing neutral and acidic components which were examined separately, the latter after methylation.

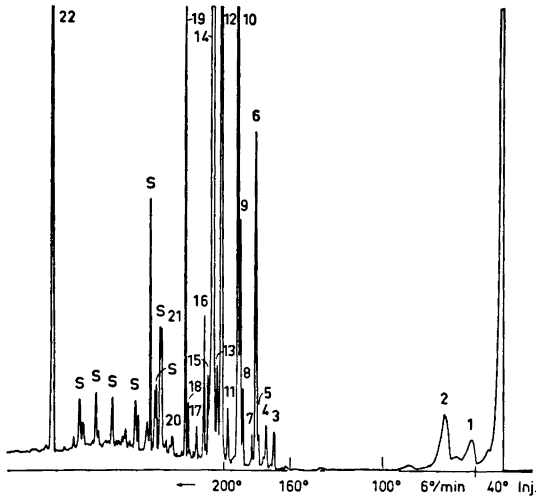

Fig. 1. Gas chromatogram of the neutral fraction. Column: Carbowax 20M 50 m × 0.5 mm.

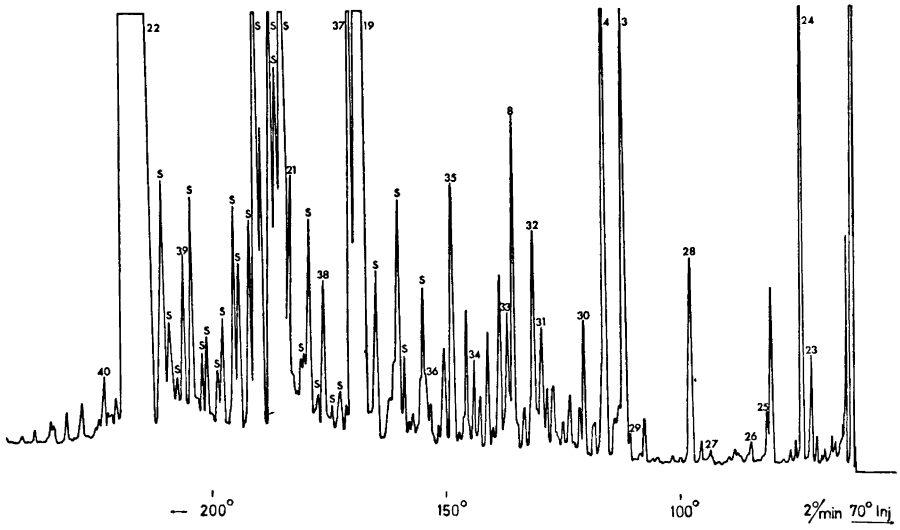

Fig. 2. Gas chromatogram of the oxygenated constituents of the neutral fraction. Column: Emulphor 50 m × 0.5 mm.

Neutrals. The neutral fraction proved to be quite complex and was therefore subjected to extensive chromatography on ordinary and $AgNO_3$-impregnated silica gel * prior to a more detailed analysis by GLC and GLC–MS. Identification of the individual components was accomplished mainly by comparison

* Precautions were taken to avoid contamination in all operational procedures.

of mass spectra and retention times with those of authentic samples. A few constituents, those present in sufficient amounts, were isolated and conclusively identified by comparison with IR and NMR data for authentic compounds. It follows from the results obtained, summarized in Table 1, that coumarin and a series of sesquiterpene hydrocarbons are the major components of the neutral fraction (Fig. 1) and that this also contains a large number of oxygenated constituents present in low concentration (Fig. 2).

Table 1. Neutral constituents.

Peak No.	Compound	Method of identification	Ref.
	Hydrocarbons		
1	α-Pinene	MS, GLC	27
2	β-Pinene	MS, GLC	27
	Limonene [b]	MS	27
	Camphene [b]	MS	27
	Myrcene [b]	MS	27
	Bicycloelemene	MS, GLC	31
	α-Ylangene	MS, GLC	30
5	β-Elemene	MS, GLC	28
	α-Copaene [b]	MS	28
	α-Bourbonene	MS, GLC	28
6	β-Bourbonene	MS, GLC	28
7	α-*trans*-Bergamotene [b]	MS	42
9	β-Ylangene	MS, GLC	[a]
10	Caryophyllene	MS, IR	32, 33
	Sativene	MS, GLC	[a]
11	β-Copaene	MS, GLC	[a]
	β-Cedrene	MS, GLC	32
12	α-Humulene	MS, IR	28, 33
	β-Santalene	MS, GLC	28
13	γ-Muurolene	MS, GLC	28
14	Germacrene D	IR, NMR	29
	α-Amorphene	MS, GLC	[a]
15	α-Muurolene	MS, GLC	28
	δ-Amorphene	MS, GLC	[a]
16	δ-Cadinene	MS, GLC	28
17	γ-Cadinene	MS, GLC	28
	4,10-Dimethyl-7-isopropyl-bicyclo-(4.4.0)deca-1,4-diene	MS, GLC	28
18	Calamenene	MS, GLC	[a]
20	α-Calacorene [b]	MS	[a]
	Cadalene	MS, GLC	[a]
	Ethers		
	1,8-Cineol [b]	MS	[a]
8	Thymol methyl ether	MS, IR, NMR	[a] 8
33	Carvacrol methyl ether	MS, GLC	[a]
19	Thymohydroquinon dimethyl ether	MS, IR, NMR	[a] 8
	Aldehydes		
23	Hexanal	MS, GLC	[a]
25	2-Hexenal	MS, GLC	[a]
	Heptanal	MS, GLC	[a]

Table 1. Continued.

27	2-Heptenal	MS	21
	Octanal	MS, GLC	[a]
	2-Octenal	MS	tentative
29	Nonanal	MS, GLC	[a]
	Decanal	MS, GLC	[a]
3	2,4-Heptadienal	MS	15, 16
4	2,4-Heptadienal	MS	15, 16
	2,4-Decadienal	MS	17
	Benzaldehyde	MS, GLC	[a]
30	α-Campholenealdehyde	MS	43
	Myrtenal	MS	34
	β-Cyclocitral	MS, GLC	[a]
	Ketones		
24	Mesityloxide [d]	MS, GLC	[a]
26	5-Methylhex-3-en-2-one	MS	22
	Hept-3-en-2-one	MS	22
28	6-Methylhept-5-en-2-one	MS, GLC	22
	6-Methylheptanone	MS, GLC	[a]
31	6-Methylhepta-3,5-dien-2-one	MS, GLC	[a]
	Oct-3-en-2-one	MS	22
	Octa-3,5-dien-2-one	MS	19
	Non-3-en-2-one	MS	22
	Pinocamphone	MS	28
32	Isophorone	MS, GLC	41
34	Verbenone	MS, GLC	34
35	2,2,6-Trimethylcyclohex-5-enone	MS	tentative
36	Tetrahydrogeranylacetone	MS, GLC	[a]
37	Geranylacetone	MS, GLC	36
	Damascenone	MS, GLC	37
38	β-Ionone	MS, GLC	35
21	Humuladienone	MS, GLC	6
39	Hexahydrofarnesylacetone	MS, GLC	38
40	Farnesylacetone	MS, GLC	[a]
	Esters and lactones		
	Dihydroactinidiolide	MS, GLC	40
22	Coumarin	MS, IR, NMR	2
	Methyl palmitate	MS, GLC	[a]
	Ethyl palmitate	MS, GLC	[a]
	Alcohols		
	Borneol	MS	39
	Phytol	MS, GLC [c]	[a]

[a] Mass spectrum of an authentic sample. [b] The fraction was too small to permit repeated injections for GLC-retention time studies. [c] The GLC-retention time measurements of this compound was performed on a 2 m steel column (i.d. 3.2 mm) packed with Carbowax 20M on Cromosorb support. [d] Probably an artefact formed from the acetone used in the extraction.

In contrast to the previous investigation,[2] no trace of 2,3-benzofuran could be detected and dihydrocoumarin, encountered in the acidic fraction, was present in modest quantity only. Although the reason for this discrepancy is not fully clear, it should be noted that dried intact leaves were used in the present study while previously a ground commercial sample had been examined.

The three macrocyclic compounds germacrene D, humulene, and caryophyllene proved to be the major constituents of the sesquiterpene hydrocarbons. The first two of these compounds are important biological precursors according to current views on the biogenesis of sesquiterpenoids, recently summarized by Herout.[4] It is therefore of interest to note that, while humulene and caryophyllene are the only representatives of the humulane class encountered, the majority of the sesquiterpenes found belong to the germacrane class, *i.e.* germacrene D, γ- and δ-cadinene, α- and γ-muurolene, α- and δ-amorphene, calamenene, calacorene, cadalene, α- and β-copaene, α- and β-ylangene, α- and β-bourbonene, β-elemene, bicycloelemene, and 4,10-dimethyl-7-isopropylbicyclo(4,4,0)deca-1,4-diene. In addition to these sesquiterpenes, three representatives of the bisabolane class, α-bergamotene, β-santalene, and β-cedrene, were also detected.

Although germacrene D is regarded as a biogenetic precursor of many of the above compounds, it should be noted that this labile compound has been shown to give rise to α- and (+)-γ-muurolene, (−)-α-amorphene, (+)-γ- and (+)-δ-cadinene on treatment with silica gel, acid or heat, and to (−)-β- and α-bourbonene and β-copaene on irradiation.[5] It follows therefore, particularly since the absolute stereochemistry of the compounds encountered in deertongue could not be established due to the lack of material, that some of them may be artefacts.

Several of the oxygenated neutral constituents could tentatively be recognised as sesquiterpene epoxides, ketones, aldehydes, and alcohols (peaks marked S in Figs. 1 and 2). However, since none of them was present in isolable quantities and since proper reference material was lacking in most cases, the identity of humuladienone [6] alone could be established with certainty.

Only a quantitatively small part of the distillate proved to consist of monoterpenoids, and of those identified several are present in trace amounts, *i.e.* myrcene, camphene, limonene, 1,8-cineol, myrtenal, verbenone, pinocamphone, α-campholene aldehyde, β-cyclocitral and borneol. Somewhat more abundant are α- and β-pinene. In addition to these components, three aromatic monoterpenoids, thymol methyl ether, thymohydroquinone dimethyl ether, and carvacrol methyl ether, were identified. It is interesting to note that the first two compounds (as well as other thymol derivatives) have previously been encountered in several species belonging to the Compositae.[7–9]

Several of the compounds identified are nor-isoprenoid ketones structurally related to carotenoids and evidence is now accumulating that they may be formed by degradation of higher isoprenoids. Thus, β-ionone, dihydroactinidiolide, and 6-hydroxy-2,2,6-trimethylcyclohexanone have been obtained from β-carotene,[10] and loliolide, 4(1′,2′-epoxy-4′-hydroxy-2′,6′,6′-trimethyl-1-cyclohexyl)-*trans*-3-buten-2-one and abscisal from violaxanthin [11] on photo-oxidation. Moreover, β-carotene is also degraded to β-ionone in a coupled oxidation reaction with linoleate catalyzed by the enzyme lipoxidase, and lycopene gives rise to citral and 6-methylhept-5-en-2-one under similar conditions.[12] In a study of five different tomato lines, Stevens has demonstrated that there is a relationship between the concentrations of certain volatile components (6-methylhept-5-en-2-one, neral, geranial, geranylacetone, farnesylacetone, α-

ionone, and β-ionone) and those of corresponding carotenoids, suggesting that the volatiles are formed by enzymatic degradation of the latter compounds. The results indicated that the carotenoids are preferentially oxidized at the first conjugated diene bonds and that bonds extending from methyl-substituted carbons are oxidised preferentially.[12]

Our results are consistent with these findings, since the acyclic methyl ketones 6-methylhept-5-en-2-one (I), geranylacetone (II), and farnesylacetone (III) but not the aldehydes farnesal and citral were detected. Moreover, the corresponding saturated components 6-methylheptanone (IV), tetrahydrogeranylacetone (V), hexahydrofarnesylacetone (VI) and several cyclic compounds – β-ionone (VII), dihydroactinidiolide (VIII), β-cyclocitral (IX), 2,2,6-trimethylcyclohex-5-enone (X), damascenone (XI), and isophorone (XII) – were also encountered.

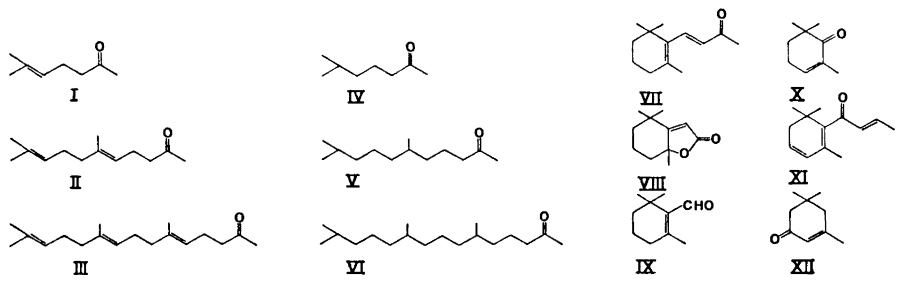

Fig. 3.

Several of these nor-isoprenoids are typical perfume components and they have been shown to be present in a number of essential oils. All of them, with the exception of isophorone, and 2,2,6-trimethylcyclohex-5-enone, have also been encountered in tobacco,[13,14] indicating that the effect of adding deertongue leaf to tobacco, besides providing a coumarin note, may be strengthening of that part of the tobacco aroma attributable to these compounds.

In addition to the above oxo-nor-isoprenoids, a number of straight-chain α,β-unsaturated aldehydes and ketones were encountered in *C. odoratissimus* (*cf.* Table 1). Thus two isomers of hepta-2,4-dienal, displaying only minor intensity differences mass spectrometrically,[15,16] and one isomer of deca-2,4-dienal [16,17] were found. Since no reference samples were available for studies of GLC retention times, it was impossible to determine which stereoisomers are present. However, it is worth noting that all-*trans*-hepta-2,4-dienal and the corresponding 4-*cis*-isomer have previously been identified in plant materials, *e.g.* tomatoes [18] and tea,[15] and that all-*trans*-deca-2,4-dienal is a tomato constituent.[18] A compound having a mass spectrum identical to that of the di-unsaturated ketone all-*trans*-octa-3,5-dien-2-one, recently isolated from tea, was also encountered.[15,19] Both decadienal and octadienone have interesting aroma properties. The former compound is often used to "beautify a citrus topnote",[20] and the latter is reported to have a woody and pleasant aroma.[19]

Seven compounds gave mass spectra in agreement with those of 2-hexenal, 2-heptenal,[21] 2-octenal, hept-3-en-2-one,[22] oct-3-en-2-one,[22] non-3-en-2-one,[22] and 5-methylhex-3-en-2-one,[22] respectively. Of these only the presence of 2-hexenal, a compound widely distributed in nature and known to possess certain aroma properties, could be verified by co-chromatography due to the lack of reference material.

The five C_6-C_{10} saturated straight-chain aldehydes encountered, evidently also contribute to the aroma of the distillate and several of them are well-known perfume constituents.

Acids. The acidic fraction, examined after methylation with diazomethane, proved to be less complex than the neutral fraction, and subsequent separation on silica gel furnished five relatively simple fractions. These were examined by GLC and GLC–MS and the results are presented in Table 2.

Table 2. Acidic constituents.

Compound	Method of identification	Ref.
Hexanoic acid	MS, GLC	[a]
Heptanoic acid	MS, GLC	[a]
Octanoic acid	MS, GLC	[a]
Nonanoic acid	MS, GLC	[a]
Decanoic acid	MS, GLC	[a]
Undecanoic acid	MS, GLC	[a]
Dodecanoic acid	MS, GLC	[a]
Tridecanoic acid	MS, GLC	[a]
Tetradecanoic acid	MS, GLC	[a]
Pentadecanoic acid	MS, GLC	[a]
Hexadecanoic acid	MS, GLC	[a]
Heptadecanoic acid	MS, GLC	[a]
2-Pentenoic acid	MS	23
2-Hexenoic acid	MS	16
3-Hexenoic acid	MS	16
2-Octenoic acid	MS	16
Benzoic acid	MS, GLC	[a]
o-Methoxycinnamic acid	MS	[a]
o-Methoxydihydrocinnamic acid	MS	[a]
Thymol	MS	[a]
Dihydrocoumarin	MS, IR, NMR	2
Coumarin	MS, IR, NMR	2

[a] Mass spectrum of an authentic sample.

The presence of C_6-C_{17} saturated straight-chain fatty acids, of which palmitic acid is the main component, was readily established. In addition, mass spectral evidence for the occurrence of 2-pentenoic acid,[23] 2- and 3-hexenoic acids,[24] and 2-octenoic acid [24] was obtained. A few aromatic acids – benzoic acid, *o*-methoxycinnamic acid and *o*-methoxydihydrocinnamic acid – were also encountered. The more polar fractions contained thymol, dihydrocoumarin (*vide supra*) and coumarin.

EXPERIMENTAL

Infrared spectra were recorded on a Perkin-Elmer 257 instrument and NMR spectra in $CDCl_3$ on a Varian A-60A spectrometer at 60 MHz.

Analytical GLC was performed on a Varian 1700 instrument equipped with a capillary injector and a flame ionization detector. The temperatures of the injection block and the detector were kept at 275°. The carrier gas flow was 3 ml N_2/min and the samples were injected with a split ratio of 1:20. Stainless steel columns (50 m × 0.5 mm) coated with Apiezon L, Carbowax 20M, and Emulphor were used in the experiments. GLC in combination with mass spectrometry was carried out on an LKB 9000 instrument, incorporating a home-made gas chromatograph equipped with a capillary injector, a device for introduction of make-up gas, a splitter, and a flame ionization detector.[25] The columns described above were used also in the GLC–MS studies but helium was substituted for nitrogen as a carrier gas. The temperature of the separator was 260° and that of the ion source 290°. An electron energy of 70 eV was employed.

Liquid chromatography was carried out on silica gel (Merck 0.05–0.20 mm, activity 1) and on $AgNO_3$-impregnated silica gel (23 %). The silica gel was washed with ethanol and ether followed by reactivation prior to use, whereas the aqueous $AgNO_3$-solution used for impregnation was purified by extraction with ether.

The solvents used in all operations, pentane, ether, and acetone, were carefully purified. Thus, the pentane was successively treated with fuming H_2SO_4, a saturated solution of $KMnO_4$ in aqueous H_2SO_4 (10 %), and water. After treatment with a drying agent (Na_2SO_4), the pentane was distilled twice using a packed column (60 × 3.5 cm, reflux ratio 0.5). The ether was dried ($CaCl_2$) and distilled over a small amount of $LiAlH_4$. The acetone was distilled three times. The solvents purified by these techniques were shown to be free from impurities by GLC-analysis of samples concentrated 10 000-fold.

Extraction and isolation. Dried leaves of *Carphephorus odoratissimus* * (1650 g) were crumbled and extracted with acetone in a Soxhlet apparatus for 24 h. Most of the solvent was carefully removed yielding a green viscous liquid (169 g).** This was distilled at reduced pressure (90°, 0.1–0.01 mmHg), using CO_2 as a carrier gas,[26] furnishing a small amount of distillate (8 g) and a distillation residue (161 g) which was set aside. The distillate, containing residual acetone, was diluted with pentane (500 ml) and subsequently extracted with water. The polar material (3.1 g) present in the aqueous phase was transferred into ether and shown to consist almost exclusively of coumarin. The pentane phase (4.9 g) was separated into acids (1.1 g) and neutral constituents (3.8 g). No bases were present.

Neutrals. A portion of the pentane-soluble neutral material (3 g) was chromatographed on silica gel (pentane–ether, 1:0→1:4) to give fractions 1–15, which were studied by GLC and GLC–MS.

Fractions 1 and 4–14 were small and complex and only studied as described above.

Fraction 2 (2 g), consisting of unsaturated hydrocarbons, was rechromatographed on $AgNO_3$-impregnated silica gel (pentane-ether) to give 25 fractions, all of which were examined by GLC and GLC–MS. Of these, fractions 2-18, 2-21, and 2-25 contained pure caryophyllene (MS,[32] IR,[33]), germacrene D (IR,[29] NMR,[29]) and humulene (MS,[28] IR,[33]) respectively.

Fraction 3 was separated by chromatography on silica gel into thymol methyl ether (4 mg), and thymohydroquinone dimethyl ether (20 mg), which were identified by comparison of their IR, NMR [8] and mass spectra with those of authentic specimens. However, the thymol methyl ether isolated was later found to contain ~10 % of a compound, having retention time and mass spectrum identical to those of carvacrol methyl ether.

Acids. A portion of the pentane-soluble acidic material (1 g) was treated with ethereal diazomethane for 24 h and subsequently subjected to chromatography on silica gel (pentane-ether) to give five fractions which were analysed by GLC and GLC–MS. Fractions 1 and 2 were essentially mixtures of methyl esters of fatty acids, containing

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* We are grateful to Mr. Robert Lazor, Department of Biological Science, Florida State University, USA, for the collection and botanical classification of the plant material.

** Since the solvent was not removed completely to avoid losses of volatile material, the weights given for the various fractions are necessarily inexact.

also some methyl benzoate. Fraction 3 contained thymol, methyl *o*-methoxycinnamate and methyl *o*-methoxydihydrocinnamate.

Fraction 4 was a mixture of dihydrocoumarin, coumarin, and one incompletely identified component.

Acknowledgements. We are grateful to Miss Pia Nyberg for valuable technical assistance. Generous gifts of reference samples were received from Drs. Y. Hirose and Y. Naya, The Institute of Food Chemistry, Osaka, Japan; Drs. V. Herout and L. Novotny, Czechoslovak Academy of Science, Praha, Czechoslovakia; Dr. E. Klein, Dragoco-Werk, West Germany; Dr. T. Anthonsen, Norway Institute of Technology, Trondheim, Norway; Drs. S. Sundin and L. Westfelt, Forest Products Research Laboratory, Stockholm, Sweden; Dr. G. Karlsson, Swedish Institute for Food Preservation Research, Göteborg, Sweden; Prof. George Büchi, Massachusetts Institute of Technology, Cambridge, Massachusetts, U.S.A.; Dr. A. R. Pinder, Clemson University, South Carolina, U.S.A.; Dr. Sukh Dev, National Chemical Laboratory, Poona, India; Dr. V. Rautenstrauch, Firmenich & Cie, Genève, Switzerland.

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Received December 20, 1971.