(2 H, *m*, $-C^{\beta}H_2-$). Hydrolysis: 20 mg of **2** were dissolved in 1 ml 1.5 N HCl and heated in a sealed ampoule at 100° for 21 hr. On cooling crystals separated, mp 120–123°. No depression of mp was observed in a mixture with benzoic acid and IR and UV spectra also agreed with those for benzoic acid.

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VOLATILE COMPONENTS OF SUGAR BEET LEAVES

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Key Word Index—*Beta vulgaris*; Chenopodiaceae; sugar beet leaves; volatile components.

Abstract—Extracts of both young and old sugar beet plants were obtained using a modified Likens and Nickerson apparatus. Constituents were identified by GC/MS, and using selected ion monitoring it was shown that the previously determined phenylacetonitrile was probably not of glucosinolate origin. Some unsaturated aldehydes, alcohols and derivatives (enzymic lipid degradation products) were formed to greater extents by the younger leaves, but otherwise such quantitative differences were relatively few and generally random. An interesting range of chlorinated compounds was obtained only from the older plants; a pesticide origin is suggested.

INTRODUCTION

During a recent examination of sugar beet leaves for constituents possessing auxin-like activity [1], an interesting array of volatile components was observed. These have now been studied in more detail and results are reported here. The previously determined growth substance, phenylacetonitrile [1], could conceivably originate from a glucosinolate precursor, and this would be noteworthy since such occurrence would be rather unexpected in a plant so distant from the Cruciferae. Therefore, a specific search was undertaken in appropriate extracts of sugar beet leaves for other products of glucosinolate degradation, in particular isothiocyanates. The previous study had also shown the nitrile to be present to a greater extent in extracts of young leaves compared with those of mature leaves [1], and indeed this might be expected with a growth-promoting substance. This finding was further tested in this work, and other volatile components were also assessed for this behaviour.

RESULTS AND DISCUSSION

Table 1 lists the components identified in extracts of both young and old sugar beet plants. The relative percentage abundance of each component in a sample is given, together with an assessment of absolute concentration of a fr. wt basis. Where no quantitative data are given in the table this means that the component in question was not detected in that sample. Most constituents were positively identified from their mass spectra, and their spectra agreed with the literature within experimental error (i.e. instrumental variability). All spectra have been adequately reported before so none is given here. Some constituents were only partially characterized and a few remain unidentified.

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Table 1. Volatile constituents of sugar beet leaves

Peak No.	Component	R_t	Young plants		Old plants	
		(min)	(%)	(μg/g)	(%)	(μg/g)
1.	A branched C_7 hydrocarbon	1.1	3.9	3.0		
2.	Cyclohexane	1.4	1.0	0.8	1.1	0.8
3.	Heptane	1.6	0.5	0.4	1.8	1.4
4.	Unknown	1.7			0.4	0.3
5.	Dimethyl sulphide	2.1			0.9	0.7
6.	Octane	2.6	21.0	16.1	26.2	20.1
7.	An ethylhexane	3.2	1.9	1.5	4.8	3.7
8.	A dimethylcyclohexane	3.7	0.3	0.2	0.4	0.3
9.	A C_3 cyclohexane (?)	4.0			0.9	0.7
10.	Unknown	4.6	1.5	1.1	0.2	0.2
11.	Carbon tetrachloride	5.6	0.1	0.1		
12.	Ethylcyclohexane	5.8	0.4	0.3	0.2	0.2
13.	Dichloromethane	6.0	0.8	0.6	1.1	0.8
14.	A methyl-2,4-hexadiene	7.0	1.0	0.8	0.6	0.5
15.	Butanedione	7.3	0.1	0.1		
16.	Pentanal	7.8	0.2	0.2	0.4	0.3
17.	Unknown	8.0			0.1	0.1
18.	Butan-2-ol	8.3			0.8	0.6
19.	Chloroform	8.6	5.4	4.1	0.4	0.3
20.	Toluene	9.4	1.1	0.8	4.8	3.7
21.	Unknown	9.8			0.1	0.1
22.	Hexanal	10.1	0.4	0.3	1.5	1.1
23.	Unknown	10.7	0.2	0.2		
24.	Unknown	11.2	0.6	0.5	0.5	0.4
25.	Ethylbenzene	11.5	0.4	0.3	0.2	0.2
26.	A xylene	11.8			1.0	0.8
27.	Pyridine	12.4	0.3	0.2	1.3	1.0
28.	A xylene	12.6	1.0	0.8	0.4	0.3
29.	(*E*)-Hex-2-enal	12.8	2.2	1.7	0.5	0.4
30.	Chlorobenzene	13.1			10.0	7.7
31.	Unknown	13.4	0.1	0.1		
32.	Styrene	13.8			0.1	0.1
33.	Unknown	13.8	1.1	0.8	0.1	0.1
34.	Unknown	14.0	0.4	0.3	0.1	0.1
35.	(*Z*)-Hex-3-en-1-yl acetate	14.4	11.1	8.5	0.1	0.1
36.	Unknown	14.6	0.5	0.4		
37.	Hexan-1-ol	14.8			0.2	0.2
38.	Unknown	15.0			0.1	0.1
39.	(*Z*)-Hex-3-en-1-ol	15.6	2.7	2.1	1.5	1.1
40.	Hex-2-en-1-ol (*Z* or *E*)	16.0	1.0	0.8	0.8	0.6
41.	Unknown	16.2	0.3	0.2	3.1	2.4
42.	Unknown	17.4	1.8	1.4		
43.	Unknown	18.2			0.1	0.1
44.	*p*-Dichlorobenzene	18.8			0.8	0.6
45.	Unknown	18.8	3.9	3.0		
46.	Unknown	19.4	1.3	1.0	8.0	6.1
47.	Benzaldehyde	20.4	0.9	0.7	1.1	0.8
48.	Unknown	21.7			1.2	0.9
49.	Unknown	21.9	0.2	0.2		
50.	Unknown	22.1	2.9	2.2		
51.	Unknown	22.6	0.2	0.2	0.3	0.2
52.	Unknown	23.4	0.1	0.1	0.3	0.2
53.	Phenylacetaldehyde	24.4	5.1	3.9	4.6	3.5
54.	Phenylacetonitrile	25.0	5.3	4.1	1.0	0.8
55.	Unknown	25.7	0.5	0.4	0.1	0.1
56.	Unknown	26.1	12.0	9.2		
57.	A methylpyrrolidone	26.2			1.7	1.3
58.	Unknown	26.8	0.3	0.2		
59.	Terpene ester	27.6	1.3	1.0	7.8	6.0
60.	A dimethylbenzaldehyde	29.2			0.5	0.4
61.	A γ-lactone	31.2			2.0	1.5

Table 1. (*Continued*)

Peak No.	Component	R_t (min)	Young plants (%)	Young plants (μg/g)	Old plants (%)	Old plants (μg/g)
62.	Unknown	32.0	1.1	0.8		
63.	Unknown	33.1	0.7	0.5	1.3	1.0
64.	Unknown	33.5			1.8	1.4
65.	A chlorobenzaldehyde	34.8			2.3	1.8
66.	Unknown	36.1			0.1	0.1
67.	Unknown	39.3	0.8	0.6		

Most sugar beet components were common to both young and old plants, as would be expected, except for an interesting series of chlorinated compounds which was only located in samples from old leaves. Generally, components were present in *ca* the same concentrations in both types of leaves, although there were some exceptions such as toluene, hexanal and pyridine. In particular, however, (*Z*)-hex-3-en-1-yl acetate and (*E*)-hex-2-enal were produced in larger amounts by the young plants. The related (*Z*)-hex-3-en-1-ol and hex-2-en-1-ol were also formed in slightly larger quantities by these plants. These data agree very well with previous work on cabbage leaves where most cultivars examined were also found to produce larger amounts of such unsaturated alcohols and aldehydes from younger plants compared with older ones [2]. These compounds are well-known products of enzymic lipid degradations, e.g. [3]. The suspected auxin-like component, phenylacetonitrile [1], was also produced in greater amounts by the younger leaves, as expected, and this confirms the previous findings [1]. The chemically related phenylacetaldehyde was also detected in sugar beet leaves, and in quite large amounts, but for this compound there was no significant difference between quantities in old and young plants.

The specific searches (using SIM and MPM techniques) for benzyl isothiocyanate and benzyl thiocyanate proved negative, so there is no evidence that phenylacetonitrile is of glucosinolate origin in sugar beet. Since the sensitivity of these searches was high, it can be reasonably deduced that there is no benzylglucosinolate in sugar beet leaves. Based on the presence of phenylacetaldehyde, similar searches were conducted for phenylpropionitrile and 2-phenethyl isothiocyanate [4], but again these were not detected in any samples, so 2-phenethylglucosinolate is also not present in sugar beet leaves.

As well as phenylacetaldehyde, other aromatic aldehydes, namely benzaldehyde, a dimethylbenzaldehyde and a chlorobenzaldehyde, were also positively identified in extracts of sugar beet leaves, and their origin is of interest. The latter compound is a member of a group of chlorinated components which were only located in the older plants; some were present in quite large amounts, e.g. chlorobenzene at 7.7 μg/g. This is the major difference between the two samples and the only explanation that can be suggested is that these compounds originated from routine and regular treatment of the plants with pesticides. Clearly the older plants had received many more applications than the younger ones and this would account for the components being detected only in samples prepared from the former. Three pesticides were employed, namely dicofol, pirimicarb and permethrin, but only the acaricide dicofol could be the possible cause. It is well known that this compound [1,1-bis(4-chlorophenyl)2,2,2-trichloroethanol], which has a benzylic hydroxyl group adjacent to an electron-deficient carbon atom, readily eliminates this carbon atom under a variety of conditions to give 4,4'-dichlorobenzophenone. This decomposition will certainly occur in a GC column. However, no evidence has yet been presented that dicofol is either metabolized to the three chloro compounds reported here or that it or its known metabolic products decompose to give such compounds during analytical procedures. There is, nevertheless, some chemical similarity between these three chloro compounds (particularly the chlorobenzaldehyde) and the pesticide or its known metabolites, so the origin is a possibility. No other reasonable explanation can be offered.

EXPERIMENTAL

Sample preparation. Fresh leaves of sugar beet (*Beta vulgaris* cv Bush Mono G, 400 g) were chopped, mixed with H_2O (650 ml) and extracted for 4 hr in a Likens and Nickerson apparatus [5] as modified in ref. [6]. 2-Methylbutane (25 ml) was the solvent, and extracts were then concd to 1 ml using a low temp.–high vacuum distillation method [6].

GC. Concentrates were examined by temp. programmed FID GC. A 5.5 m × 4 mm i.d. glass column packed with 10% PEG 20M coated on 100–120 BSS mesh acid-washed Diatomite C was mainly used, with N_2 carrier (60 ml/min). The best temp. programme was 60° for 5 min, followed by an increase at 12°/min to 180° for the remainder of the run. Detector and injection temps were 250° and typically 4 μl of sample were injected.

GC/MS. A Kratos MS 25 instrument was used, linked on-line to a DS 50S data processing system. The same GC conditions as given above were employed, but using He carrier at a slightly lower flow rate (40 ml/min). A single-stage, all-glass jet separator was used at 250°. Both electron impact (EI) and chemical ionization (CI) MS were performed, and at various times (particularly when searching for specific nitriles or isothiocyanates) the MPM unit and the retrospective single ion monitoring facility of the data system were extensively employed. Significant operating parameters of the mass spectrometer during EI work were: ionization potential, 70 eV; ionization current; 100 μA; source temp., 200°; accelerating voltage, 1.5 kV; resolution, 600; scan speed, 1 sec/decade (repetitive throughout runs). Identical conditions were employed during CI MS except for the following: reagent gas, methane; ionization potential, 100–110 eV; emission current, 5 mA.

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TRACE CONSTITUENTS IN THE ESSENTIAL OIL OF *THYMUS CAPITATUS*

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Key Word Index—*Thymus capitatus*; Labiatae; essential oil; terpenes; bornyl chloride; GC/MS analysis.

Abstract—The essential oil of *Thymus capitatus* was investigated by glass capillary gas chromatography in combination with mass spectrometry. In the analysis, 22 hitherto unreported trace constituents were isolated, of which 20 were identified.

INTRODUCTION

In the past, the essential oil of *Thymus capitatus* (origanum oil) has often been confused with the essential oil of *Thymus vulgaris* (thyme oil). However, origanum oil is in reality an entirely different type, distilled from the flowering herb *Thymus capitatus*, Hoffmag et Link, which is now considered a separate genus, viz. *Coridothymus capitatus* Rchb. [1–5].

The essential oil content of *Thymus capitatus* has been extensively investigated [6–8]. Nevertheless, it was decided that a more thorough analysis was warranted, particularly as it is not known which constituents are significant from a biosynthetic and phyllogenetic point of view. Therefore, the analysis was run with the intention of including the characterization on trace (less than 0.1 %) components, so that more light could be shed upon the terpenoid distribution pattern. Also, the data obtained should prove useful in the recognition and identification of a genuine oil.

RESULTS AND DISCUSSION

The oil used in this study was obtained by steam distillation from the aerial parts of the plant, collected from the Hellenic island Thassos. Analysis and identifications were carried out using a computerized GC/MS system. Identifications of the individual components were accomplished with the aid of various computer interpretative techniques, as well as by individual interpretation of some spectra. The computer techniques included a comparison of unknown spectra with a collection of 25 409 authentic spectra, and plotting of relative intensities of significant masses ('mass chromatograms') to reveal structural isomers of compounds, or to detect some minor components and the compounds hidden by other components in an incompletely separated gas chromatographic peak.

Table 1 lists the 22 trace compounds that have been isolated and characterized for the first time in the oil of *Thymus capitatus*. The presence of bornyl chloride for the first time in natural products should be noted. Also, because of the fact that some of the essential oil constituents are very unusual, we thought it useful to cite their mass spectral data.

The compound producing peak 15a was identified as bornyl chloride (**1**). Its MS showed important peaks at *m/z* (rel. int.): 159 (4), 157 (13), 136 (27), 121 (22), 110 (29), 95 (100), 93 (30), 81 (19), 67 (10) and 53 (6) in accordance with the expected peaks for this structure. The occurrence of bornyl chloride as a component of an essential oil is so unusual that we should not exclude the possibility that it