Composition and Content of Aroma Compounds in Dill, *Anethum graveolens* L., at Three Different Growth Stages

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Aroma compounds of dill (*Anethum graveolens* L.) were isolated by the solvent extraction at three different growth stages. The aroma extracts were analyzed by the glass capillary gas chromatography–mass spectrometry method. Twenty-two volatile compounds were identified, including α-phellandrene, limonene, β-phellandrene, *p*-cymene, and 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran as the major components. These five compounds comprised together 65–80% of all the identified compounds. During the growth of dill, the content of limonene, 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran, and carvone increased, while the α-phellandrene, β-pellandrene, myristicin, and apiol contents decreased. The total amount of aroma compounds varied widely during the growth.

Dill (*Anethum graveolens L.*) is an important aromatic herb, which is used for flavoring and seasoning of various foods such as salads, sauces, soups, sea foods, and especially pickled vegetables. In Europa and the United States, essential dill oil distilled from fresh whole herb or ripe seeds is preferred by the food industry. On the other hand in Finland the whole fresh dill is mainly used before the bud formation or at the flowering stage, while small amounts of chopped dill leaves are also used as dried.

The aroma composition of dill has been the subject of numerous investigations, which, however, have resulted in controversial conclusions (Adhikari, 1965; Virmani and Datta, 1970; Baslas and Baslas, 1972; Belafi-Rethy et al., 1974; Göckerizt et al., 1979; Lawrence, 1980; Schreier et al., 1981). Three main constituents, carvone, α-phellandrene, and limonene, have been identified in dill aroma, but their amounts varied widely. It depends on different factors, such as harvesting time, i.e., the state of maturity, geographical origin, and isolation procedure of aroma compounds.

Thus carvone and limonene predominate in the seed oil (Miyazawa and Kameoka, 1974; Scheffer et al., 1977a), while α-phellandrene is the main component of the herb oil (Baslas and Baslas, 1971; Belafi-Rethy and Kerenyi, 1977). Variations of the aroma composition during the ripening of dill seeds have been reported in more detail (Kalitzki, 1954a; Luyendijk, 1954, 1957; Ihloff, 1956) than variations of the aroma composition of dill herb during the initial growth period (Zlatev, 1975, 1976; El-Gengaihi and Hornok, 1978).

The proportion of, e.g., carvone in herb oil has been reported to vary from 6 to 44% depending on the geographical origin (Kalitzki, 1954b; Virmani and Datta, 1970), while the amount of carvone in the oil from dill seeds is more constant, 40–55% (Embong et al., 1977; Scheffer et al., 1977a).

Comparison of various isolation procedures and their effects on the aroma composition of the whole dill herb were reported in our previous paper (Huopalahti et al., 1981) and in the works of Kalitzki (1954b), Chubey and Dorrel (1976), and Koedam et al. (1979).

Detailed investigations with sophisticated analytical methods on the changes of aroma compounds in dill herb during the growth period are still scarce. The present work was undertaken to study the aroma composition and content of dill at three different growth stages the herb is used in Finland by a combined glass capillary gas chromatography–mass spectrometry method (GC–MS).

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Table I. Characteristics of Dill at Various Growth Stages

stage of growth	length of growth period, days	av height of herb, cm	dry weight, %	effective[a] day deg, °C
initial stage (A)	31	10	10.6	408
before bud formation (B)	39	25	12.0	516
flowering stage (C)	66	60	16.7	907

[a] Effective day degrees is determined as the sum of all the mean temperatures of the days of the growth period exceeding +5 °C.

EXPERIMENTAL SECTION

Materials. The dill (*A. graveolens* L. "Mammut") was grown in the Central Finland (Sahalahti) in 1980 on a prefertilized light sphagnum peat, huminocity 1–3 (Finn peat, ST-400-82). The whole herb was harvested at three different stage during the growth as described in Table I. The dill samples were packed in polyethylene bags, frozen immediately, and stored at −20 °C. Analyses were carried out during the week after the harvesting.

Isolation of Aroma Compounds. Aroma compounds were isolated by extracting 100 g of chopped frozen dill for 6 h with 450 mL of the mixture of redistilled *n*-pentane and diethyl ether (1:2 v/v) by using a modified Soxhlet technique in which the side tube of the regular apparatus was replaced by a column containing glass beads to prevent the cycle of aroma compounds. The extract was concentrated at 35 °C, with a Widmer column, to the volume of 2 mL. The purification of the concentrate was carried out at 10 °C on a column (20-mm i.d.) packed with 8 g of silica gel (Merck, silica gel 60, 70–230 mesh) as described by Scheffer et al. [1977b; see also Huopalahti et al. (1981)]. The aroma compounds were eluted with 80 mL of the same solvent mixture used for the extractions. The effluent was concentrated as above to the final volume of 500 μL prior to the GC analysis.

Gas Chromatography and Mass Spectrometry. The GC analyses of the aroma concentrates were carried out on a Varian aerograph, Model 2100-20 instrument, equipped with a flame ionization detector connected to a Varian CDS 111C integrator. Self-made glass capillary columns (0.32 i.d. × 42 m) coated with FFAP and temperature programming at the rate of 2 °C/min from 60 to

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240 °C were applied for separation. The temperature of injection port was 245 °C and that of detector was 255 °C. The split ratio was 1:30 and the flow rate of carrier gas (nitrogen) was 1.5 mL/min. For the optimization of the detector response, nitrogen was used as the makeup gas with the flow rate of 30 mL/min.

The 70-eV mass spectra were obtained on an LKB-9000 instrument by using the same capillary column as in the GC analysis in order to optimize the comparison with the analytical GC data.

The total aroma content was calculated by using linalool as the internal standard and summing up 50 corresponding peaks of each chromatograms.

Reliability of the Determinations. Analytical errors arising from solvent extraction and gas chromatography were studied by assaying concecutively 10 identical dill samples under the described experimental conditions. The relative standard error of the mean (SE) was 0.13%, when 20 aroma compounds from each sample were determined. The SE was calculated from the standard deviation by dividing it by the square root of the number of analyses.

RESULTS AND DISCUSSION

The conditions of the harvesting are given in Table I, since the state of maturity has an essential effect on the aroma composition and content of dill. This information is unfortunately lacking in many recent studies concerning the chemical composition of dill herb, which makes the comparison of the results difficult.

The aroma compounds were extracted from dill herb with the organic solvent mixture after the samples have been first chopped as frozen. This is because according to Koedam et al. (1979) it is hardly ever possible to liberate all aroma compounds from untreated plant material. The results of various isolation methods on the aroma components of dill herb have been presented earlier by us (Huopalahti et al., 1981).

The concentrates obtained after the solvent extraction and Widmer concentration were analyzed by glass capillary GC. The reproducibility of the method applied in this study can be considered very good (SE = 0.13%). Earlier it has been demonstrated in the literature that the relative proportions of individual terpenes in essential oil can be determined by GC with a reproducibility of 0.1%, but the type of column and instrumentation can increase this up to 0.5–1.0% (von Rudloff, 1973).

Combined GC–MS was employed for identification of components through comparison of mass spectra with those of authentic compounds and/or with authentic spectra (Stenhagen et al., 1974).

Twenty-two identified aroma compounds from dill at the three different stages of the growth and their relative amounts are presented in Table II according to their GC elution order. The most abundant aroma compounds in the concentrates were α-phellandrene, limonene, β-phellandrene, *p*-cymene, and 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran, and they comprised together 65–80% of all the analyzed compounds and 80–90% of all the identified compounds. α-Phellandrene was the major component in the aroma concentrate isolated from the dill at the initial stage (34.4%) and before the bud formation (47.4%), whereas in the concentrate isolated from the dill at the flowering stage, 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran, was the most abundant compound (31.5%). All listed compounds are reported before as constituents of dill (Huopalahti et al., 1981).

Table II clearly shows that there are certain characteristic variations in the relative amounts of aroma compounds in dill at different stages of the growth. The

Table II. Aroma Compounds Identified from Dill Herb (A) at Initial Growth Stage, (B) before Bud Formation, and (C) at Flowering Stages

compound[a]	relative amount, %[b] A	B	C
α-pinene	2.64	2.29	1.87
terpinolene	0.06	tr	tr
β-pinene	0.32	0.21	0.11
n-undecane	0.22	0.16	0.20
Δ^3-carene	tr	tr	tr
α-phellandrene	34.43	47.38	25.95
α-terpinene	tr	tr	tr
limonene	3.70	3.47	10.21
β-phellandrene	9.43	9.42	7.34
γ-terpinene	0.17	tr	0.33
p-cymene	6.60	4.70	5.03
cis-3-hexen-1-yl acetate	0.23	0.31	tr
cis-3-hexen-1-ol	0.13	tr	0.33
trans-2-hexen-1-ol	0.60	0.13	0.20
3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	10.93	15.50	31.50
terpinen-4-ol	0.20	0.15	0.75
α-terpineol	0.47	0.39	0.16
carvone	nd	tr	2.47
thymol	0.35	0.33	0.35
carvacrol	0.07	0.13	0.09
myristicin	7.63	2.81	nd
apiol	4.32	2.85	0.70
total	82.50	90.23	87.59

[a] Compounds are listed according to their elution order on an FFAP capillary column. [b] 50 aroma compounds were analyzed. tr, in trace amounts ≤ 0.05%. nd, not detected.

content of limonene, 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran, terpinen-4-ol, and carvone increased during the growth period. Especially the strong increase of carvone content was remarkable. At the initial stage carvone was not even measurable, while at the flowering stage its relative proportion was already 2.5% and is evidently further increasing, because it is one of the major components of dill seed oil as mentioned before. This result is supported by Guenther (1950), Kalitzki (1954a), and Zlatev (1975, 1976), who found that before the bud formation the carvone content was only 2–6% but after that it increased rapidly and reached 46% at the stage of full maturity. On the other hand, Dorrell (1972) and Embong et al. (1977) have reported that the increased carvone content was balanced first by a decrease in content of α-phellandrene and later on also by that of limonene. In our studies the beginning of the decrease of α-phellandrene can be noticed at the flowering stage.

The relative amount of 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran was at the initial stage 11%, while at the flowering stage it was already 31.5%. Its MS analysis showed the major peaks at *m/e* 152 (M^+, rel intensity 11%), 137 (100), 69 (31), 109 (30), 41 (21), 91 (15), 55 (14), and 79 (13). Göckeritz et al. (1979) identified this compound first from dill herb. Our result is confirmed in reports of Chou and Iwamura (1978) and Schreier et al. (1981), who also have identified this compound and found it to be one of the major components in dill herb. 3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran has been found earlier tentatively in dill oil (5.3–8.1%) and seed oil (2.8–4.9%) by Embong et al. (1977) and in herb oil (2.9%) by Belafi-Rethy et al. (1974).

Besides α-phellandrene mentioned above, the content of β-phellandrene, α-terpineol, myristicin, and apiol is decreasing during the dill growth (Table II). It is interesting to note that at the initial stage the total amount of myristicin and apiol, which have been found insectidal and

hallucinogenic (Harborne et al., 1969; Lichtenstein et al., 1974), was rather high (12%), but it diminished rapidly during the growth of the herb being below 1% at the flowering stage. Myristicin was not even detected at that stage. In the light of above results the absence of apiol and myristicin in "European" dill (*A. graveolens*) seeds is not surprising. However, they are present in "Indian" dill (*Anethum sowa*) seeds (Betts, 1969% Harborne et al., 1969).

The total amounts of aroma compounds at three growth stages (A, B, and C) were 6.8, 2.0, and 5.6 mg/g dry weight or 0.72, 0.24, and 0.94 mg/g fresh weight, respectively. The changes of total amount of aroma compounds in dill during the growth show an exceptional trend in that the lowest value is found at the stage before bud formation. This may due to the rapid growth of dill (Table I) compared with the biosynthesis rate of aroma compounds. On the contrary, Zlatev (1975) and El-Gengaihi and Hornok (1978) have found that the essential oil content in dill increased continuously during the whole growth period.

In Finland the dill is mainly harvested at the stage before bud formation. In spite of its low aroma content the quality of the odor and flavor is considered to be optimal for the Finnish sense of taste.

According to our preliminary sensory analyses the flavor of dill changes at the flowering stage to resemble that of dill seed oil which may arise from the increased content of carvone. Guenther (1950) has suggested that the odor and flavor of dill herb oil is mainly due to its content of α-phellandrene. The herb character predominate as long as the oil contains less than 35% of carvone. Also Embong et al. (1977) have stated that herb oils preferred by food industry are characterized by a lower content of carvone and a higher content of α-phellandrene than seed oil. On the other hand 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran found in great amounts in dill herb (Table II) and somewhat in dill seed oil (Embong et al., 1977) has been reported to exhibit an odor similar to fresh dill herb (Schreier et al., 1981).

Evidently small variations in the amounts of other aroma compounds of dill herb also affect the dill flavor, and this is a topic of our further investigations.

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Registry No. α-Pinene, 80-56-8; terpinolene, 586-62-9; β-pinene, 127-91-3; *n*-undecane, 1120-21-4; Δ^3-carene, 13466-78-9; α-phellandrene, 99-83-2; α-terpinene, 99-86-5; limonene, 138-86-3; β-phellandrene, 555-10-2; γ-terpinene, 99-85-4; *p*-cymene, 99-87-6; *cis*-3-hexen-1-yl acetate, 3681-71-8; *cis*-3-hexen-1-ol, 928-96-1; *trans*-2-hexen-1-ol, 928-95-0; 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran, 70786-44-6; terpinen-4-ol, 562-74-3; α-terpineol, 98-55-5; carvone, 99-49-0; thymol, 89-83-8; carvacrol, 499-75-2; myristicin, 607-91-0; apiol, 523-80-8.

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