Sensory study on the character-impact flavour compounds of dill herb (*Anethum graveolens* L.)

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The dependence of the characteristic odour note of dill herb on the concentration levels of five compounds, having the highest odour units in an extract obtained from the fresh material, was studied. (S)-a-Phellandrene was evaluated as the character-impact compound of the dill flavour which was rounded off by an additive effect of (3R, 4S, 8S)-3,9-epoxy-1-p-menthene (dill ether). The contributions of myristicin, methyl 2-methylbutanoate and (R)-limonene to the dill flavour appeared less significant.

INTRODUCTION

The volatile fraction of fresh dill herb has been analyzed by several authors to clarify the composition of the aroma. Among more than 120 compounds identified (review by Maarse & Visscher, 1989) (3R, 4S, 8S)-3,9-epoxy-1-p-menthene (dill ether) was assessed as the typical odour compound of the dill herb on the basis of its dill-like, floral and herbaceous aroma and its high odour activity value (Drawert et al., 1981; Huopalahti, 1986). The odour activity value (OAV) is defined as the ratio of the concentration of a compound in the food or food extract, to its odour threshold (Rothe & Thomas, 1963; Guadagni et al., 1966). Calculation of OAV for numerous compounds identified in dill herb had indicated a-phellandrene, myristicin and limonene, in addition to dill ether, as significant odorants (Huopalahti, 1986).

The determination of the odour activity of the dill herb volatiles by gas chromatography-olfactometry confirmed dill ether, (S)-alpha-phellandrene and myristicin as major odorants and, in addition, methyl 2-methylbutanoate instead of (R)-limonene (Blank & Grosch, 1991).

To clarify the actual contribution of each of these five compounds to the typical flavour of dill herb, the odour qualities of mixtures containing the five compounds in varying concentrations were described and compared to that of the dill herb. The details are reported in the present paper.

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MATERIAL AND METHODS

Materials

Fresh dill herb (*Anethum graveolens* L.), harvested in the beginning of July 1989, was supplied from a local farmer. N-Methyl-N-nitroso-p-toluenesulphonamide (MNSA), diethylene glycol monoethyl ether (carbitol) and Eugenol were from Merck (Darmstadt, FRG) and (+)-2-methylbutyric acid, (-)-2-butyric acid methyl ester, (+)-1-p-menthen-9-ol, (R)(+)-limonene, (+)-1-p-menthen-9-ol, (R)(-)carvone, sodium dithionite, potassium nitrosodisulphonate (Fremy's salt), and dodecane were from Aldrich (Steinheim, FRG). (R)(-)alpha-Phellandrene was supplied from Fluka (Buchs, Switzerland) and menthofuran was from Roth (Karlruhe, FRG).

The solvents were purified according to Schieberle and Grosch (1983). An ethereal extract was prepared from fresh dill herb as recently reported (Blank & Grosch, 1991).

Syntheses

Dill ether

A mixture of (3R, 4S, 8R)- and (3R, 4S, 8S)-3,9-epoxy-1-p-menthene was prepared by photooxidation of (+)-1-p-menthen-9-ol (Ohloff et al., 1966; Blank et al., 1989). The two isomers, formed in a ratio of 2 (8R-isomer) to 1 (8S; dill ether), were separated by preparative gas chromatography on an OV-11 column (3 m × 2 mm; 3% w/w of OV-11 on Chromosorb G-AWDMCS, 80–100 mesh) at 120°C. The purified dill ether was dissolved in diethyl ether.
(S) (+)-α-Phellandrene
The compound was prepared from (R)(-)carvone as recently reported (Sen & Grosch, 1990). It was purified by distillation in vacuo.

Myristicin
The compound was synthesized in a 67% overall yield following the work of Dallacker and Sluysmans (1969). Eugenol was oxidized with potassium nitrosodisulphonate (Fremy’s salt, Teuber & Tellinek, 1952) and the 3-methoxy-5-allyl-o-benzoquinone obtained was reduced to 3-methoxy-5-allylpyrocatechol with sodium dithionite. Methylolation with ClCH₂Br/K₂CO₃ did afford the target compound.

The crude product was applied onto a silica gel column (Blank & Grosch, 1991) and myristicin was eluted with diethyl ether-pentane (5:95, v/v; 150 ml).

Methyl (S)(+)-2-methylbutanoate
(S)(+)-2-methylbutyric acid was methylated with diazomethane (Schlenk & Gellermann, 1960). The exceeding acid was extracted with aqueous Na₂CO₃ (2M, 20 ml). The ester solution was washed with water (3 x 20 ml) and dried over anhydrous Na₂SO₄.

Quantitative analysis
The concentrations of the following compounds were determined by high resolution gas chromatography (HRGC) using the internal (i.st) or external (e.st.) standard substance given in brackets: dill ether (menthofuran, i.st.), (S)-α-phellandrene ((R)-α-phellandrene, e.st.), myristicin (eugenol, e.st.), methyl (+)-2-methylbutanoate (methyl (+)-2-methylbutanoate, e.st.).

The potent odorants of dill herb were quantified as described by Blank and Grosch (1991).

HRGC
HRGC was performed on capillaries SE-54 and OV-1701 using the conditions earlier reported (Blank et al., 1989).

Sensory evaluation
The sensory properties of the compounds listed in Table 1 were studied.

Sample preparation
To prepare stock solutions the compounds in the concentrations (mg/ml) reported in brackets were dissolved in diethyl ether: dill ether (5.75), (S)-α-phellandrene (45), myristicin (1.6), methyl (±)-2-methylbutanoate (0-117), and (R)-limonene (14-1). Mixtures of the compounds were prepared from the stock solutions, as exemplified in the following for the model presented in Table 2. Dill ether (80 µl = 460 µg), (S)-α-phellandrene (50 µl = 2250 µg), myristicin (15 µl = 24 µg), and methyl (±)-2-methylbutanoate (12 µl = 1.4 µg) were added to odourless tap water (200 ml). The mixture was stirred for 10 min at room temperature and then two sets of samples were prepared for nasal and retronasal perception of the odour. Set I: aliquots (20 ml each) were pipetted into glass vials (height 65 mm, volume 45 ml) which were closed with a glass cap. Set II: glass vials (volume: 30 ml) were completely filled with aliquots of the aqueous mixture and then sealed with a screw cap. The compositions of the other mixtures are reported in Tables 3–5.

An aliquot of the stock solution was diluted with odourless tap water and stirred for 10 min to prepare a Table 1. Sensory properties

<table>
<thead>
<tr>
<th>Compound</th>
<th>Odour description</th>
<th>Odour threshold³</th>
<th>Data from literature²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nasal</td>
<td>Retronasal</td>
</tr>
<tr>
<td>(S)(+)-α-Phellandrene</td>
<td>Dill-like, weak herbaceous</td>
<td>100-300 (200)</td>
<td>100-300 (200)</td>
</tr>
<tr>
<td>(R)(-)-α-Phellandrene</td>
<td>Terpeny, medicinal</td>
<td>300-700 (500)</td>
<td>nd</td>
</tr>
<tr>
<td>(R)(-)-Limonene</td>
<td>Citrus-like</td>
<td>100-200 (150)</td>
<td>500-2000 (1250)</td>
</tr>
<tr>
<td>Eugenol</td>
<td>Spicy, clove-like</td>
<td>100-200 (150)</td>
<td>nd</td>
</tr>
<tr>
<td>Methyl (±)-2-methylbutanoate</td>
<td>Fruity</td>
<td>0-3-0-5 (0-4)</td>
<td>0-1-0-3 (0-2)</td>
</tr>
<tr>
<td>Methyl (S)(+)-2-methylbutanoate</td>
<td>Fruity</td>
<td>0-2-0-4 (0-3)</td>
<td>nd</td>
</tr>
</tbody>
</table>

³ Odour threshold in water (µg/kg) evaluated in two replicates; the lower value of the range was perceived by 67% and the higher value by 100% of the assessor.

² The data in parenthesis show the mean values of the odour threshold ranges.


nd, Not determined.
Character-impact flavour compounds of dill herb

Table 2. Composition of the model mixture containing the four major odorants of dill herb

<table>
<thead>
<tr>
<th>Compound</th>
<th>Composition concentration level</th>
<th>Nasal perception</th>
<th>Retronasal perception</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount (mg/kg)</td>
<td>Multiple of the odour threshold&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I&lt;sup&gt;b&lt;/sup&gt;</td>
<td>II&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Dill ether</td>
<td>240</td>
<td>77</td>
<td>92</td>
</tr>
<tr>
<td>(S)-α-Phellandrene</td>
<td>1050</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>Myristicin</td>
<td>11</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Methyl 2-methylbutanoate</td>
<td>0.7</td>
<td>18</td>
<td>35</td>
</tr>
</tbody>
</table>

Odour quality of the mixture (no. of judges)

<table>
<thead>
<tr>
<th>Main</th>
<th>Additional</th>
<th>Main</th>
<th>Additional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbaceous (3)</td>
<td></td>
<td>Herbaceous (2)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Multiple of the odour threshold of the compound in the model mixture.

<sup>b</sup> Concentration in fresh dill herb (Blank & Grosch, 1991).

<sup>c</sup> Concentration in the model mixture (in water).

Sample for the determination of odour threshold values. The most concentrated sample was 15- to 20-fold above the threshold value of the compound. The concentration of diethyl ether was, even in the most concentrated samples, less than 0.1% (v/v), and not perceptible by the assessors.

Table 3. Odour profile of the mixture containing the major aroma compounds of dill herb as a function of the concentration of each component

<table>
<thead>
<tr>
<th>Component&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Odour notes (no. of judges)&lt;sup&gt;h&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture no. Name</td>
<td>(mg/kg)</td>
</tr>
<tr>
<td>1a (S)-α-Phellandrene</td>
<td>0</td>
</tr>
<tr>
<td>1b</td>
<td>2.2</td>
</tr>
<tr>
<td>1c</td>
<td>11.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>1d</td>
<td>22.6</td>
</tr>
<tr>
<td>1e</td>
<td>45.6&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>2a Dill ether</td>
<td>0</td>
</tr>
<tr>
<td>2b</td>
<td>2.3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>2c</td>
<td>4.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>2d</td>
<td>9.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>2e</td>
<td>18.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3a Myristicin</td>
<td>0.12&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3b</td>
<td>0.5</td>
</tr>
<tr>
<td>3c</td>
<td>1.9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3d</td>
<td>7.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3e</td>
<td>30.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3a Methyl 2- methylbutanoate</td>
<td>0.007&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3b</td>
<td>0.03</td>
</tr>
<tr>
<td>3c</td>
<td>0.12&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3d</td>
<td>0.29</td>
</tr>
</tbody>
</table>

<sup>a</sup> The concentration of the component was varied in the mixture while the concentration of the other three components remained constant and agreed with that occurring in the sample of dill herb (cf. Table 2).

<sup>h</sup> The odour quality was evaluated by nasal perception.

<sup>c</sup> Multiple of the mean odour threshold (OT, nasal perception) detailed in Table 1.

<sup>d</sup> The intensity of the odour impression was low.

<sup>e</sup> Composition of the model mixture presented in Table 2.
Table 4. Influence of (R)-limonene on the dill aroma

<table>
<thead>
<tr>
<th>Mixture no.</th>
<th>Concentration of (R)-limonene (mg/kg)</th>
<th>Odour note (no. of judges)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nasal perception</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Main</td>
</tr>
<tr>
<td>5a</td>
<td>0.2</td>
<td>Dill-like (8)</td>
</tr>
<tr>
<td>5b</td>
<td>0.4</td>
<td>Dill-like (8)</td>
</tr>
<tr>
<td>5c</td>
<td>1</td>
<td>Dill-like (8)</td>
</tr>
<tr>
<td>5d</td>
<td>2</td>
<td>Dill-like (8)</td>
</tr>
<tr>
<td>5e</td>
<td>5</td>
<td>Citrus-like (8)</td>
</tr>
<tr>
<td>5f</td>
<td>10</td>
<td>Citrus-like (8)</td>
</tr>
</tbody>
</table>

(R)-limonene was added to an aqueous solution of the model mixture reported in Table 2.

Rinsed into the mouth and the odour was then retronasally perceived.

Odour threshold values were determined by the triangle-test using odourless tap water as blank. The samples were presented in order of decreasing concentrations. The judges first rated each dilution as to whether or not it was distinguishable from the blanks. Once the threshold level was determined, the assessors were instructed to describe the odour quality individually and spontaneously. Descriptive terms obtained from different concentrated samples were recorded. For each compound the number of descriptors was reduced to three terms by deletion of rarely used descriptive terms and combination of synonymous descriptors.

The panellists were instructed to describe the odour quality of the mixtures as well as to specify differences from the odour of an extract which was prepared from fresh dill herb.

Table 5. Odour quality of (S)-α-phellandrene and dill ether

<table>
<thead>
<tr>
<th>Compound</th>
<th>Level (mg/kg)</th>
<th>Odour note (no. of judges)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nasal perception</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Main</td>
</tr>
<tr>
<td>(S)-α-Phellandrene</td>
<td>0.6</td>
<td>Dill-like (8)</td>
</tr>
<tr>
<td>2.8</td>
<td>Dill-like (8)</td>
<td>Herbaceous (2)</td>
</tr>
<tr>
<td>5.6</td>
<td>Dill-like (8)</td>
<td>Herbaceous (4)</td>
</tr>
<tr>
<td>11.2</td>
<td>Dill-like (8)</td>
<td>Herbaceous (4)</td>
</tr>
<tr>
<td>22.0</td>
<td>Dill-like (8), pungent (5), herbaceous (3)</td>
<td>Dill-like, burning (5), herbaceous, terpeny (2)</td>
</tr>
<tr>
<td>Dill ether</td>
<td>0.13</td>
<td>Dill-like (8)</td>
</tr>
<tr>
<td>0.52</td>
<td>Dill-like (8), ethereal (2)</td>
<td>Dill-like (8)</td>
</tr>
<tr>
<td>1.35</td>
<td>Minty-sweet (5), ethereal (3)</td>
<td>Dill-like (3)</td>
</tr>
<tr>
<td>5.7</td>
<td>Sweet-minty (6), ethereal-terpeny (2)</td>
<td>Dill-like (3), terpeny (2)</td>
</tr>
<tr>
<td>11.3</td>
<td>Ethereal-terpeny (7), pungent (1)</td>
<td>Minty-sweet (6)</td>
</tr>
</tbody>
</table>

Concentration level in water.
Low intensity.
High intensity.
Not tested.
RESULTS AND DISCUSSION

Odour threshold

At first the sensory purities of dill ether, (S)-α-phellandrene, myristicin, methyl (±)-2-methylbutanoate and (R)-limonene were checked by capillary gas chromatography-olfactometry using capillary OV-1701 (Blank et al., 1989). The odour of the major peak was identical with the overall odour description of the corresponding compound presented in Table 1 and, with the exception of myristicin, no impurities were perceived in the retention index range 700–2000. The impurity of the myristicin sample was identified as eugenol, the starting material of the synthesis. However, in solution the spicy odour of eugenol was completely masked by the myristicin odour, as the concentration of the impurity was very low (less than 1% in relation to myristicin) and its odour threshold was 2.5-fold higher than that of myristicin (Table 1).

The sensory properties of all the compounds investigated are listed in Table 1. In the tests to determine the threshold values, no significant difference was found for all the compounds investigated between the detection and the recognition threshold. The methyl 2-methylbutanoates (racemic mixture and S-enantiomer) had the lowest odour threshold values followed by myristicin and dill ether. The highest threshold values were found for α-phellandrene and (R)-limonene. The odour thresholds were determined by nasal and retronasal perception. Differences were only observed for (R)-limonene and, to a lesser extent, for methyl (±)-2-methylbutanoate (Table 1).

The enantiomers of α-phellandrene differed in the odour threshold values and qualities. The (S)-form, occurring in the dill herb (Blank & Grosch, 1991), smelled dill-like, weak herbaceous; and the (R)-form terpeny, medicinal. The stereochemistry of methyl 2-methylbutanoate present in dill herb was unknown (Blank & Grosch, 1991). As the odour threshold values and qualities of the racemic mixture and of the (S)-enantiomer were virtually identical (Table 1), it was suggested that the stereochemistry did not affect the sensory properties of the ester. In further experiments the racemic mixture of the ester was used.

A comparison of the threshold data found in this study with those published in the literature (Table 1) indicated an agreement for dill ether, (R) (+)-limonene and myristicin. However, 5-fold higher values were evaluated in the study for (S)-α-phellandrene and eugenol.

Simulation of the ‘dill-like’ odour note

A model mixture was prepared from the four compounds which, on the basis of the highest flavour dilution (FD) factors, were identified as the major odorants of dill herb (Blank & Grosch, 1991). The concentration of each compound dissolved in 1 kg of water was approximated to that occurring in 10 g of dill herb. Consequently, as shown in Table 2, the concentrations of the compounds were 4–77 times and 4–92 times stronger than their thresholds (Table 1) determined by nasal and retronasal perception, respectively.

The model mixture smelled dill-like with ethereal-terpeny and herbaceous (nasal perception) or with minty-terpeny and herbaceous by-notes (retronasal perception). The odour of the model was compared with that of an extract prepared from fresh dill herb. The extract was diluted with tap water as far as the concentrations of the four major odorants agreed with those of the model. In a triangle-test, four of the eight assessors were not able to distinguish the diluted dill herb extract from the model. The remaining four assessors described the odours of the two samples as very similar dill-like, but they could differentiate them. This experiment indicated that the model mixture met the odour of dill herb very well and that the odorants which occurred in addition to the four major compounds in the aroma extract and were recently identified (Blank & Grosch, 1991), did not contribute significantly to the characteristic odour note of dill herb.

Variation of the concentration

The concentration of each component in the model reported in Table 2 was varied, while that of the other three remained constant. The changes in the odour quality were evaluated and compared with the odour of the model. To facilitate this comparison, the odour description of the model is included (footnote 'e') in the results presented in Table 3.

The mixture smelled ethereal-terpeny, minty-sweet with a weak dill-note, when (S)-α-phellandrene was lacking (no. 1a in Table 3).

The addition of 2 mg/kg of (S)-α-phellandrene (1b) did change the odour of the mixture into a pleasant dill-like impression. The dill odour was enhanced by higher amounts of (S)-α-phellandrene (1c), but above a concentration range of 25–40 mg/kg it altered more and more into a pungent, terpeny quality (1d and 1e).

Compared to α-phellandrene the influence of dill ether on the dill odour was less strong as, in the absence of (2a) and in the presence of increasing concentrations of this compound in the mixture (2b and 2e), the dill odour note did still predominate. A comparison of no. 2a with no. 2b indicated that the addition of dill ether to the model reduced the herbaceous note in the odour profile. High concentrations of dill ether (2d and 2e) changed the dill into an ethereal-terpeny odour.

The spicy, nutmeg-like odour of myristicin and the fruity odour of methyl 2-methylbutanoate (Table 1) were completely masked by the other components of
the model. Only a 15-fold increase of myristicin (3c) and a 5-fold increase of the ester (4b) in the mixture changed the ethereal-terpeny and herbaceous into spicy and fruity notes, respectively. However, the dill-like main odour impression was only replaced by spicy and fruity odours, when much higher concentrations of both odorants were added to the model mixture (3e and 4d).

**Effect of (R)-limonene**

Huopalahti (1986) has suggested (R)-limonene as a potent odorant of dill herb. Therefore, the influence of this compound on the aroma of the model mixture was studied.

At low amounts of limonene the intense dill-like odour of the model was not changed. Some assessors detected additional minty-sweet and terpeny notes (nos 5a and 5b in Table 4). The typical citrus-note of limonene was only perceived when the concentration increased to 1 mg/kg (5c) and was predominant at levels of 5 mg/kg and higher (5e and 5f).

As discussed above the composition of the model mixture was related to the amounts of the four flavour compounds in 10 g of dill herb. As the (R)-limonone concentration in 10 g of the fresh material was only 0.49 mg (Blank & Grosch, 1991), the data reported in Table 4 allowed the conclusion that the character-impact odour note of dill was not significantly affected by this monoterpene.

**Sensory properties of α-phellandrene and dill ether**

As the experiments reported in Table 3 showed that α-phellandrene and dill ether were of major importance and myristicin and methyl 2-methylbutanoate of minor importance for the dill odour note, the sensory properties of the two first compounds were studied in more detail.

The odour qualities of both compounds in dependence on their concentrations in water are compiled in Table 5.

The dill-like odour quality of (S)-α-phellandrene did not change in the concentration range of 0.6-11.2 mg/kg, but the additional herbaceous (nasal perception), burning and terpeny notes (retronasal perception) increased with an increase of the concentration. At the high level of 22 mg/kg, the dill-like major odour impression became associated with herbaceous, pungent and burning odour notes.

Dill ether caused a weak dill-like odour only in a small concentration range. The most similar impression to the dill herb was obtained, when it was retronasally perceived in a concentration of 0.52 mg/kg. At higher concentrations the dill-note was more and more replaced by minty-sweet and ethereal-terpeny odour notes.

<table>
<thead>
<tr>
<th>Table 6. Sensory properties of a mixture of (S)-α-phellandrene and dill ether</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nasal perception of odour</strong></td>
</tr>
<tr>
<td><strong>Description</strong></td>
</tr>
<tr>
<td>Dill-like (8)</td>
</tr>
<tr>
<td>Herbaceous (3)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

A mixture of (S)-α-phellandrene (11.3 mg) and dill ether (2.3 mg) was dissolved in water (1 kg).

The sensory properties of a mixture of (S)-α-phellandrene and dill ether in concentrations equal to the model were evaluated. The results presented in Table 6 show that it smelled intensely dill-like with weak herbaceous and terpeny-minty by-notes established by only 3-4 of the 8 panellists. This result confirmed that (S)-α-phellandrene, in combination with dill ether, but not myristicin and methyl 2-methylbutanoate, contributed significantly to the dill odour note of dill herb.

**CONCLUSION**

The results obtained in this study show that (S)-α-phellandrene is the character-impact compound of the dill flavour which is rounded off by the additive effect of dill ether. They confirm the observation of Guenther (1972) that the flavour of dill herb oil is mainly due to its α-phellandrene content.

The results of this study are of interest also in regard to the processing of dill herb, e.g. the drying process has to be performed in such a way that only small losses of α-phellandrene and dill ether should occur.

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**REFERENCES**


