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Production of Volatile Organic Compounds by the Yeast Fungus *Dipodascus aggregatus*

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Summary. The yeast fungus *Dipodascus aggregatus* was grown aerobically in a synthetic nutrient solution and the volatile compounds produced were concentrated. Identification of the volatiles was performed by combined gas chromatography—mass spectrometry or by one of these methods. The compounds identified were 11 esters, 9 alcohols, 5 acids and 3 carbonyls.

The time course production of volatile neutral compounds was followed. During the phase of no apparent growth only a few substances were formed (mostly alcohols). The rapid phase of growth was characterized by an intense synthesis of many compounds in relatively high concentrations and later a sudden decrease in the number and amounts of substances. A slow successive decline in the number and amounts of volatile components was observed during the phase of no net growth.

The volatiles emitted by the fungus were concentrated, when most of the compounds were most abundant and the relative amounts of the major volatile neutral compounds were determined. The main components were ethyl acetate, ethyl propionate and ethanol.

Few systematic investigations of volatile products emitted by fungi have been performed. When comprehensive studies have been made, the aim has usually been to identify the aroma-giving compounds in a commercially important product, produced with the aid of a special fungus. As an example, many volatile compounds formed in alcoholic fermentation by *Saccharomyces cerevisiae* have been reported (NORDSTRÖM, 1967).

Biologists have been interested in volatiles emitted by fungi for another reason. They have observed physiological effects on the same or another organism caused by a volatile product of a fungus and often tried to isolate the active factor. DICK and HUTCHINSON (1966) reported that volatile metabolites emitted by 41 out of 62 tested fungi significantly stimulated or inhibited the linear growth of at least one other fungus. A volatile constituent of *Fomes annosus* strongly inhibited the growth of *Aspergillus niger* and *Chaetomium globosum*. The active component was later identified as hexa-1,3,5-triyne (GLEN *et al.*, 1966). In a further

investigation GLEN and HUTCHINSON (1969) examined the effects of volatile compounds produced in cultures of *Saccharomyces cerevisiae* on growth and sporulation of *Aspergillus niger* and seed germination of *Lepidium sativum*. The test organisms were inhibited by the volatile metabolites. Of 7 volatile organic substances identified, acetaldehyde and ethanol inhibited the growth of *Aspergillus niger* in concentrations equal to those found above cultures of *Saccharomyces cerevisiae*. Inhibition of spore production of *Aspergillus niger* was probably caused by CO_2 accumulation. The most active inhibitors of *Lepidium sativum* seed germination were ethanol and 3-methyl butanol-1.

In the present investigation a systematic identification of volatile constituents of *Dipodascus aggregatus* was performed. A suitable assay fungus, *Pestalotia rhododendri*, was then exposed to the vapours of the compounds identified and the effects of the treatment observed. The latter part of the investigation has already been published (NORRMAN, 1968).

Material and Methods

The strain of *Dipodascus aggregatus* Francke-Grosmann used in this study was obtained from Dr. Nyman, who isolated the fungus from slime flux (NYMAN, 1961).

The medium and cultivation methods of NYMAN (1966) were used with a few exceptions, e.g. the glucose concentration was increased from 1% to 2%.

The fungus was aseptically grown in rectangular culture flasks with a capacity of 1400 ml. Each flask was supplied with 500 ml of nutrient solution and was stoppered with a cotton-wool plug.

In some experiments the medium was strongly buffered and then had the following composition: Glucose 20.0 g; L-asparagine 1.0 g; KH_2PO_4 1.0 g; Na_2SO_4 0.28 g; biotin 2 µg; citrate buffer (GOMORI, 1955); micro nutrients according to NYMAN (1966) and distilled water 1000 ml. To obtain a pH value of about 3.2 (4.6) citric acid · H_2O 9.18 g (5.36 g) and trisodium citrate · 2 H_2O 1.85 g (7.21 g) were added to the medium per liter.

The experimental flasks (usually 9) were inoculated as described by NYMAN (1966), but the method was scaled up. A culture intended for inoculation was grown in a 300 ml Erlenmeyer flask containing 100 ml medium. After 25—29 h the culture had an absorbance of 0.010 to 0.055 at 610 nm when measured turbidimetrically and 10 ml were distributed to each experimental flask. The flasks were mounted in an upright position on a shaker with a reciprocating motion at 25° C in the dark.

Growth was measured as the dry weight of the fungus. From each experimental flask 10 ml fungal cells and medium were harvested and centrifuged. After washing three times with distilled water the cells were dried at 102° C for 24 h and weighed. The supernatant solutions after the first centrifugation were pooled for determination of final pH.

Glucose determinations were performed by the glucose oxidase method as described by LINDBERG (1963).

Concentration of Fungal Volatiles

One experimental flask was harvested and 400 ml of the medium and fungus diluted with 300 ml distilled water and extracted with 450 ml diethyl ether (analytical reagent or recycled) in a conventional type liquid/liquid extractor for about

17 h. The ether was always distilled through a column (50×3 cm) packed with small glass cylinders shortly before use. At the end of the extraction anhydrous Na_2SO_4 was added to the ether phase and the extract was placed overnight at 4° C. On a few occasions the ether phase was contaminated with small amounts of emulsions at the end of the extraction and these were removed by decanting and filtering. The ether was removed by fractional distillation through a short column packed with glass cylinders and the volume of the residue measured (about 1.5 ml).

Analysis

The distillation residues were directly used as samples for gas chromatographic examination on a Perkin-Elmer 880 chromatograph equipped with a flame ionization detector. Hamilton syringes of 1,10 and 100 µl volume were used for sample introduction.

Columns:

1. A 4 m×4.75-mm I.D. copper tube filled with 15% UCON HB 2000 on Chromosorb W AW, 60—80 mesh and a carrier gas flow rate of about 50 ml N_2/min.

2. A supported coated open tubular column, 50 feet×0.020-inch I.D. stainless steel, coated with Carbowax 20 M. Flow rate about 3.5 ml N_2/min.

3. A 4 m×1/8-inch O.D. stainless steel tube packed with 20% diiso-decyl phthalate on Chromosorb P, 60—80 mesh. Flow rate about 24 ml N_2/min.

4. A 4.8 m×1/8-inch O.D. copper tube filled with Porapak Q, 100—120 mesh. Flow rate about 29 ml N_2/min.

5. A 2.6 m×1/8-inch O.D. stainless steel tube packed with 25% Carbowax 20 M on Chromosorb W AW DMCS, 80—100 mesh. Flow rate: 25 ml He/min.

6. A 2.5 m×1/8-inch O.D. stainless steel tube filled with 5% FFAP on Chromosorb G AW DMCS, 80—100 mesh. Flow rate about 29 ml N_2/min.

When column No. 2 was used an input sample splitter of about 14:1 was installed in the chromatograph (Golay Column Adapter 008-0544 and needle restriction 6, Perkin Elmer).

Identification was performed by comparisons of retention time data on column No. 1—4 with known standards; by standard addition, by component subtraction (Hefendehl, 1964; Ikeda *et al.*, 1964; Hoff and Feit, 1963, 1964; Fredricks and Taylor, 1966) and by sample distribution between two layers of different polarity (Suffis and Dean, 1962). Some of the components could be tentatively identified by their typical smell, which was apparent at the column exit.

Furthermore a concentrate of volatiles was preparatively separated on column No. 1 and the main peaks were trapped in tubes containing 1—2 ml chilled acetone (analytical reagent). Ten successive runs were made, each time 100 µl sample were injected, and the peaks collected in the same traps every time. Approximately 1/5 of the column effluent was led to the flame ionization detector, the rest to a trap. The fractions collected were analyzed on other columns and those containing the main components examined in a combined gas chromatograph—mass spectrometer (LKB 9000). Column No. 5 was used and 5 µl sample injected. The mass spectra were recorded at 70 eV. Before analysis some of the fractions were concentrated about 10 times in a micro-distillation apparatus including a Vigreux column.

A concentrate of volatile constituents of the fungus was separated according to acidity (cf. Ryder, 1966). The separation scheme is shown in Fig.1. Small glass tubes with screw caps were used for the separations and the upper phases carefully collected with a 100 µl syringe.

Preliminary quantitative determinations were made of the components in one concentrate of fungal volatiles. For the area measurements an electronic integrator, Perkin-Elmer D 24, was used. No correction factors were determined.

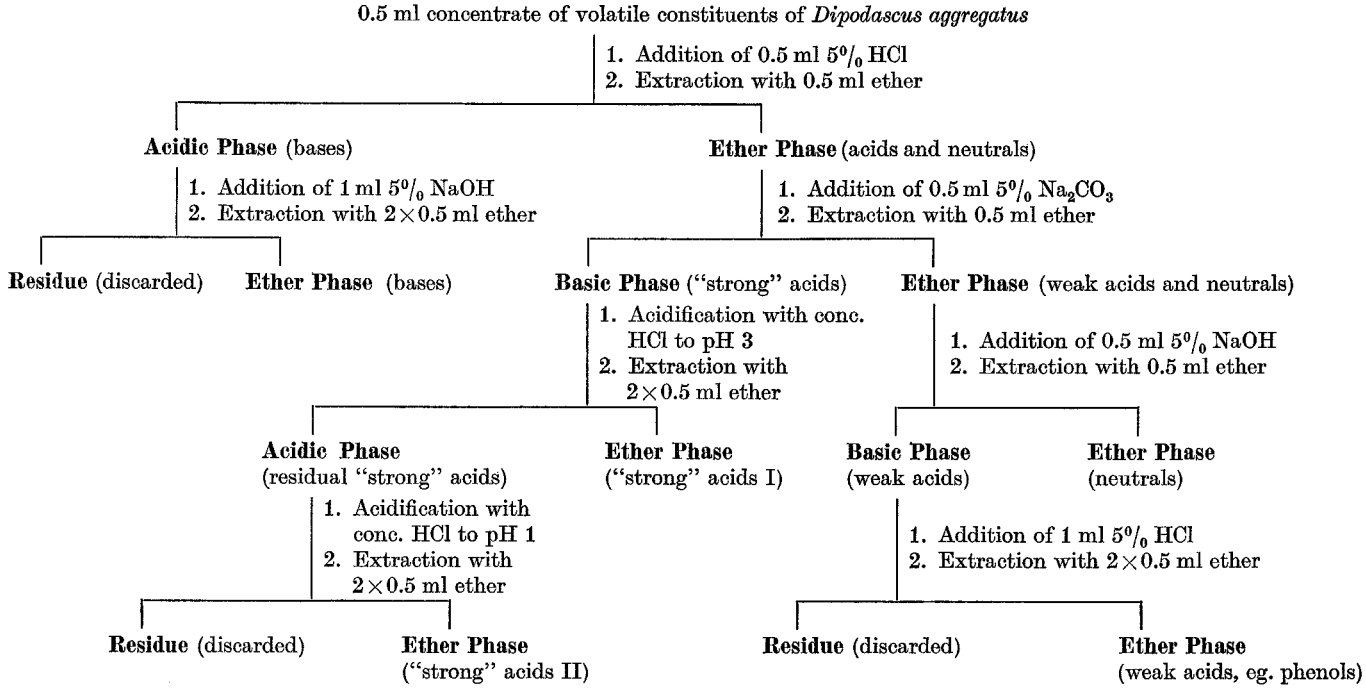


Fig. 1. A scheme for the separation of volatile constituents of *Dipodascus aggregatus* according to acidity

Results

When *Dipodascus aggregatus* was growing in the nutrient solution described by NYMAN (1966) a pleasant aroma reminiscent of apple was produced. The first experiment aimed at a time course study of the production of volatile substances in a culture of the fungus. The growth curve is shown in Fig. 2. Simultaneously with the dry weight determinations, one experimental flask was withdrawn and the contents extracted. It was considered satisfactory to extract only one flask each time, as the growth was uniform within each experiment. The standard errors of the mean as described by FISHER (1946) rarely exceeded 5% and never 10% of the mean dry weight values (4—9 replicates). At this stage of the study, the main interest was to determine when the production of volatiles was most intensive. As shown in Fig. 3 this occurred when the fungus had grown for about 88 h. The number of compounds detected was then at an optimum and the concentrations were higher than earlier or later for most of the compounds.

The hydrogen ion concentration was considerably increased during the experimental time and this change could be expected to influence the production of volatile substances. Ethyl acetate formation in aerobic

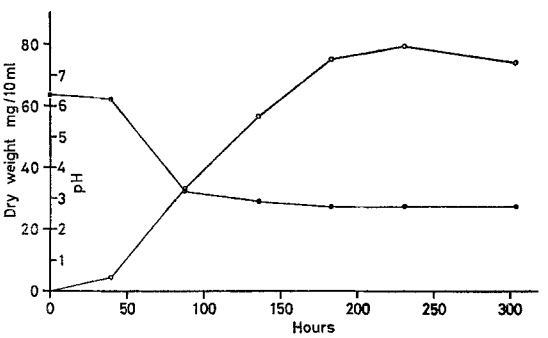
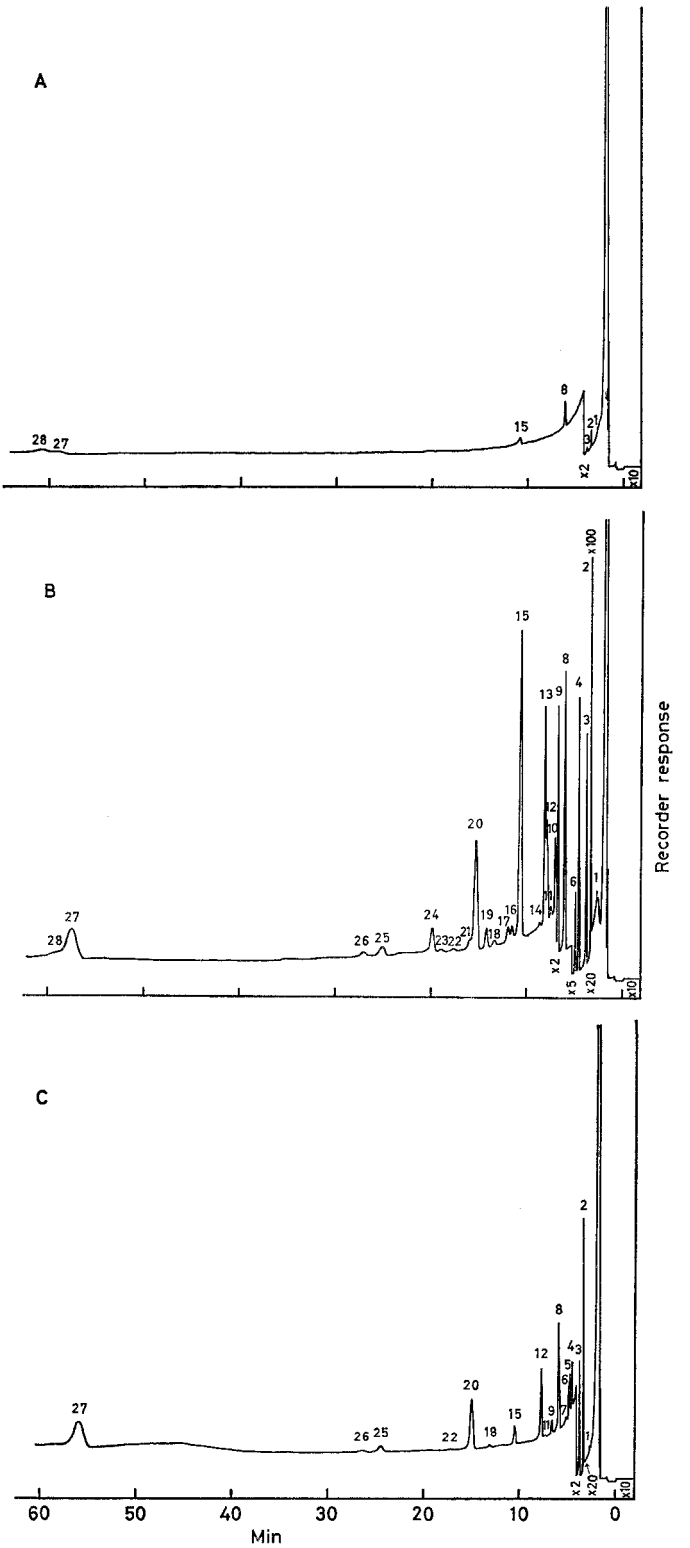


Fig. 2. The growth of and pH changes in the medium by *Dipodascus aggregatus*. Open circles = dry weight, closed circles = pH

Fig. 3. Production of volatile compounds by *Dipodascus aggregatus*. Cultures of the fungus were extracted at an age of A 39.5, B 87.5, C 135.5, D 183, E 231 and F 303.5 h. The growth curve is shown in Fig. 2. Gas chromatographic analysis was performed on column No. 2 at 75° C. One µl of sample was always injected and an input sample splitter of 14:1 used. The attenuation positions are indicated in the chromatograms. The following components, all eluted later than the big ether peak, were identified: 1 ethyl formate, 2 ethyl acetate, methanol, 3 ethanol, 4 ethyl propionate, 6 n-propyl acetate, 8 n-propanol, n-butyl formate, 9 ethyl n-butyrate, 12 n-butyl acetate, iso-butanol, 15 n-butanol, iso-amyl acetate, n-propyl n-butyrate, 19 n-amyl acetate, 20 iso-amyl alcohol, active amyl alcohol, and 24 n-amyl alcohol. If a peak contained many compounds, the major component is mentioned first


A
28
27
15
8
21
x2
x10
B
x100
2
15
8
13
9
4
3
12
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6
1
27
28
24
26
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17
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14
x2
x5
x20
x10
Recorder response
C
27
26
25
22
20
18
15
12
8
9
4
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6
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x2
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60
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40
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0
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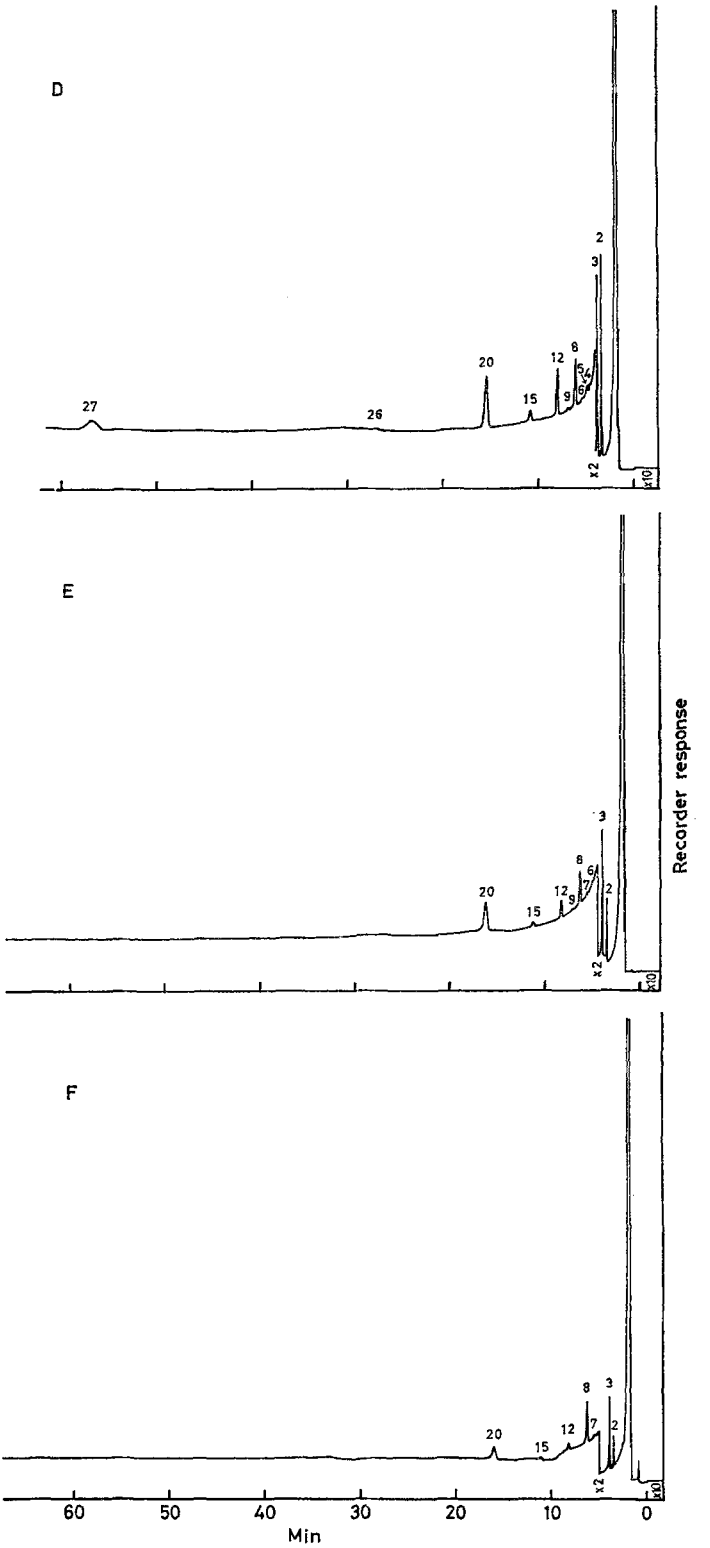
Figs. 3 A—F



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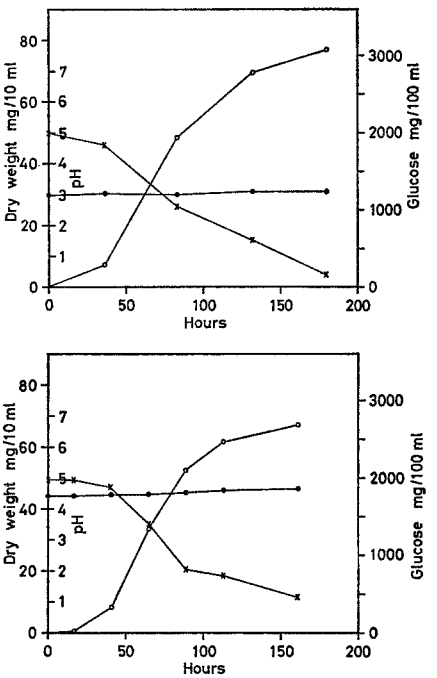

Fig. 4. The growth of *Dipodascus aggregatus* in well buffered media. Above, pH 3; below, pH 4.5. Open circles = dry weight, closed circles = pH, crosses = glucose concentration in the medium

cell suspensions of *Hansenula anomala* varied with pH and an optimum was obtained at a pH of 4.5 to 5.0, when the medium contained ethanol and acetic acid (PEEL, 1951). A pH optimum of 2.1—2.6 for production of ethyl acetate from ethanol was reported for *H. anomala* by TABACHNICK and JOSLYN (1953b). *Dipodascus aggregatus* was grown in well-buffered media at pH 3 and 4.5 and the growth curves are illustrated in Fig. 4. The growth rate was higher than in the former experiment because of a somewhat increased shaking motion. The formation of volatile substances was similar at both pH values tested and principally followed the same pattern as in the poorly-buffered medium according to NYMAN (1966). When extractions were made each day, an optimum in production of the volatiles was found at a culture age of 65 h. After this time the number and amounts of volatiles rapidly decreased. In comparison with the production of volatiles in the medium described by NYMAN (1966), fewer substances were formed and they occurred in lower concentrations. A pH below 2.90 was not tried as NYMAN (1961) showed that *D. aggregatus* was unable to grow at pH values about 2.

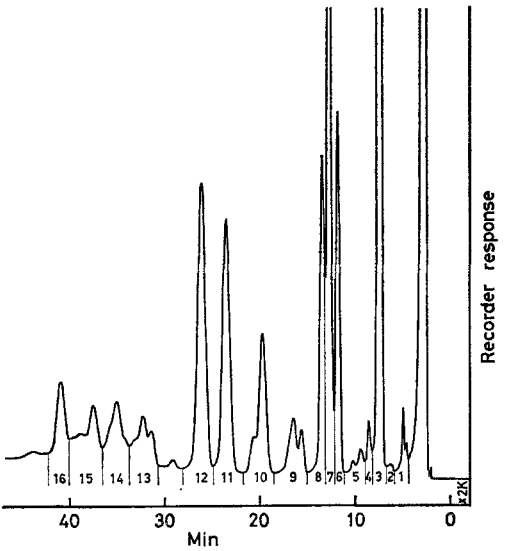

Fig. 5. Separation of a concentrate of volatile compounds produced by *Dipodascus aggregatus*. The gas chromatographic analysis was performed on column No. 1 and 10 µl sample injected. The column temperature was isothermal at 75°C for 24 min and then programmed at 4°C/min to 100°C. Preparative separations of the sample were made under the same conditions and the fractions indicated in the chromatogram trapped

To determine whether the sudden decrease in fungal volatiles could be explained by an exhausted carbon supply or not, glucose determinations were made as shown in Fig. 4. The decline was not due to a lack of carbon, since there was glucose left at the end of the experimental time.

In consequence with these results, an experiment was started aiming at identification of the main volatile compounds produced. Cultures were grown in the poorly-buffered medium and after 65 h, 700 ml medium and fungus were extracted with 450 ml ether. Altogether 2800 ml medium and fungus were extracted in 4 runs, every time with the same ether (losses were replaced). The ether phase was dried overnight with anhydrous Na_2SO_4 and the ether removed. The distillation residue, 2.6 ml, was used for gas chromatographic and mass spectrometric analysis. Fig. 5 illustrates the separation achieved on the column used for a preparative fractionation of the concentrate of volatiles. The results obtained are presented in Table 1. The main neutral, volatile products were alcohols and esters. Fraction No. 14 was analyzed on a glycerol column and the presence of both iso-amyl alcohol (3-methyl butanol-1) and active amyl alcohol (2-methyl butanol-1) in a ratio of 3:1 was revealed.

Table 1. *Identification of compounds produced by* Dipodascus aggregatus *and separated by preparative gas chromatography. The fraction nos. refer to Fig. 5. Many minor components, which remained unidentified, are not included in the Table.* + *Identity confirmed by the method indicated.* — *Unsuccessful attempts to identify the component*

Fraction No.	Identification by means of		Compound
	Mass spectrometry	Gas chromatography	
1		—	unknown
		+	ethyl formate
2		+	methanol
3	+	+	ethyl acetate
	+	+	ethanol
4		+	iso-propyl acetate
5		+	diacetyl
		—	unknown
6	+	+	ethyl propionate
7	+	+	n-propyl acetate
8	+	+	n-propanol
9	+	+	n-butyl formate
	—	—	unknown
10	+	+	ethyl n-butyrate
	+	+	iso-butanol
	(+)		(4-methyl 3-pentene-2-one)
11	+	+	n-butyl acetate
12	+	+	n-butanol
	+		4-methyl 2,3-pentanedione
13	+	+	iso-amyl acetate
	+	+	n-propyl n-butyrate
	—	—	unknown
14	—	—	unknown
	+	+	iso-amyl alcohol
	—	+	active amyl alcohol
15	+	+	n-amyl acetate
	—	—	unknown
16	+	+	n-amyl alcohol

A part of the same concentrate of volatiles from the fungus was separated according to acidity (Fig. 1). The two fractions designated "strong" acids were analyzed on column No. 6 and both contained the same acids, but the concentrations were much higher in fraction I. This fraction was examined by combined gas chromatography (column No. 6) and mass spectrometry and the following acids were identified: Acetic acid, propionic acid, iso-butyric acid, butyric acid and iso-valeric acid.

Only small amounts of weak acids and bases were found and therefore these fractions were not further examined.

The fraction which contained neutral compounds was analyzed on column No. 2 and the resulting chromatogram very much resembled a

chromatogram of the original sample obtained under the same gas chromatographic conditions. The alcohol peaks had increased a little in comparison with the ester peaks, which indicated a certain degree of hydrolysis of the esters in the sample.

In Table 2 all the compounds identified are summarized.

Table 2. *Volatile constituents of* Dipodascus aggregatus. *The identity of most compounds was confirmed by mass spectrometry (= MS) and gas chromatographic data (= GC). If only one method was used, this is indicated in the Table and the identification must then be considered as tentative*

Compound	Identification method	Compound	Identification method
Methanol	GC	iso-Propyl acetate	GC
Ethanol		iso-Amyl acetate	
n-Propanol		Ethyl propionate	
n-Butanol		Ethyl n-butyrate	
n-Amyl alcohol		n-Propyl n-butyrate	
iso-Butanol		Furfural	
iso-Amyl alcohol		Diacetyl[a]	GC
active Amyl alcohol	GC	4-Methyl 2,3-pentane-dione	MS
2-Phenethyl alcohol			
Ethyl formate	GC	Alkyl thiophene[b]	MS
n-Butyl formate		Acetic acid	
Ethyl acetate		Propionic acid	
n-Propyl acetate		Butyric acid	
n-Butyl acetate		iso-Butyric acid	
n-Amyl acetate		iso-Valeric acid	

[a] Identity confirmed by the typical smell.
[b] Probably n-propyl thiophene.

With most of the main volatile products identified, it was interesting to examine the earlier experiments anew. In Fig. 3 it is possible to follow the appearance and disappearance of many compounds; not the acids however, as they are eluted very late from the column used. About the end of the phase of no apparent growth, only a few compounds could be detected and most of them were alcohols. The relatively high-boiling components in peak No. 27 (probably a 2,3-diketone) and No. 28 (unknown) were already formed. During the first half of the rapid phase of growth, at a culture age of 88 h, nearly every neutral compound ever found in this investigation was detected and many of them occurred in their optimum concentrations. After this time a successive decline in number and amounts of volatile products was noticed. The following compounds were found in nearly every sample: Ethyl acetate, ethanol, n-propyl acetate, n-propanol, ethyl n-butyrate, n-butyl acetate, n-butanol, iso-

amyl alcohol + active amyl alcohol and the compound in peak 27. In a 300 h old culture many esters had disappeared and there were more alcohols left.

A coarse picture of the time course production of acids by *Dipodascus* is given by the pH curve in Fig. 2. To begin with the production of acids followed the same pattern as for the neutral compounds, but after 90 h the acids or the total amount of acidic substances remained unchanged in contrast to the neutral compounds.

Table 3. *Relative amounts of the major, neutral components in a concentrate of volatile constituents of* Dipodascus aggregatus. *A chromatogram of this concentrate is shown in Fig. 3B. For the quantitative separations column No. 1 was mainly used. The amount (= area) of n-amyl alcohol was set = 1 and the relative amounts of the other compounds estimated. If a peak contained more than one substance the main component is mentioned first. The nos. in the table refer to the corresponding fraction nos. in Fig. 5. The measured peaks constituted major parts of these fractions*

No.	Compound	Relative amount
3	ethyl acetate, ethanol	59, 10
5	diacetyl	0.2
6	ethyl propionate	16
7	n-propyl acetate	5.5
8	n-propanol	6.5
9	n-butyl formate, unknown	0.8
10	ethyl n-butyrate, iso-butanol	8.2
11	n-butyl acetate	3.1
12	n-butanol, 4-methyl 2,3-pentanedione	5.1
13	iso-amyl acetate, n-propyl n-butyrate	2.1
14	iso-amyl alcohol, active amyl alcohol	4.3
15	n-amyl acetate, unknown	0.9
16	n-amyl alcohol	1
—	a 2,3-diketone ?[a]	2.0

[a] Peak No. 27 in Fig. 3.

The relative amounts of the main volatile neutral compounds in the richest concentrate were determined and the results are presented in Table 3. Ethyl acetate was the entirely dominating substance and occurred in nearly the same amount as all the other compounds together.

Two control experiments were performed. The direct ether extraction method used to concentrate the volatile constituents of the fungus was not ideal, as the emulsions formed sometimes contaminated the ether extracts. To determine whether this was deleterious to the extracts or not, the first control experiment was carried out. *Dipodascus* was grown as usual and fungus and medium were distilled. The pH of the distillate

was adjusted to 8.2 (to bind the acids formed) and the usual ether extraction and concentration procedure followed. Gas chromatographic analysis showed that this sample was qualitatively very alike those prepared by direct extraction. Obviously, the emulsions did not damage the ether extracts seriously.

In the other control experiment uninoculated culture flasks containing autoclaved medium were handled as if they had been inoculated; yet with one exception. The flasks were placed on the usual shaker at 25°C in the dark, but the incubation time was prolonged to 14 days, to permit any volatile substances formed in the medium to accumulate. The medium was then ether extracted and the volatiles concentrated in the usual way. Gas chromatographic analysis detected 7 components, all in low concentrations. Retention time data indicated that ethyl formate, ethyl acetate and furfural were present.

Discussion

The volatile organic compounds produced in an aerobic culture of *Dipodascus aggregatus* were mainly esters (especially ethyl acetate), alcohols and acids. Many of these compounds have agreeable odours, which explains the pleasant smell emitted by a growing culture of the fungus. Most of the compounds formed by *D. aggregatus* have earlier been reported as by-products of alcoholic fermentation (Drawert and Rapp, 1966; Nordström, 1967), but there were quantitative differences. Typical by-products of alcoholic fermentation are iso- and active amyl alcohol, 2-phenethyl alcohol, ethyl acetate, iso-butanol, n-propanol, n-butanol and iso-butyl acetate. All of these except for iso-butyl acetate were detected in cultures of *D. aggregatus* and probably this ester was present too as a minor component. Some esters, e.g. ethyl acetate, ethyl propionate, n-propyl acetate, ethyl n-butyrate and n-butyl acetate, were relatively more abundant than in typical fusel oils. In *Saccharomyces*-fermentations ethyl propionate is rarely produced (Nordström, 1964), but in aerobic cultures of *D. aggregatus* it was a major product.

The effects of gentle aeration on *Saccharomyces*-fermentation was studied by Nordström (1966). A small increase in the total amount of volatile esters was obtained, while the formation of fusel alcohols was not influenced. Obviously, there is a fundamental difference in the production of volatile compounds between *S. cerevisiae* and *D. aggregatus*, which is not surprising since they belong to different families. The ability to form esters is even strain specific in the genera *Saccharomyces* and *Ceratocystis* (Nordström, 1964; Sprecher, 1968). The composition of the medium is of course important. Drawert and Rapp (1964, 1966) cultured *S. cerevisiae* strain H_2 in a synthetic medium on different

nitrogen sources and obtained wide quantitative variations in the formation of volatile products.

Two of the compounds identified in this investigation have not earlier (to my knowledge) been reported as produced by fungi, namely n-butyl formate and n-propyl n-butyrate. The latter ester has earlier been detected in apple aroma (FLATH *et al.*, 1967).

In a gas chromatographic study of dicarbonyls in beer HARRISON *et al.* (1965) observed a small component in the chromatograms, which they proposed was 4-methyl 2,3-pentanedione. Their assumption was probably right as the same dicarbonyl was present in a concentrate of volatiles of *D. aggregatus*.

The alkyl thiophene detected, which probably was n-propyl thiophene, very likely originated from the metabolic activity of the fungus on biotin, as this vitamin was included in the nutrient solution.

Furfural (2-furaldehyde) has often been reported as a fungal metabolite (e.g. COLLINS and KALNINS, 1966), though it might be an artefact. If a medium containing pentoses is heated, furfural is formed (SIEFKER and POLLOCK, 1956). A small amount of furfural was also formed in an autoclaved, uninoculated medium with no other carbon source than glucose, as shown in the second control experiment related above. In many studies of volatiles emitted by fungi, complex natural media have been used and no attempt made to distinguish the substances produced by the fungus from those originating directly from the medium or formed by heating of the medium.

Biosynthesis of the main by-products of *Saccharomyces*-fermentation has been reviewed by STEVENS (1960), WEBB and INGRAHAM (1963) and NORDSTRÖM (1967). Whether the synthesis of volatile compounds in an aerobic culture of *D. aggregatus* follows the same pathways was not investigated. However, the striking qualitative resemblance in the formation of volatile products between *Saccharomyces* and *Dipodascus* indicates a near relationship and they have probably many biosynthetic pathways in common.

The production of volatile neutral compounds in a shake culture of *D. aggregatus* was, as already mentioned, characterized by a real outburst of many substances during the first half of the phase of rapid growth and then a sudden decline in the number and amounts of the substances. When the rapid phase of growth began, it was accompanied by an intense synthesis of volatile products. This was not the case in an aerobic culture of the yeast fungus *Hansenula anomala*, in which the production of ethyl acetate started later and reached a peak at the beginning of the autolysis phase of growth (TABACHNICK and JOSLYN, 1953a). In a shake culture of *Ceratocystis coerulescens* the production of volatile substances similarly reached an optimum at the beginning of the phase of no net

growth (SPRECHER, 1964). The cause of the surprisingly small amounts of volatile products in the older cultures of *D. aggregatus* was not investigated and there are at least two possible explanations. Either the synthesis of volatiles decreased and the volatile products in the medium evaporated, or the synthesis went on at the same rate, but the substances were utilized in the metabolism of the fungus. The second hypothesis is most probable as it has been shown that ethyl acetate formed in aerobic cultures of *H. anomala* is hydrolyzed by an esterase at pH values above 3 (TABACHNICK and JOSLYN, 1953b). Furthermore, the same authors studied the evaporation of ethanol and ethyl acetate from a shaken water solution and concluded that the losses were too small to explain the rapid decline of these substances in a shake culture of *H. anomala* (TABACHNICK and JOSLYN, 1953a).

Volatile metabolites emitted by fungi are often considered as secondary products, the function of which, if any, is rather obscure. Ethyl acetate formation in aerobic cultures of *H. anomala* is possibly a detoxification of acetic acid until it can be used in the rate-limiting citric acid cycle (TABACHNICK and JOSLYN, 1953a). NORDSTRÖM (1964) proposed the following functions of ester formation in *Saccharomyces*: a) "a regulation of the concentration of various acyl-CoA-compounds, similar to the manner in which ATP-ases regulate the level of ATP;" b) A reduction of the amounts of acyl-CoA-compounds in the cell as they may be toxic in high concentrations; c) Esters are formed as by-products in reactions catalyzed by rather unspecific enzymes; d) The aroma of esters may be attractive to insects, which are important in the dispersal of the fungus.

The last hypothesis might be applicable to *D. aggregatus* as the fungus has been isolated from channels in pinewood made by the bark beetle *Ips acuminatus* (FRANCKE-GROSMANN, 1952).

Many of the compounds identified in this study stimulate the production of conidia in *Pestalotia rhododendri* (NORRMAN, 1968). In a preliminary test *D. aggregatus* and *Pestalotia rhododendri* were inoculated in different compartments of the same Petri dish with a common atmosphere. Seven days later sporulation and linear growth of *P. rhododendri* were somewhat inhibited, but the production of aerial hyphae slightly stimulated. These results are in keeping with those obtained with *Aspergillus niger* exposed to volatiles emitted by *S. cerevisiae* (GLEN and HUTCHINSON, 1969). Whether the volatiles produced by *D. aggregatus* could affect the development of other fungi in nature is nevertheless doubtful.

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