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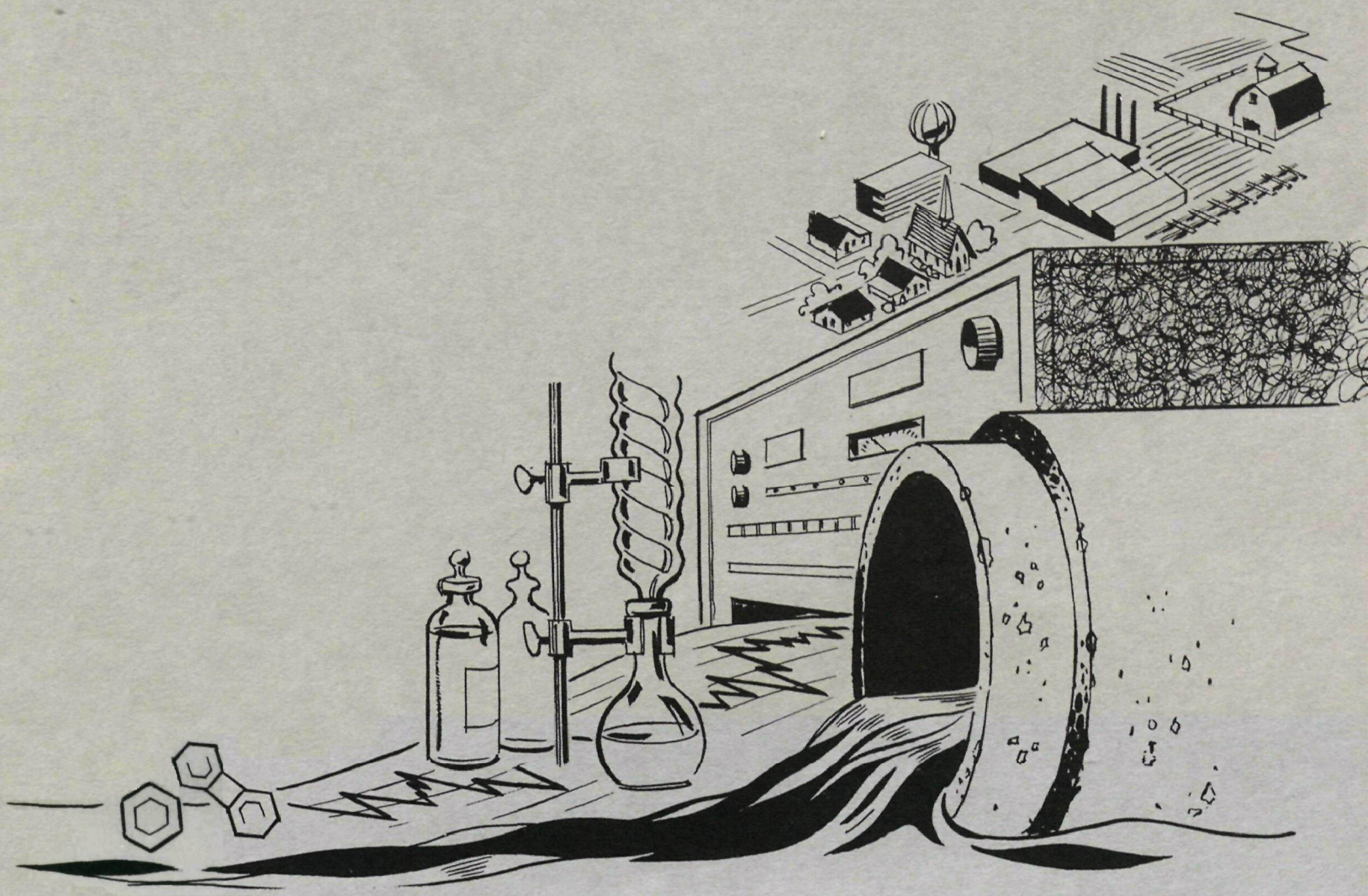
# CHARACTERIZATION OF TASTE AND ODORS IN WATER SUPPLIES



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# CHARACTERIZATION OF TASTE AND ODORS IN WATER SUPPLIES

by

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for the

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## ABSTRACT

An examination of the odorous constituents produced by microorganisms which are involved in taste and odor problems in public water supplies was conducted. The organisms used in the study were the actinomycete *Streptomyces odorifer*, the alga *Synura petersenii*, and the fungus *Trichoderma viride*.

The odorous constituents produced by these organisms were obtained by steam distillation of the culture medium containing the cells. The aqueous distillates were extracted with an organic solvent and these extracts were then reduced to a small volume before analysis.

Analysis of the extracts was made by gas chromatography, infrared, mass and nuclear magnetic spectroscopy. The major odorous constituents produced by *Streptomyces odorifer* have been identified and the compounds are trans-1, 10-dimethyl-trans-9-decalol (geosmin), 2-exo-hydroxy-2-methylbornane and cadin-4-ene-1-ol. A large number of odorous constituents were also identified from the culture filtrates *Synura petersenii* and heptanal was the one constituent having an odor similar to that of the organism. The major odorous compound produced by *Trichoderma viride* was 6-pentyl- $\alpha$-pyrone.

This report was submitted in fulfillment of project number 16040 DGH.

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## SECTION I

## CONCLUSIONS

1. Satisfactory analytical procedures for the analysis of small samples of taste and odor constituents have been developed.

2. Large scale cultural and extraction procedures have been developed for taste and odor microorganisms.

3. The major earthy-musty odor constituents produced by *Streptomyces odorifer* have now been identified.

4. The predominant odor constituents produced by *Synura petersenii* have been identified.

5. The major odor constituent of *Trichoderma viride* was identified.

## SECTION II

## RECOMMENDATIONS

It seems quite apparent that the technology for the identification of taste and odor constituents is now present in most of the laboratories engaged in this activity around the country. The techniques developed have been particularly successful in the elucidation of the earthy-musty odor complex.

There has not been as much success in identifying the odor constituents produced by algae. This is not because the analytical procedures are not satisfactory but rather is due to a lack of information on the isolation and mass culture of these organisms. It is recommended that nutritional studies of taste and odor algae be conducted so that these organisms can be studied by the interested analyst.

While the identification of taste and odor constituents has rapidly progressed, it is also true that our knowledge of those factors which lead to the accumulation of these constituents in public water supplies is sadly lacking. It is recommended that the microbiologists and chemists cooperate in a study of those conditions which lead to taste and odors in a natural environment.

The final aim of taste and odor studies is more than an identification of the compounds responsible for the taste and odors and should lead to suggestions for their control. It seems clear that this has not happened and it is recommended that studies which would lead to the control of taste and odor microorganisms be initiated.

One of the difficulties in the taste and odor field is the subjective terms used for the description of tastes and odors. It is recommended that a taste and odor kit be assembled and distributed to water works operators so that communication between the scientist and the person involved with a practical taste and odor problem can be enhanced.

## SECTION III

## INTRODUCTION

The purpose of the present investigation was to isolate and identify those compounds, produced by selected microorganisms, which give rise to taste and odor.

The problem of taste and odor in water supplies is an old one and it is generally conceded that most tastes and odors are microbial in origin. Only in recent years has there been real progress in the chemical identification of taste and odor constituents produced by microorganisms. Most of the effort and progress has centered around the identification of those compounds which give rise to the earthy-musty odor complex. Several laboratories have contributed to a solution of this problem and most of their findings have now been published (1), (2), (3), (4), and Morris 1966 and Collins, Knaak and Soboslai 1970.

## SECTION IV

## MATERIALS AND METHODS

This section will be divided into three parts: the first part dealing with Streptomyces odorifer, the second with Synura petersenii, and the third with Trichoderma viride.

S. odorifer, an actinomycete, was originally isolated from the Thames River in London and it was felt that this organism was responsible for the earthy odor of salmon that were caught from that river.

The organism was grown in potato dextrose (10%) broth containing $CaCO_3$ 0.2 g/liter either fermentation vessels containing 12 liters or large flasks (2500 ml) containing 700 ml of the medium. After inoculation the fermentation vessels were placed in a New Brunswick fermentor and incubated at 25°C for 48 hr. The large flasks were incubated, after inoculation, at 25°C for 48 hr on a reciprocal shaker.

At the end of the growth period the cells and culture medium were steam distilled. The steam distillate was freeze-concentrated following the method of Whitaker (6). The concentrated material was extracted for 72 hr with petroleum ether (lower boiling fraction) in a liquid-liquid extractor. The petroleum ether solution was dried, carefully concentrated to a final volume of approximately 3 ml and the extract was then analyzed by gas chromatography (glc).

The gas chromatograph used was a Hewlett-Packard Model 5750B and was equipped with a dual flame ionization detector. The column (6 ft stainless steel) used 80-100 mesh, acid washed silanized Chromosorb W as the solid support and this was coated with 10% SE-30. Other operating conditions were: injection port 200°C, collector 200°C; temperature programmed from 100-285°C at 6°C/min; helium flow rate, 30 ml/min; hydrogen flow rate, 40 ml/min; air flow rate 500 ml/min; inlet pressure 60 psi; outlet pressure, atmospheric. The instrument was equipped with an effluent splitter (approximate ratio 1:10) to permit collection of compounds.

In order to collect sufficient material for infrared and mass spectroscopy the compounds of interest were trapped by means of glass U tubes as they exited from the gas chromatograph. The glass tubes were 12 in. x 1/16 in. i.d. and were connected to the exit port by means of a Swagelok fitting. To insure a leak-proof system, one end of the U tube was passed through a rubber septum inserted into the fitting. The tubes were cooled in a bath of dry ice and acetone.

Infrared spectra were obtained on a Perkin-Elmer 137 Infracord (KBr disks) and mass spectra were obtained on an AEI M.S. 12 mass spectrometer, using the gas inlet system, ion source operated at 250°C.

Previous to the present study a detailed examination of the odorous constituents produced by Synura petersenii has been conducted and a series of compounds had been identified. In the present work the volatile compounds were reexamined employing the newer techniques then available.

Synura petersenii is a member of the Chrysophyta and is found in the plankton of many lakes and pools. Synura sp are known to produce an odor in water, when present in small numbers, resembling that of a ripe cucumber or watermelon. In larger numbers the organism produces a fishy odor.

The organisms were grown in the defined medium of Provasoli and Pintner (7) and was maintained at 16°C in a lighted refrigerator (400 ft-c).

In order to obtain enough material for analytical work the organism was grown in large (25 liter) carboys containing 15 liters of medium. The contents of three large test tubes containing 300 ml of the medium served as the inoculum for each large carboy. The carboys were incubated without agitation in a constant temperature room maintained at 15 $\pm$ 1°C. The fluorescent light present in the room provided sufficient illumination for good growth (200 ft-c). The incubation period varied between ten and fourteen days.

The volatile constituents were obtained by steam distillation of the culture medium followed by liquid-liquid extraction of the aqueous distillate with ether. The ether fraction was dried, carefully concentrated to a small volume and analyzed by glc. The analytical procedures were essentially the same as those described for S. odorifer except that a Carbowax 20M column was used.

Trichoderma viride is a fungus that was isolated from a soil sample collected near Coventry Lake in Coventry, Connecticut. The organism was grown in a potato dextrose (10%) liquid medium in quart milk bottles containing 60 ml of the medium. After inoculation the milk bottles were placed on their side and incubated at room temperature for three to four days. At the end of this period the surface of the medium was covered with the dark green conidia characteristic of this organism.

At the end of the growth period the cells and culture medium were steam distilled at atmospheric pressure. The aqueous distillate was dried and extracted with ether. The ether extract was concentrated under reduced pressure leaving 3.3 g of a yellow oil having a strong coconut-like aroma. The oil was separated into its components by glc (Hewlett-Packard Model 7620A) utilizing a flame ionization detector equipped with an effluent splitter. The operating conditions were the same as those described for Streptomyces odorifer except that the column used was Carbowax 20M (19%) plus PDEAS (1%). For further purification of the compound a column packed with W98 (19%) was used.

Infrared and mass spectra were obtained by the same procedures outlined for *S. odorifer*. The (nmr) spectra were obtained on a Varian A-60 nuclear magnetic resonance spectrometer.

## SECTION V

## EXPERIMENTAL RESULTS

As in the Materials and Methods section the results for each of the organisms studied will be discussed separately.

In order to determine which compounds gave rise to the earthy or musty odor in Streptomyces odorifer the odor of each compound was noted as it exited from the gas chromatograph. Three compounds had a definite earthy or musty odor. The compound having the shortest retention time on glc had a definite musty odor and its ir was identical to that of the published spectrum of 2-exo-hydroxy-2-methylbornane (2). This compound was the major constituent in the mixture and was identified as 2-exo-hydroxy-2-methylbornane. The second compound had a definite earthy odor and its ir spectrum corresponded with the published spectrum of geosmin (1).

The mass spectrum for the suspected 2-exo-hydroxy-2-methylbornane gave the empirical formula $C_{11}H_{12}O$ with the parent peak at m/e 168 and the base peak at m/e 95. The fragmentation agreed with that published by Medsker et al (2). The mass spectrum for the suspected geosmin was compared with the spectrum published for this compound (1) and the agreement was excellent. The mass spectrum showed a parent peak at 182 and a base peak at 112.

The third compound was present in very small amounts and it was difficult to obtain amounts large enough for good analytical work, but this has been accomplished and the compound identified as cadin-4-ene-1-ol. The mass spectrum of the compound indicated a $C_{15}H_{26}O$ compound and it contained a small parent peak at 222 as well as typical sesquiterpene alcohol peaks. The ir spectrum was also consistent with the structure suggested above.

A recent paper by Gerber (8) had described the formation of cadin-4-ene-1-ol by another species of Streptomyces. An ir spectrum of the compound isolated from S. odorifer was sent to Dr. Gerber for comparison and the two spectra were identical. A small amount of the authentic compound was also obtained and its retention time (glc) was identical with the compound produced by Streptomyces odorifer. It is concluded, therefore, that the third odorous constituent produced by S. odorifer is cadin-4-ene-1-ol.

As has been previously discussed, the Synura petersenii work was essentially a repetition of earlier work except that more modern instrumentation was available for analytical purposes. All of the compounds previously identified by classical procedures were confirmed in the present study. All of the identified compounds were presented to a taste panel comprised of laboratory personnel to see which compound or compounds comprosed a typical Synura-like odor. The only compound which

some of the taste panel agreed on as having an odor somewhat like that of Synura petersenii was n-heptanal. In the author's opinion, while this compound undoubtedly is part of the Synura odor, there are other compounds present and as yet unidentified which are responsible for the typical odor produced by this organism.

The major component of the oil (over 90%) in Trichoderma viride had a retention time of approximately 24 min. The isolated compound was a colorless liquid having a strong coconut-like odor.

The infrared spectrum showed two C=O absorption bands at 1740 and 1725 $Cm^{-1}$ and two strong C=C stretching bands at 1636 and 1553 $Cm^{-1}$ suggesting an $\alpha$-pyrone ring.

The mass spectrum of the compound showed a parent peak at m/e 166. The molecular formula was assigned as $C_{10}H_{14}O_2$ on the basis of the mass spectral data and elemental analysis (Found C, 72.84%; H, 8.39%; O, 18.77%; Calculated, C, 72.9%, H, 8.5%; O, 19.21%). The mass spectrum also revealed a base ion at m/e 95 attributable to $M-C_5-H_{11}$.

The (nmr) showed signals at $\delta$ 0.94 (3H, triplet), 1.4 (6H, complex multiplet) and 2.48 (2H, triplet) all consistent with an n-pentyl group. The three $\alpha$-pyrone ring protons formed an ABX pattern with $\delta$A 5.83, $\delta$B 6.00 and $\delta$X 7.19 and $J_{AX}$ = 6.5 and $J_{BX}$ = 9.5 HZ. The ABX protons represent those attached to positions 5, 3, and 4, respectively.

As an additional proof the compound was hydrogenated over Adams platinum oxide in ethanol. When the reduction product was subjected to glc (19% W98 column) two peak areas were revealed with an approximate peak area ratio of 1:5. The minor peak was identified as n-decanoic acid (infrared, retention time) and the major peak was identified as $\delta$-decalactone (infrared, retention time, odor).

## SECTION VI

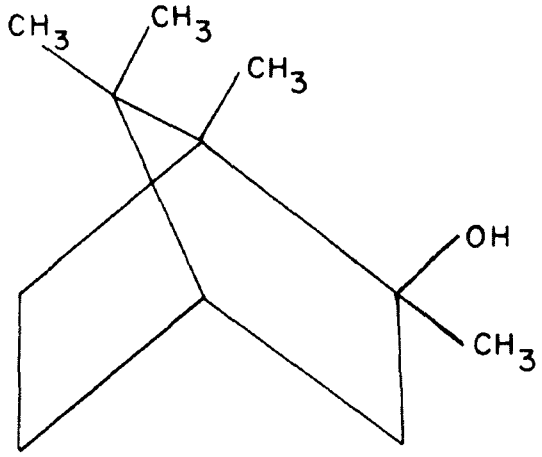
## DISCUSSION

It is quite clear that all of the major odorous constituents produced by Streptomyces odorifer have now been identified. There are, however, two additional compounds having longer retention times (glc) than the three discussed above. Both of these compounds have faint but discernable "woody" odors. A preliminary analysis of these compounds (ir) indicates that both are sesquiterpene alcohols. An investigation of these compounds is now being conducted.

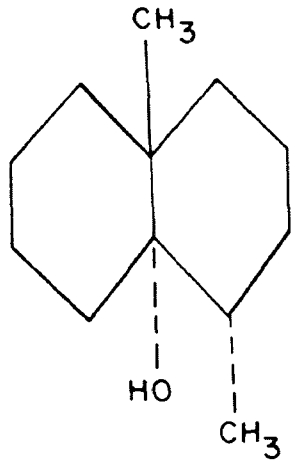
The reinvestigation of Synura petersenii failed to unearth any new compounds and n-heptanal is regarded as the compound primarily responsible for the characteristic odor produced by this organism.

The major odor compound of Trichoderma viride was identified as 6-pentyl-$\alpha$-pyrone. This is a new fungal metabolic product and the only other isolation of this compound from a natural source has been from peach fruits. The compound has interest not only as a new fungal metabolite but also as a potentially useful new compound in the flavor industry.

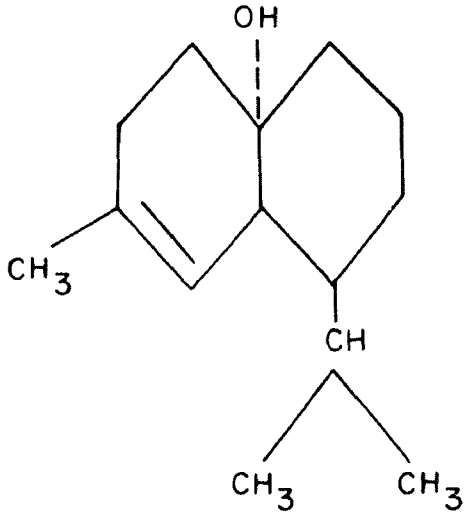
A series of techniques have been developed, as described above, which permit the isolation and characterization of the small amounts of material normally found in research involving taste and odor. This does not mean that other techniques are not available which would permit a more rapid identification, but it does mean that a laboratory with only moderate facilities can carry out this sort of analysis.



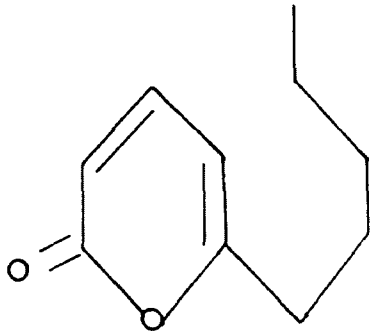

2-EXO-HYDROXY-2-METHYLBORNANE




TRANS-
1,10-DIMETHYL-TRANS-
9-DECALOL
(GEOSMIN)




CADIN-4-ENE-1-OL




α-PENTYL PYRONE

FIGURE 1. STRUCTURAL FORMULAS OF EARTHY-MUSTY METABOLITES

## SECTION VII

## ACKNOWLEDGMENTS

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The support and encouragement of Dr. Ronald Webb, the Grant Project Officer, was also invaluable during the course of this investigation.

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2. Collins, R. P., and Halim, A. F., "Characterization of the Major Aroma Constituent of the Fungus Trichoderma viride (Pers.)," Journal of Agriculture and Food Chemistry (manuscript in press).

3. Collins, R. P., and Halim, A. F., "The Production of Cadin-4-Ene-1-Ol by Streptomyces odorifer," Lloydia (manuscript in press).

## SECTION X

## GLOSSARY

Actinomycete - A filamentous bacterium. *Streptomyces odorifer* is an example.

Chrysophyta - A group of algae characterized by the formation of a yellow brown pigment. *Synura petersenii* is an example.

Fungus - A group of microorganisms lacking chlorophyll and generally filamentous. *Trichoderma viride* is an example.

ir - Abbreviation for infrared spectroscopy.

glc - Abbreviation for gas-liquid chromatography.

nmr - Abbreviation for nuclear magnetic spectroscopy.

PDEAS - Abbreviation for phenyldiethanolamine.

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27 Abstract


The taste and odor constituents produced by the actinomycete Streptomyces odorifer, the alga Synura petersenii and the mold Trichoderma viride were examined.

The odorous constituents were obtained by steam distillation of the culture medium. The odorous constituents were identified by means of gas-chromatography, infrared, mass and nuclear magnetic spectroscopy. The major odorous constituents produced by Streptomyces odorifer have been identified as trans-1, 10-dimethyl-trans-9-decalol (geosmin), 2-exo-hydroxy-2-methylbornane and cadin-4-ene-1-ol. A large number of odorous materials were also identified from the culture filtrates Synura petersenii and heptanal was the one compound having an odor similar to that of the organism. The major odorous compound produced by Trichoderma viride was 6-pentyl-α-pyrone. (Collins--U. Conn.)


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