Phytochemistry, 1973, Vol. 12, pp. 387 to 390. Pergamon Press. Printed in England.

# VOLATILE TERPENOIDS FROM AECIOSPORES OF *CRONARTIUM FUSIFORME*

J. L. LASETER
Department of Biological Sciences, Louisiana State University in New Orleans,
New Orleans, LA 70122, U.S.A.

J. D. WEETE
Lunar Science Institute, Houston, TX 77058, U.S.A.

and

C. H. WALKINSHAW
National Aeronautics and Space Administration, Lunar Receiving Laboratory,
Houston, TX 77058, U.S.A.

(*Received* 4 *July* 1972. *Accepted* 9 *September* 1972)

**Key Word Index**—*Cronartium fusiforme*; Uredinales; Fungi; mono- and sesqui-terpenes; *o*-cresol; comparison with host plant *Pinus*.

**Abstract**—Aeciospores of *Cronartium fusiforme* isolated from slash pine (*Pinus elliottii*) trees were analyzed for volatile terpenoids by GLC and GLC–MS. α-Pinene, β-pinene, $\Delta^3$-carene, myrcene, linonene, β-phellandrene, and δ-terpinene were the major monoterpenoid hydrocarbons present with only traces of camphene. A number of monoterpenoid alcohols were also present of which terpinen-4-ol predominated. Among the various acyclic sesquiterpenes present, β-farnesene and β-citronellol were identified. Several aromatic compounds were also observed, including *o*-cresol.

## INTRODUCTION

WHEN the steam volatile constituents of *Ceratocystis*, *Penicillium*, and *Fusarium* species grown on synthetic media were investigated by Sprecher,[1] no detectable quantities of monoterpenes were present. He further demonstrated that the lack of monoterpenes was not a result of their preferential degradation in the culture media by the fungi investigated. More recently, however, simple terpenoids have been reported as constituents of the volatile fraction of two wood-rotting species of *Ceratocystis* and *Trametes* grown in culture, but no cyclic monoterpenes were detected among the volatile products reported.[2,3] Little work has been reported which deals with volatile hydrocarbons from other fungal organisms and no attention has been given to those fungi which are pathogenic on members of the terpenoid-producing Pinaceae.

In recent years, considerable information has been published by Smith,[4] Roberts,[5] and

---

[1] VON E. SPRECHER, *Planta Med.* **13**, 418 (1965).
[2] R. P. COLLINS and A. F. HALIM, *Lloydia* **33**, 481 (1970).
[3] A. F. HALIM and R. P. COLLINS, *Lloydia* **34**, 451 (1971).
[4] R. H. SMITH, *U.S. Forest Serv. Res. Note PSW*-135 (1967).
[5] D. R. ROBERTS, *Phytochem.* **9**, 809 (1970).

others[6–8] on the volatile terpenoids of various *Pinus* species. Predominate monoterpenoids include acyclics such as myrcene and ocimene; monocyclics like limonene, β-phellandrene, δ-terpinene, and bicyclics such as α-pinene, camphene, β-pinene, $\Delta^3$-carene. Many of the above compounds also occur in hydrated forms, e.g. as borneol and terpinen-4-ol. In addition, a large number of both acyclic and cyclic sesquiterpenes have also been described.

It is interesting here that volatile terpenoids of oleoresin are among the factors thought to be related to susceptibility of *Pinus* species to attack by wood-inhabiting fungi.[9,10] There is also some evidence that the terpenoid composition can vary in pine trunk xylem as a result of wounding.[11] For these reasons it seemed timely to determine if a pine rust disease organism such as *C. fusiforme* elaborates volatile terpenoids and if so, whether or not the terpenoids are similar to those reported for the gymnosperm host.

## RESULTS AND DISCUSSION

This paper reports the occurrence of terpenoids present in the volatile fraction from aeciospores of the gall rust fungus *C. fusiforme*. Due to small sample sizes, the common steam-distillation techniques were not employed. Volatiles were driven off by gentle heating in helium and trapped at acetone-dry ice temperatures. The same procedure was used with mature slash pine tissues and the results compared favorably with those previously reported. The isolation method was a modification of that used to monitor the volatile components of human breath and urine.[12]

TABLE 1. RELATIVE PERCENTAGE OF VOLATILES IDENTIFIED BY GLC–MS FROM *Cronartium fusiforme* AECIOSPORES

| Identified compound | Relative %* | Identified compound | Relative %* | Identified compound | Relative %* |
|---|---|---|---|---|---|
| α-Pinene | 8·9 | Myrcene | 5·5 | β-Farnesene | 4·2 |
| Camphene | >0·1 | Limonene | 3·0 | β-Citronellol | 2·3 |
| β-Pinene | 3·3 | β-Phellandrene | 4·0 | o-Cresol | 2·7 |
| $\Delta^3$-Carene | 1·4 | Terpinen-4-ol | 6·8 | | |

* Computed from gas chromatographic peak areas of total volatiles.

Table 1 lists the volatiles identified from a 0·2-g aeciospore sample with the relative concentration of each computed from GLC peak areas. As can be seen, not only are a number of common monoterpenes present but oxygenated monoterpenes and sesquiterpenes are also found. MS data of the monoterpene hydrocarbons compared favorably with those previously published.[13,14] Even though differences between the spectra of the monoterpenes

---

[6] A. B. ANDERSON, R. RIFFER and A. WONG. *Phytochem.* **8**, 869 (1969); *Phytochem.* **8**, 873 (1969); *Phytochem.* **8**, 1999 (1969); *Phytochem.* **8**, 2401 (1969); *Holzforschung* **24**, 182 (1970).

[7] E. ZAVARIN and F. W. COBB, JR., *Phytochem.* **9**, 2509 (1970).

[8] E. ZAVARIN, K. SNAJBERK C. J. LEE, M. HENLEY and N. T. MICOV, *Phytochem.* **10**, 1857 (1971).

[9] R. H. SMITH, in *Breeding Pest Resistant Trees*, p. 189, Pergamon Press, Oxford (1966).

[10] F. W. COBB, JR., M. KRISTIC, E. ZAVARIN and H. W. BARBER, JR., *Phytopathol.* **58**, 1327 (1969).

[11] D. R. ROBERTS, *Assoc. Southeast. Biol. Bull.* **15**, 53 (1968).

[12] R. TERANISHI, T. R. MOM, A. B. ROBINSON, P. CARY and L. PAULING, *Analyt. Chem.* **44**, 18 (1972).

[13] R. RYHAGE and E. VON SYDOW, *Acta Chem. Scand.* **17**, 2025 (1963).

[14] A. F. THOMAS and B. WILLHALM, *Helv. Chem. Acta* **47**, 176 (1964).

are not great, the contrast is sufficient to allow for easy differentiation. As an added precaution, however, the volatiles from young slash pine needles and stems were also extracted and analyzed. The electron impact data coupled with information from authentic standards provided confirmation for most of the terpenoid structures reported. α-Pinene is the major monoterpene and is over 2·5 times the concentration of β-pinene. Roberts[5] observed that the α-pinene percentage was greater than that of β-pinene in all xylem oleoresin originating from 19-year-old *Pinus elliottii* trees. In no instance did he observe, on a relative basis, myrcene values above 18% of the total monoterpene hydrocarbon content. Myrcene, however, is a major component of the xylem monoterpenes of *P. monophylla* and is frequently present in low quantities in other pine species.[6,8] On the other hand, the fact that the bicyclics such as $\Delta^3$-carene and camphene as well as the monocyclic limonene are present in small amounts relative to the other monoterpenes seems to be in good agreement with previous reports for *P. elliottii*.[5] Taken as a class, however, the monoterpenes appear to be lower in concentration than the higher-boiling volatiles when compared to slash pine needles, stem cortex and stem xylem tissues extracted and analyzed by the same technique.

Molecular ions of *m/e* 156 were common in scans made of compounds with relative retention volumes higher than δ-terpinene and were taken as indicative of monoterpene alcohols. Terpinen-4-ol was positively identified and the resulting spectrum agreed with that previously published.[15] A compound with a base peak at *m/e* 69 and major fragments at *m/e* 41 and 55 with a molecular ion at *m/e* 156 was also observed and identified as β-citronellol.[16] Several other acyclic alcohols were also present, but in lower concentrations. MS of these alcohol components were typical of acyclic compounds in having high intensity fragmentation at low mass. Additional evidence was provided by meta-stable ions at *m/e* 89·2, 78·1 and 37·2 which are characteristic of acylcic monoterpene alcohols. Citronellol has been reported by Collins and Halim[2] as the major monoterpene in *Ceratocystis variospora* and *Trametes odorata*. β-Farnesene appears to be present in relatively high concentrations in the aeciospores of this study and produced a molecular ion at *m/e* 204 and fragments characteristic of a 3,7,11-methyl branched structure. This acyclic sesquiterpene has been previously reported as a minor component of the higher-boiling terpenoids of *P. monophylla*.[5]

A compound having a relatively high retention volume and a molecular ion at *m/e* 108 was identified as *o*-cresol; other fragments present at *m/e* 79 and 77 correspond to the benzenium and $C_6H_5^+$ ions respectively. Fragments at *m/e* 90, corresponding to the loss of water, were very evident and typical of the *ortho* isomer.[17] Scans of other less well resolved chromatographic peaks with strong molecular ions at *m/e* 108 were also observed and associated with intense peaks at *m/e* 78 corresponding to $C_6H_6^{+\cdot}$ fragments. Such fragments are known to be present with aromatic ethers like anisole.[18] In no instance were fragments characteristic of methyl esters (*m/e* 74, 87 and 143) or ethyl esters (*m/e* 88 or 101) observed in any spectra. Likewise, if there were any normal alkanes present, they were below the limits of detectability.

## DISCUSSION

The present results show that aeciospores of *C. fusiforme* isolated from its natural host contain many of the common volatile terpenoids present in *Pinus*. However, the amounts

---

[15] E. von Sydow, *Acta Chem. Scand.* **17**, 2504 (1963).

[16] F. W. Cobb, Jr., E. Zavarin and J. Bergot, *Phytochem.* **11**, 1815 (1971).

[17] *Atlas of Mass Spectral Data* (edited by E. Stenhagen, S. Abrahamson and F. W. McLafferty), Vol. I, pp. 337–338, Interscience–Wiley, New York (1969).

[18] E. Zavarin, *Phytochem.* **9**, 1049 (1970).

of monoterpenes in the rust spores appear to be lower than might be expected in terms of the higher-boiling terpenoids when compared to the pattern in *Pinus*. Even though Fungi are known to synthesize oxygenated monoterpenes, the evidence available suggest that they lack the ability to either form and/or accumulate cyclic monoterpenes.[2,3] This may be due to their inability to form the postulated 1-*p*-methane-8-carbonium ion intermediate leading to compounds such as $\alpha$- and $\beta$-pinene, $\Delta^3$-carene and limonene.[7] It has been suggested[18] that myrcene formation does not proceed through the carbonium ion but rather is formed directly from the common precursor, neryl-pyrophosphate. If this latter pathway is active in wood-inhabiting fungi then it could explain the relatively high myrcene values observed in the aeciospores.

The mono- and bi-cyclic terpenoids as well as the acyclic types may, however, simply be accumulated from the pine host. If such is the case, then the fungus does so on a selective basis exhibiting a marked tendency to accumulate the higher-boiling terpenoids. This fact is supported by a recent observation on the total fatty acids of both the rust spores and slash pine tissues.[19] Total fatty acids present in the aeciospores and slash pine stem tissues were extracted and analyzed by GLC and MS methods and the results indicate that fatty acids from these two sources are qualitatively and quantitatively distinct.[20] For example, if lipids are simply accumulated then one would expect to see fatty acids occurring in rust tissues such as the 3-methyl branched hexadecanoic acid, which is common in the pine host tissues. However, no methyl branched fatty acids were observed in the aeciospores used in this study.

## EXPERIMENTAL

Aeciospores of *C. fusiforme* were collected in the field from infected slash pine (*Pinus elliottii*) trees averaging 15 years of age. The aeciospores, collected in 1972, were maintained at 5° until analyzed. Pine tissues used in this study were collected locally from 5- to 6-year-old trees.

Volatiles were collected by passing a stream of ultrapure He across *ca.* 0·2–0·3 g of sample in a 6 mm × 10 cm Pyrex tube at a rate of 10 ml/min. After purging for 5 min at room temp. the glass tube containing the spores was heated to *ca.* 40° by means of a heat gun for 20 min. The effluent containing the volatile components passed directly through a cold trap emmersed in an acetone-dry ice bath. The trapping apparatus was 61 cm in length and constructed of stainless steel. The inlet segment was 0·31 cm o.d., 0·25 cm i.d. tubing silver soldered to an outlet segment of 0·15 cm o.d., 0·10 cm i.d. stainless steel tubing. The trapping apparatus is a modification of that employed by Teranishi *et al.*[12] for evaluation of volatiles from breath and urine. The trap was vented to the atmosphere during sample collection. Injection onto the gas chromatograph column was accomplished by means of a stainless steel capillary 6-way gas sampling value (Varian Aerograph, Walnut Creek, Calif.) as the acetone-dry ice was removed and the trap heated by a heat gun. The sampling value and all transfer lines were maintained at a constant 85° with the injection port of the gas chromatograph held at 100°.

Chromatographic separation was achieved by means of a Hewlett–Packard Model 5750 gas chromatograph equipped with a 1·52 × 0·025 cm stainless steel capillary column coated with IGEPAL CO-880 and programmed from room temp. Approximately 90% of the chromatographic effluent was allowed to enter a duPont 21-491 double focusing mass spectrometer by means of jet-type separator. The separator and transfer lines were held at 125°. The ion source was 100° with a filament current of 40 $\mu$A. All spectra were obtained at 70 eV with scan speeds of 2 sec/decade.

*Acknowledgements*—This work was supported in part by the Cancer Association of Greater New Orleans, by contract NAS-9-11339 from the National Aeronautics and Space Administration to LSUNO (JLL), and the Lunar Science Institute which is operated by the Universities Space Research Association under Contract No. NSR 09-051-001 with the National Aeronautics and Space Administration. This paper constitutes the Lunar Science Institute Contribution No. 114. We are indebted to Mrs. S. Baxter for technical assistance.

[19] Unpublished data.

[20] J. L. Laseter, J. Weete and D. J. Weber, *Phytochem.* 7, 1177 (1968).