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IDENTIFICATION OF TWO NEW TERPENE ALCOHOLS FROM FRASS PRODUCED BY *IPS CONFUSUS* IN PONDEROSA PINE

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Abstract—Two new terpene alcohols have been isolated from frass produced by *Ips confusus* feeding in ponderosa pine. They are (−)-2-methyl-6-methylene-7-octen-4-ol and *trans*-2-methyl-6-methylene-3,7-octadien-2-ol as determined by spectral analyses; they serve as internal markers in the isolation of the sex pheromone.

THE presence of very effective pheromone communication systems has been established for many of the bark beetles (family Scolytidae). The behavior of *Ips confusus* (LeConte) in particular has been the subject of an intensive investigation in these laboratories. This and previous work has been reviewed and summarized.[1] The male beetle initiates an entrance gallery in ponderosa pine, and produces frass (primarily a mixture of phloem fragments and excrement pellets) that contains a pheromone attractive to both sexes but much more so to the female. The massive invasion of beetles attracted by this pheromone frequently kills the tree. The successful development of a laboratory bioassay for the response of *I. confusus* to the attractant[2] has furnished the chemist with an indispensable tool for monitoring isolation procedures. A detectable response on bioassay with females was evoked by dilutions of a benzene extract equivalent to 3×10^{-8} g of frass.

The mass rearing program[1] furnished about 3 kg of frass from about 21,000 male beetles boring in freshly cut logs of ponderosa pine (*Pinus ponderosa* Laws). A highly active fraction obtained from this frass by the sequence of extraction, short-path distillation, column chromatography, and gas chromatography amounted to 0·0007% of the wt of the starting frass. The first broad gas chromatography cut on SE 30 contained two conspicuous peaks bracketing the active fraction. The compounds responsible for these were isolated and identified as nonanal and geranyl acetate.[3] Further fractionation on Carbowax 20M produced a fairly narrow cut that showed sharp peaks at the leading and trailing edges of the active material. The identification of these compounds, which serve as internal markers for the active fraction, is the subject of this paper. The compound at the leading edge is designated Compound 1; that at the trailing edge, Compound 2. Gas chromatography was performed as follows: 8% Carbowax 20M on Chromosorb G, 6′ × ¼″, column 120°, detector 155°,

[1] D. L. Wood, L. E. Browne, R. M. Silverstein and J. O. Rodin. *J. Insect Physiol.* In press (1966).
[2] D. L. Wood and R. W. Bushing, *Canad. Entomol.* **95**, 1066 (1963).
[3] R. M. Silverstein and J. O. Rodin, *Microchem. J.* **9**, 301 (1965).

41 ml He/min, on-column injection, Aerograph A90P3. The retention time for compound 1 was 17–22 min, that for compound 2 was 26–29 min. About 260 mg of compound 1 and 26 mg of compound 2 were collected. About 20 mg of each sample was rechromatographed as follows for spectrometric analysis: 4% phenyldiethanolamine succinate on Chromosorb G, 10′ × ¼″, column 110°, 55 ml He/min for compound 1 (retention time 10–14 min), and 30 ml He/min for compound 2 (retention time 24–29 min).

We do not know whether these compounds are present as such in the tree resins or whether they are products of beetle metabolism.

Identification of compound 1

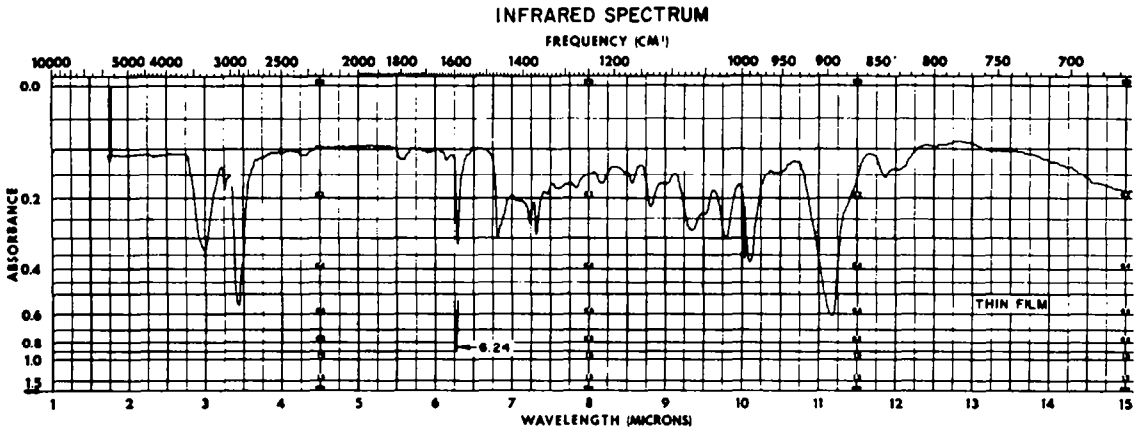
Figure 1 presents the IR, mass, UV and NMR spectra of compound 1. The mol. wt. is 154 (parent peak in mass spectrum). The P + 1 and P + 2 peaks are too weak to be accurately measured. The IR spectrum shows an O—H stretching band at 2·96 μ, an olefinic C—H stretching band at 3·23 μ, a conjugated C=C stretching band at 6·25 μ, and characteristic "vinyl" absorption at 10·1 μ and 11·1 μ. The presence of a geminal dimethyl group is suggested by the double peak at 7·21 μ and 7·29 μ. The UV spectrum suggests a conjugated double bond system (λ_{max}^{hexane} 226 mμ, ε 20,000). The vinyl proton peaks in the NMR spectra show a 1:4 ratio, and the downfield proton (3·6 τ) is split only by two geminal protons (J_{trans} 18 c/s, J_{cis} 12 c/s). The following moiety can be written.

The remaining protons are distributed in the ratio 1:6:6. The molecular formula must be $C_{10}H_{18}O$. Given the context, we think in terms of a terpene alcohol.

Shaking the deuteriochloroform solution with deuterium oxide caused the peak at 8·40 τ in the NMR spectrum to disappear. The deshielded multiplet at 6·2 τ (1 proton) can be tentatively ascribed to a proton geminal to the hydroxyl group. This assignment was confirmed by the shift of this proton to 4·9 τ on acetylation with acetic anhydride in pyridine at room temperature. The mass, IR, UV and NMR spectra of the acetate were in accord with acetylation without rearrangement of the rest of the molecule. The parent peak in the mass spectrum of the acetate was 196, and the P + 1 and P + 2 peaks were 13·8% and 1·7% respectively of the parent peak; this is in satisfactory agreement with the calculated[4] P + 1 and P + 2 values of 13·4 and 1·2 for $C_{12}H_{20}O_2$ which provides confirmation for the assigned molecular formula of the alcohol, $C_{10}H_{18}O$.

The presence of an isopropyl group can be surmised from the strong peak in the mass spectrum at *m/e* 43, and from the double peak in the IR spectrum at 7·21 μ and 7·29 μ. The upfield doublets in the NMR spectrum peaks (6 protons) must represent the geminal methyl groups which have slightly different shift positions (9·08 τ and 9·10 τ); each methyl group is split by the vicinal CH, giving rise to two overlapping doublets (J 7c/s). The five protons (excluding the OH proton) between ~7·4 τ and ~8·9 τ occur as two multiplets of two protons each, separated by a multiplet of one

[4] R. M. Silverstein and G. C. Bassler, *Spectrometric Identification of Organic Compounds* p. 35. Wiley, N.Y. (1963).



Mass Spectral Data (Relative Intensities)

m/e	% of base peak	m/e	% of base peak	m/e	% of base peak
38	4	53	25	77	3
39	37	55	6	79	6
40	12	57	17	80	4
41	70	58	7	85	15
42	10	65	4	87	10
43	66	66	5	91	3
44	18	67	35	93	8
45	26	68	100	97	2
50	3	69	64	98	2
51	6	70	5	121	2
52	4	71	8	136	2
				154(P)	1·1

UV data

λ_{max} 226 mμ
ε_{max} 20,000
Solvent
Hexane

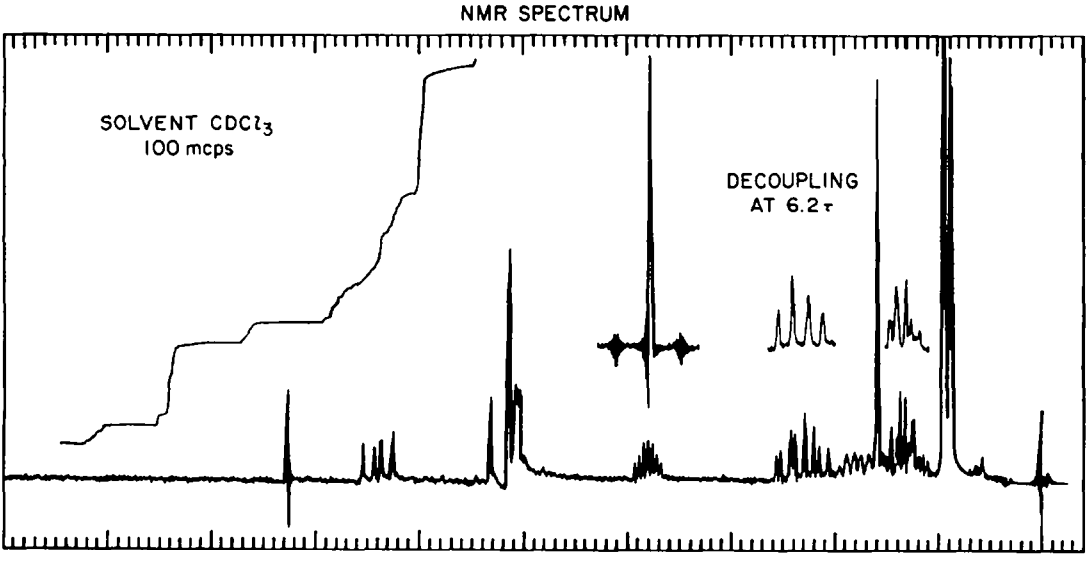
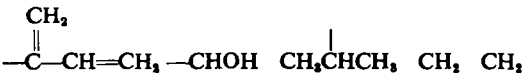


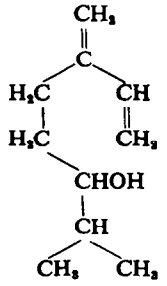
FIG. 1 Spectral data for compound 1.

proton which must represent the methine proton of the isopropyl group. We thus have the following fragments which account for the molecular formula



The vinylidene out-of-plane bending absorption could be accommodated under the broad IR band at 11·15 μ.

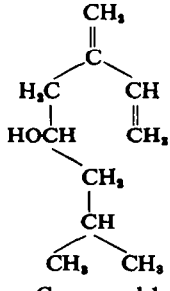
To account for the different shift positions of the methyl groups, the hydroxy-bearing carbon atom was placed adjacent to the isopropyl group thus furnishing an asymmetric center within effective range of the methyl proton. The following tentative structure was written.



Discrepancies were apparent. This configuration would not account for the wide separation in the NMR spectrum of the CH_2 groups, or for the first-order pattern of the downfield CH_2 group.

Decoupling the C*H*OH proton (6·2 τ) collapsed the downfield CH_2 pattern to a pair of doublets and also affected the upfield CH_2 pattern (Fig. 1); the CHOH group must therefore lie between the CH_2 groups. Attempts to decouple the very broadly split CH_3CHCH_3 proton were only partly successful; however, the methyl pattern collapsed to two single peaks, and the upfield methylene pattern was changed. Thus, the general character of the methyl groups was confirmed, and the upfield CH_2 group was located adjacent to the isopropyl group.

The optical rotation ($[\alpha]_D^{25°}$, *c*, 1 EtOH) was $-17·5° \pm 0·7°$. The structure of compound 1, therefore, is (−)-2-methyl-6-methylene-7-octen-4-ol.



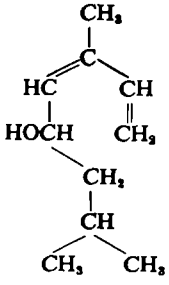
Compound 1

This structure satisfies all the spectrometric requirements. Deshielding of one of the methylene groups by a neighboring double bond and a hydroxy group accounts for its downfield position in the NMR spectrum. The protons of this deshielded methylene group are not equivalent because of the adjacent asymmetric center; one

proton is at 7·53 τ, the other at 7·82 τ. Each is split by the other, J_{gem} 14 c/s, and by the neighboring proton, J_{vic} 9 c/s and J_{vic} 4 c/s. The upfield methylene multiplet also comprises two non-equivalent methylene protons but the couplings are too complex for first-order interpretation. The non-equivalence of the methyl groups can be rationalized even though the asymmetric center is separated from the isopropyl moiety by a methylene group. Since the two protons of this methylene group are not equivalent because of their relation to the optically active center, the methylene group constitutes a "magnetically asymmetric" center. The methyl groups see three different (i.e. magnetically non-equivalent) substituents on the carbon atom adjacent to the carbon atom to which they are attached. It is possible that the influence of the optically active center is felt directly by the methyl groups; a recent study,[5] showed that isopropyl group nonequivalence was manifested over 4 bonds, including a C—O—C bond, from the asymmetric center.

The principal cleavages in the mass fragmentation pattern might be expected on either side of the CHOH group. One of these cleavages (*m/e* 87) does account for a major peak. The base peak is *m/e* 68, which presumably arises from allylic cleavage with rearrangement of a hydrogen atom. The moderately intense peaks at *m/e* 136 and *m/e* 121 represent consecutive elimination of water and a methyl group, This sequence, commonly found in mass spectra of alcohols, confirms *m/e* 154 as the parent peak.

The name tagetol has been given to the reduction product of tagetone found in several species of *Tagetes*. Tagetol has been assigned the following structure.[6,7]



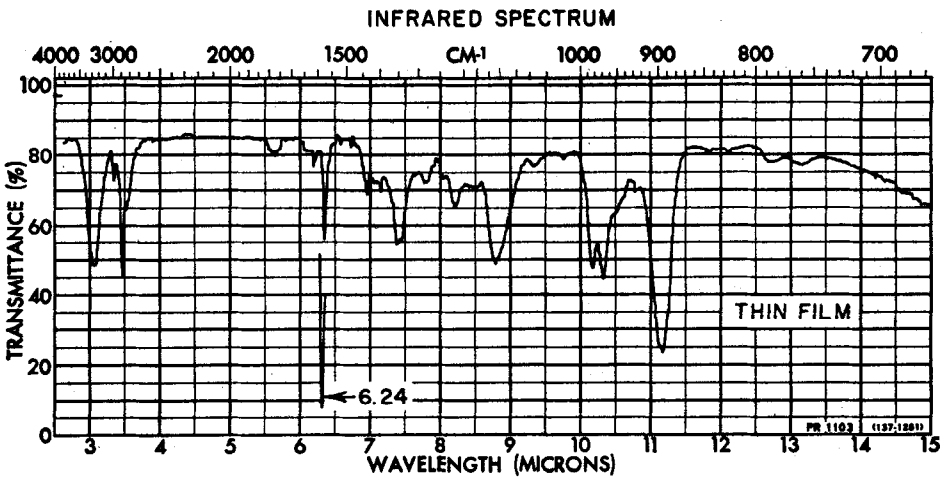
Tagetol

Compound 1 is the methylene isomer of tagetol.

Identification of compound 2

Figure 2 presents the IR, mass, UV, and NMR spectra of compound 2. The IR spectrum of compound 2 is similar in major respects to that of compound 1. We note the O—H stretch at 2·97 μ, the olefinic C—H stretch at 3·28 μ, the strong conjugated C=C stretch at 6·28 μ, a weak C=C stretch at 6·13 μ, the geminal dimethyl double peak at 7·32 μ and 7·37 μ and the vinyl and vinylidene bands at 10·1 μ and 11·1 μ. There is an additional sharp band at 10·25 μ. The UV spectrum is similar to that of compound 1 ($\lambda_{max}^{cyclohexane}$ 225 mμ, ε 20,000). The compound is optically inactive.

[5] G. M. Whitesides, D. Holtz and J. D. Roberts, *J. Amer. Chem. Soc.* **86**, 2628 (1964).
[6] T. G. H. Jones, *Univ. Queensland Papers, Dept. Chem.* **1**, No. 11 (1939); *Chem. Abstr.* **34**, 2353 (1940).
[7] E. E. Boehm, V. Thaller and M. C. Whiting, *J. Chem. Soc.* 2535 (1963).



Mass Spectral Data (Relative Intensities)

m/e	% of base peak	m/e	% of base peak	m/e	% of base peak
29	12	54	3	91	64
31	4	55	12	92	22
32	(27)	56	3	93	17
38	5	57	6	94	2
39	42	63	6	103	6
40	9	65	12	104	5
41	47	66	7	105	21
42	5	67	8	106	8
43	15	68	4	107	3
44	37	77	33	115	3
45	9	78	19	117	6
50	6	79	100	119	35
51	14	80	12	120	4
52	7	81	13	133	2
53	27	85	2	134	13
				135	2

UV data

λ_{max} 225 mμ
ε_{max} 20,000
Solvent
Cyclohexane

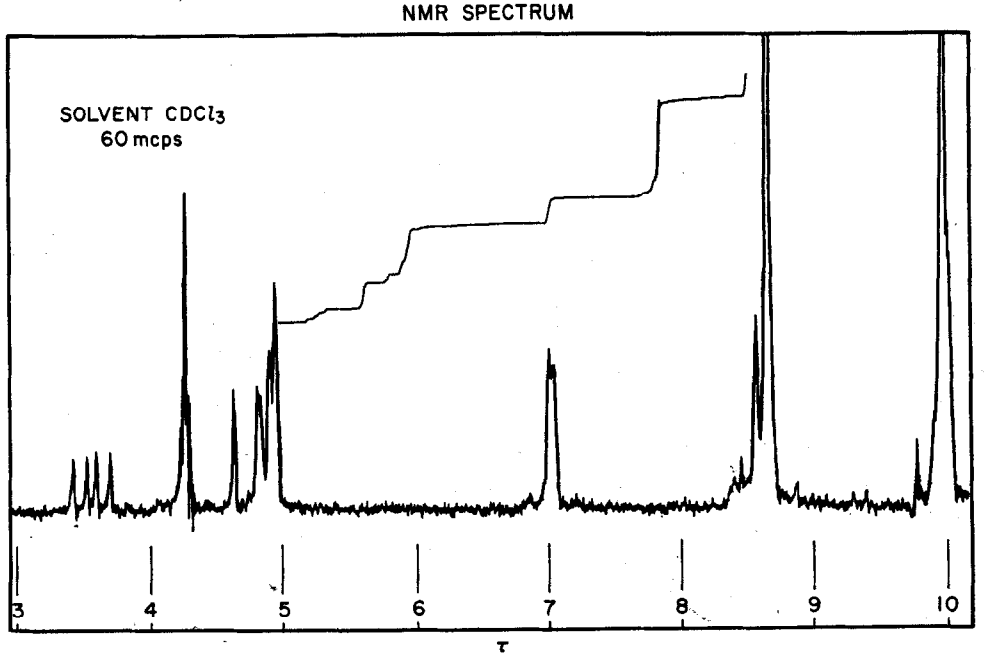
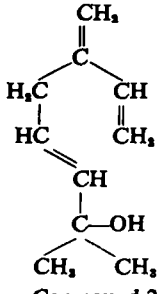


FIG. 2 Spectral data for compound 2.

The peak of highest mass in the mass spectrum of compound 2 is *m/e* 134. This is not the parent peak, however, because we are dealing with an alcohol (IR spectrum), and the next lower prominent peak is 15, rather than 18, units less. The *m/e* 134 peak must therefore result from elimination of water from a compound of mol. wt. 152. The proton count in the NMR spectrum is in accord with this assumption.

The NMR spectrum shows seven olefinic protons of three types in the ratio 1:2:4. As in compound 1, the downfield olefinic proton at 3·57 τ is a pair of doublets (J_{trans} 10 c/s, J_{cis} 7 c/s). Apparently we have the same conjugated system as in compound 1 plus another isolated double bond containing two protons which absorbs at 4·27 τ. There are two protons probably methylene, at 7·02 τ, and 7 protons upfield. The peak at 8·57 τ disappeared on shaking with deuterium oxide; this located the OH absorption. The sharp peak (6 protons at 8·67 τ must correspond to two equivalent methyl groups attached to a fully substituted carbon atom).

The molecular formula can be written $C_{10}H_{16}O$, and a terpene alcohol is a likely candidate. Since one end of the molecule is $-\overset{\overset{CH_2}{\|}}{C}-CH=CH_2$ and the other end is $\begin{matrix} & CH_3 & \\ \diagdown & & \diagup \\ & C & \\ \diagup & & \diagdown \\ & CH_3 & \end{matrix}$, the two olefinic protons that absorbed at 4·27 τ must be —CH=CH—. A CH_2 group and an OH group must be fitted together with these moieties. The CH_2 group must be inserted between the conjugated moiety and the isolated olefinic group, and the OH group can be placed only on the same carbon atom to which the geminal methyl groups are attached. The presence of a strong sharp band in the IR spectrum at 10·25 μ and the virtual absence of absorption beyond 12 μ argue for the *trans* configuration. Compound 2 is therefore *trans*-2-methyl-6-methylene-3,7-octadiene-2-ol.



Compound 2

Several confirmatory tests were carried out. The NMR spectrum was obtained on a deuterioacetone solution. The OH peak was shifted downfield and remained as a singlet; none of the other peaks showed additional splitting. A primary or a secondary alcohol would be expected to show coupling under these conditions.[8,9] However the recent note[10] that such considerations are not valid for all types of alcohols

[8] D. E. McGreer and M. M. Mocek, *J. Chem. Educ.* **40**, 358 (1963).
[9] O. L. Chapman and R. W. King, *J. Amer. Chem. Soc.* **86**, 1256 (1964).
[10] J. G. Traynham and G. A. Knesel, *J. Amer. Chem. Soc.* **87**, 4220 (1965).

dictates a conservative interpretation. Decoupling of the CH_2 proton resulted in sharpening of the —C*H*=C*H*— absorption. Evidently both protons have the same chemical shift, and no information can be obtained about the —C*H*=C*H*— coupling constant from which the *cis-trans* configuration might have been determined. When the —C*H*=C*H*— absorptions was decoupled, the slightly broadened CH_2 doublet collapsed to a singlet slightly broadened by coupling to the allylic protons.

The major cleavage in the mass spectrum occurs between C_3 and C_4 with transfer of a hydrogen atom to give the base peak *m/e* 79. Another major cleavage occurs between C_2 and C_3 with the charge remaining in the hydrocarbon fragment (*m/e* 93) which eliminates one and two atoms of hydrogen to give the large peaks at *m/e* 92 and *m/e* 91.

An alternative structure, *trans*-2-methyl-6-methylene-4,7-octadiene-2-ol would give a cross-conjugated structure. This is ruled out by the shift position of the protons of the double bond in question.

Instrumentation—The IR spectra were run on a Perkin–Elmer 221 and 137, the NMR on a Varian HR60 and HA100 the UV spectra on a Cary 14M, the mass spectra on a CEC21–103C, and the optical rotation on a Perkin–Elmer 141.

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The NMR spectra were run by Mr. W. R. Anderson, Jr., the optical rotation by Dr. P. Lim, and the mass spectra by Mrs. J. S. Whittick. Mr. Paul Skelton assisted with many of the laboratory operations. We are indebted to Dr. D. W. Henry for many helpful discussions.