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MONO-OXYGENATED MONOTERPENES FROM THE FRASS OF THE WOOD-BORING BEETLE *HYLOTRUPES BAJULUS (L)*

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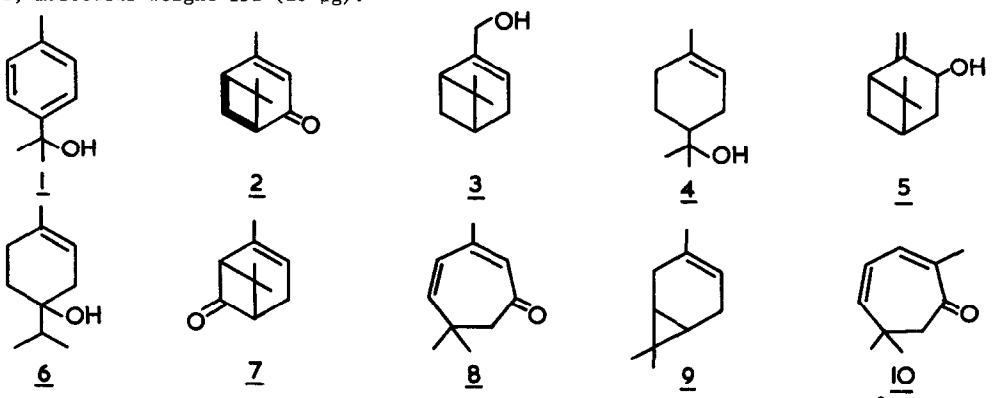
The wood-boring beetle *Hylotrupes bajulus (L)*, (Coleoptera: Cerambycidae) is a pest of coniferous soft woods, inflicting serious damage to structural timbers in many countries.

We have found that the hydrocarbons present in host wood *Pinus sylvestris* (e.g. α- and β-pinene, 3-carene) are converted mainly into mono-oxygenated monoterpenes on passing through the digestive tract of the larvae.[1] The extraction and fractionation of frass (consisting predominantly of fecal pellets) has allowed microscale identification of eight monoterpenes. The two major components, p-cymene-8-ol (1, 570 µg), and (-)-verbenone (2, 450 µg) show oviposition activity. The minor components are myrtenol (3, 90 µg), α-terpineol (4, 70 µg), *trans*-pinocarveol (5, 60 µg), terpinen-4-ol (6, 30 µg), chrysanthenone (7, 30 µg) derived as an artifact from verbenone, 3,6,6-trimethylcyclohepta-2,4-dienone (8, 55 µg) and an unidentified alcohol, molecular weight 152 (20 µg).**



1 2 3 4 5

6 7 8 9 10

The frass (300 g) was obtained from culture blocks of unseasoned *P. sylvestris*[2] and Soxhlet extracted for three days with methylene chloride, which on evaporation of the solvent yielded a viscous brown oil (6.84 g). This oil was stirred vigorously for two hours in hexane, the resulting pale yellow solution (containing 1.7 g solutes) decanted and washed with saturated aqueous sodium bicarbonate, followed by 10% aqueous sodium hydroxide. The extraction to this

** Small sample sizes have limited determination of absolute configuration to (2)

point was monitored by electroantennography[3] (EAG: excised antennae, glass electrodes) in order to follow physiological activity. Only the fraction remaining after base extraction (120 mg) showed EAG activity.

Gas liquid chromatography (GLC) on column A[4] showed this fraction to contain a group of mono-oxygenated monoterpenes and neutral high molecular weight compounds. Preparative GLC was carried out on column A, the resulting mono-oxygenated monoterpene fraction (1.4 mg) being the only source of EAG activity. This fraction was separated into individual components by preparative GLC using columns B and C,[4] held isothermally at 120° and 100° respectively. The compounds were identified on a microscale by GLC including on-column reaction chromatography, mass spectrometry, FT-PMR and UV spectroscopy, the latter two techniques being used in conjunction with an efficient GLC micropreparative system.[5] A combination of these techniques was particularly effective in the elucidation of the minor component 8 (55 µg), which on the basis of mass and UV spectra was thought to be eucarvone (10) (m/e: 107 (100%), 150 (M, 50), 91 (47), 135 (27), 79 (26), 108 (25), 41 (18), 77 (15); $\lambda_{max.}$ 294 nm, ε = 7,400). The concentration of UV solutions was determined by comparison of GLC response with a standard solution of eucarvone and confirmed by use of a synthetic sample of (8). However, the retention times on column B (15.2 min) and column C (16.0 min) were considerably longer than those of eucarvone (7.6 and 8.1 min respectively). After retrapping into 300 µl of $CDCl_3$[6] an FT-PMR spectrum (10850 pulses) was obtained on 50 µg [100 MHz in $CDCl_3$, internal TMS; τ8.88 (s, 6H), 8.02 (s, 3H), 7.52 (s, 2H), 4.35 and 4.02 (AB q, 2H, J = 11Hz), 4.09 (s, 1H)]. The coupling pattern of the vinyl protons was inconsistent with the eucarvone structure, but could be interpreted in terms of 8 or the 4,6,6-trimethyl substituted isomer.

A synthetic sample of 8 was obtained by aerial oxidation of 3-carene (9), (18 h, 40°C, continuous bubbling of air), followed by reductive work-up with aqueous alkaline sodium sulphite.[7] The product was then extracted with diethyl ether, dried (Na_2SO_4) and distilled (85°C/1 mmHg). Compound 8, obtained pure by preparative GLC in 12% overall yield, had UV, PMR and mass spectra, and 2,4 DNP (m.p. 176.5°-177°C) identical to published data[8] and to those of the naturally occurring compound. It is interesting to note that compound 1 was also isolated from the mixture of products in 14% yield.

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References and notes

1. D.A. Evans and M.D. Higgs, manuscript in preparation.
2. We are particularly grateful to Mr. R. Berry, Building Research Establishment, Princes Risborough, Bucks, U.K., for assistance with materials and culture methods.
3. D. Schneider, Z. vergl. Physiol, 1957, 40, 8.
4. GLC conditions: Diatomite C support (DMCS treated, 80-100 mesh); column A: 3/8" x 5' 5% OV-1, column B: 1/4" x 5' 10% PPGA, column C: 1/4" x 5' 5% XE-60.
5. R. Baker, J.W.S. Bradshaw, D.A. Evans, M.D. Higgs and L.J. Wadhams, manuscript in preparation.
6. 99.8% isotopically pure, dried by refluxing over activated molecular sieves (13X) for 12 h.
7. British Patent 761686.
8. W.D.P. Burns, (Miss) M.S. Carson, W. Cocker, and P.V.R. Shannon, J. Chem. Soc. (C), 1968, 3073. We thank Dr. Cocker for generous gifts of samples and data.