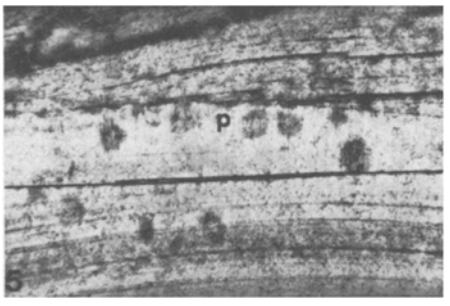



Fig. 5. Longitudinal section. p, normal prismatic (calcitic) layer. ×200.

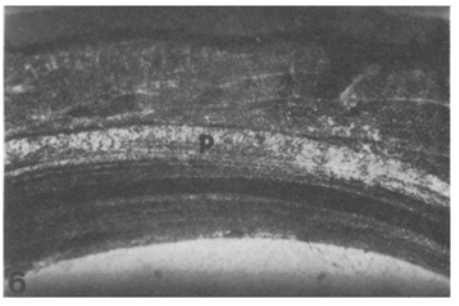



Fig. 6. Same section as Figure 5, stained for aragonite. p, unstained calcite. ×75.

Investigations on shell mineralogy demonstrate that most marine gasteropod shells are aragonitic. Lowenstam[2] found only 5 genera with mixed mineralogies: Patella, Haliotis, Fissurella, Nerita, and Littorina. After Waskowiak[3], Thais, Neptunea, Purpura, Tegula, Crepidula may be added.

A further genus, comprising at least 1 species with mixed mineralogy, is recorded in this paper: the species Monodonta (Osilinus) articulata Lamark 1822 (figures 1, 2 and 3) secretes a shell containing appreciable quantities of calcite (figure 4).

In the course of an investigation on shells of Monodonta articulata from different localities of Western Sicily, percentages of calcite varying from 7% to 25% have been determined through calibration curves prepared following the procedure proposed by Turekian and Armstrong[4]. The calcitic microstructural unit, characterized by a normal prismatic structure, has been identified by mechanical separation of the structural units of the shell and by staining technique on thin section (figures 5 and 6). Moreover, it has been observed that the calcitic unit lies anomalously between 2 aragonitic layers, as figures 5 and 6 clearly show, whereas it is well known from literature[5–7] that, in shells with aragonite-calcite composition, calcite forms only the uppermost layer, the only exception to this rule being some species of Haliotidae[5].

2 H. E. Lowenstam, Proc. nat. Acad. Sci. *40*, 39 (1954).
3 J. D. Milliman, Marine carbonates. Springer, Berlin 1974.
4 K. K. Turekian and R. L. Armstrong, J. Marine Res. *18*, 133 (1960).
5 O. B. Bøggild, D. K. D. Vidensk. Selsk. Skr. naturh. Math. *9*, 231 (1930).
6 R. G. C. Bathurst, Carbonate sediments and their diagenesis. Elsevier, Amsterdam 1971.
7 O. P. Majewske, Recognition of invertebrate fossil fragments in rocks and thin section. Brill, Leiden 1974.

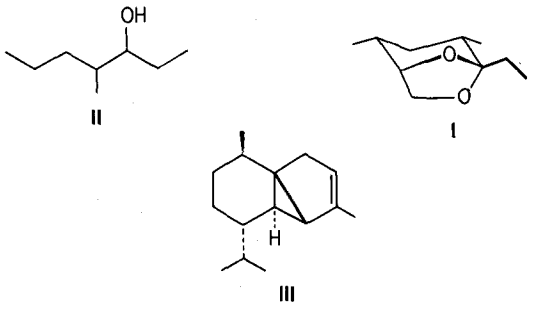
## Volatiles associated with Scolytus scolytus beetles on English elm[1]

Margaret M. Blight, F. A. Mellon, L. J. Wadhams and M. J. Wenham

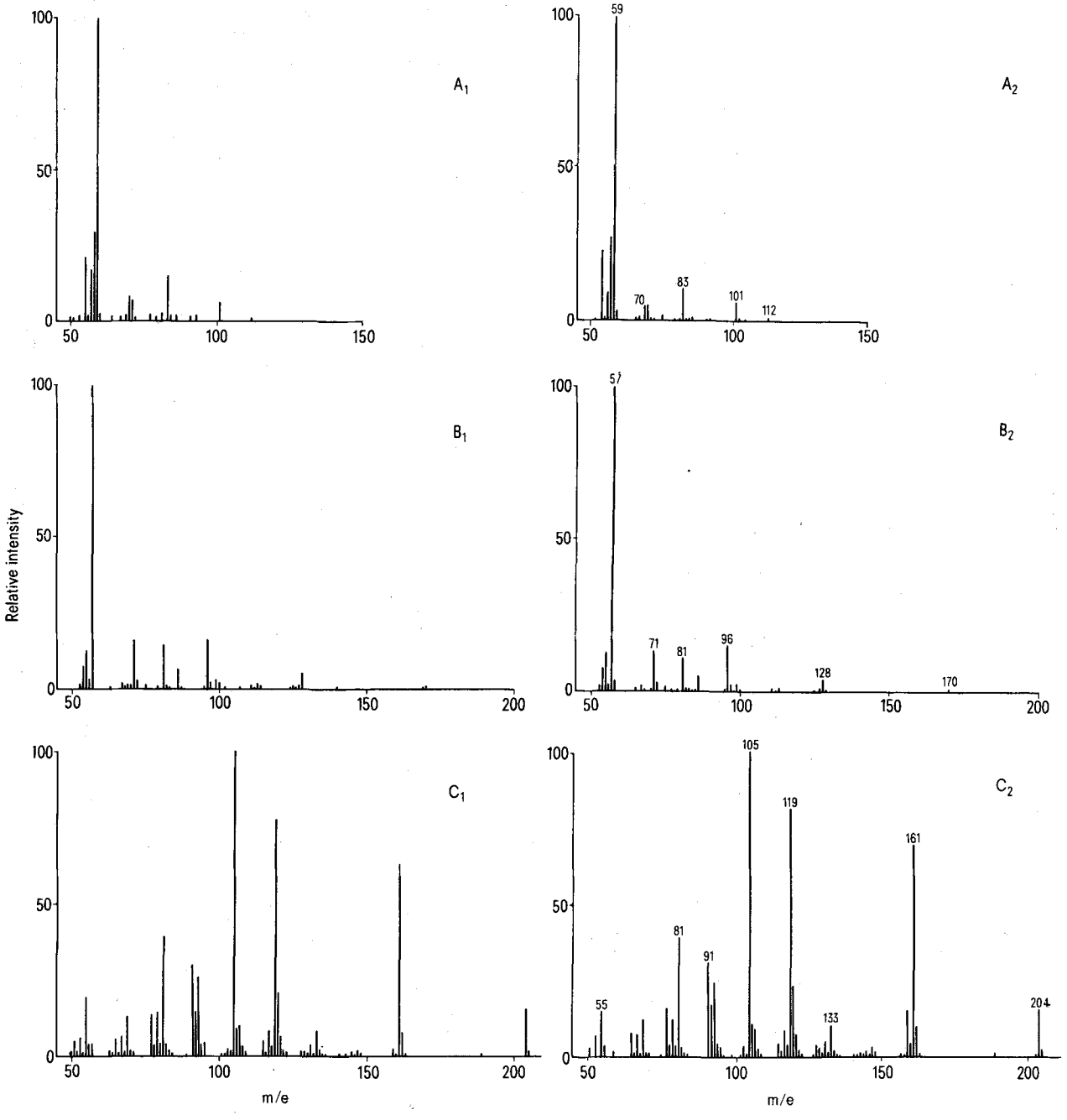
*Agricultural Research Council, Unit of Invertebrate Chemistry and Physiology, University of Sussex, Falmer, Brighton BN1 9QJ (England), 9 December 1976*

*Summary.* α-Multistriatin, 4-methyl-3-heptanol and α-cubebene, the components of the aggregation pheromone of Scolytus multistriatus are also associated with virgin female Scolytus scolytus on English elm, Ulmus procera.

In the USA the main vector of Ceratocystis ulmi, the causal fungus of Dutch elm disease, is the smaller European elm bark beetle Scolytus multistriatus[2]. When pioneer virgin females bore into American elm, Ulmus americana, an aggregation pheromone is produced which results in the secondary attraction of both sexes[3]. This attractant is now known to consist of at least 3 compounds[2]: 2,4-dimethyl-5-ethyl-6,8-dioxabicyclo(3.2.1) octane (α-multistriatin, **I**), 4-methyl-3-heptanol (**II**) and α-cubebene (**III**). **I** and **II** are beetle metabolites, while **III** is a host-produced synergist. A synthetic mixture of these substances, multilure, is now being used experimentally in the USA for population sampling and control[4]. Since the re-introduction of an aggressive strain of




1 We thank Dr J. F. Grove for advice and encouragement, Mrs J. Allsop for technical assistance, Dr J. W. Peacock for authentic samples of multistriatin, 4-methyl-3-heptanol and α-cubebene, and the Parks and Gardens Dept., Brighton Corporation, for supplies of English elm.
2 G. T. Pearce, W. E. Gore, R. M. Silverstein, J. W. Peacock, R. A. Cuthbert, G. N. Lanier and J. B. Simeone, J. chem. Ecol. *1*, 115 (1975).
3 J. W. Peacock, A. C. Lincoln, J. B. Simeone and R. M. Silverstein, Ann. ent. Soc. Am. *64*, 1143 (1971). – J. W. Peacock, R. M. Silverstein, A. C. Lincoln and J. B. Simeone, Environ. Entomol. *2*, 355 (1973).
4 R. A. Cuthbert and J. W. Peacock, Environ. Entomol. *4*, 889 (1975).




Mass spectra of authentic and natural compounds associated with S. scolytus beetles on English elm. $A_1$, natural 4-methyl-3-heptanol; $A_2$, authentic 4-methyl-3-heptanol; $B_1$, natural α-multistriatin; $B_2$, authentic α-multistriatin; $C_1$, natural α-cubebene; $C_2$, authentic α-cubebene.

C. ulmi into England in the late 1960's[5] approximately 9 million elms have died[6]. The major vector of the disease in the United Kingdom is the larger European elm bark beetle, Scolytus scolytus. As part of a study of the chemically mediated behaviour of S. scolytus we are investigating its secondary attractants. We report herein results which show that **I**, **II** and **III** are among the volatiles produced by virgin female S. scolytus boring in English elm, U. procera.

*Materials and methods.* Volatiles from a chamber containing virgin female-infested U. procera were collected on Porapak Q[7]. The Porapak was extracted with purified pentane and the extract, which induced responses from males and females in a laboratory arrestant-excitant bioassay[8], was concentrated by fractional distillation. A control extract of elm volatiles was obtained under identical conditions.

The extracts were examined by gas chromatography (GC) using a Pye 104 gas chromatograph equipped with a flame ionisation detector. The columns used were a 57 m × 0.5 mm i.d. glass OV-17 support-coated open tubular column and a 50 m × 0.5 mm i.d. glass OV-225 porous-layer open tubular column. Coupled gas chromatography-mass spectrometry (GC-MS) analyses were performed using the 57 m × 0.5 mm i.d. glass OV-17 support-coated open tubular column coupled through a

variable jet separator to a Varian MAT CH5D mass spectrometer. Spectra were processed and recorded by a Varian 620/L computer.

*Results and discussion.* α-Multistriatin and α-cubebene were identified in the extract of female-infested elm volatiles by comparison of their mass spectral fragmentation patterns with those of authentic specimens (figure ) and by accurate mass measurement of the molecular ions. 4-Methyl-3-heptanol (which shows no molecular ion) was identified in the same extract from its mass spectral fragmentation pattern (figure). These assignments were confirmed by co-injections of the Porapak Q extract with authentic samples on both the capillary GC columns. GC and GC-MS examination of the extract of U. procera volatiles showed that of the above 3 compounds only α-cubebene was present.

Although the components of the aggregation pheromone produced by S. multistriatus on U. americana are also produced by S. scolytus virgin females on U. procera it is not yet known what part they play in the aggregation behaviour of S. scolytus. Multilure does not appear to attract S. scolytus in large numbers in the field[9]. Further work on the components of the secondary attraction in S. scolytus is now in progress.

5 J. N. Gibbs and C. M. Brasier, Nature *241*, 381 (1973). – C. M. Brasier and J. N. Gibbs, Nature *242*, 607 (1973).
6 J. N. Gibbs, in: Report on Forest Research. (For. Commn., H. M. S. O., London, 1977).
7 J. W. Peacock, R. A. Cuthbert, W. E. Gore, G. N. Lanier, G. T. Pearce and R. M. Silverstein, J. chem. Ecol. *1*, 149 (1975).
8 J. H. Borden, R. G. Brownlee and R. M. Silverstein, Can. Ent. *100*, 629 (1968).
9 J. P. Vité, R. Lühl, B. Gerken and G. N. Lanier, Z. PflKrankh.-PflSchutz *83*, 166 (1976).

## Further information on the mechanism of the cystathionine-γ-synthase catalyzed reactions from the assignment of the $^1$H-NMR spectrum of homoserine

C. Fuganti and Dianella Coggiola

*Istituto di Chimica del Politecnico, Centro del CNR per la Chimica delle Sostanze Organiche Naturali, I-20133 Milano (Italy), 16 February 1976*

*Summary.* Unambiguous assignment of the $^1$H-NMR. resonances due to the hydrogen atoms in the β-position of homoserine indicates that the hydrogen which is exchanged and removed in the cystathionine-γ-synthase catalyzed reactions holds the pro-R configuration.

The knowledge of the absolute configuration of the hydrogen atom in the β-position of L-homoserine which is stereospecifically exchanged and removed in the conversion of O-succinylhomoserine into cystathionine or, in the absence of cysteine, into α-ketobutyrate by cystathionine-γ-synthase from Salmonella typhimurium[1] has been thought to be useful for a proper mechanistic interpretation of the isotopic studies[2] carried on with this pyridoxal phosphate dependent enzyme.

We have unambiguously assigned the $^1$H-NMR signals relative to the β-hydrogen atoms of homoserine using stereospecifically deuteriated materials[3], and found that the upfield absorbing β-proton, which has been reported[2] to be exchanged and removed in the cystathionine-γ-synthase catalyzed reactions, holds the pro-R configuration. This means that in the methylene interconversion occurring in the enzymic transformation of O-succinylhomoserine into α-ketobutyrate, protonation of the intermediate leading to the latter compound takes place from the same side from which the hydrogen had been removed in the homoserine-coenzyme intermediate Schiff's bases. The retention of configuration therefore supports, most economically, the previous idea schematized in the reported reaction path[4] that a single polyhydric base is present on the enzyme active side to remove both the α and the β pro-R hydrogen atoms in the formation of the enzyme-bonded vinylglycine derivative. The latter picks up a proton into the γ-methylene group from the same protonated base in the tautomerization to the (Z)-aminocrotonate derivative[5], as shown from the intramolecular hydrogen transfer from the α and β to the γ position of the $C_4$ framework. The latter intermediate is protonated in the β-position from the identical reprotonated base to give, eventually, after hydrolysis, α-ketobutyrate with overall retention of configuration in the β-methylene group.

This picture would be in line with the results of studies on the mechanism of pyridoxal phosphate dependent enzymes[6], and with recent views on the general significance of the enzyme reaction stereospecificity[7]. The assignment of the $^1$H-NMR resonances due to the β-methylene group of homoserine obtained by stereoselective deuteration is in agreement with that recently reported based on instrumental methods[8].

1 M. M. Kaplan and M. Flavin, J. biol. Chem. *241*, 4463 (1966).
2 B. I. Posner and M. Flavin, J. biol. Chem. *247*, 6412 (1972), and references therein.
3 D. Coggiola, C. Fuganti, D. Ghiringhelli and P. Grasselli, J. C. S. chem. Commun., p. 143 (1976).
4 B. I. Posner and M. Flavin, J. biol. Chem. *247*, 6418 (1972).
5 M. Flavin and C. Slaughter, Biochemistry *5*, 1340 (1966); M. Flavin and C. Slaughter, J. biol. Chem. *244*, 1434 (1969).
6 G. E. Skye, R. Potts and H. G. Floss, J. Am. chem. Soc. *96*, 1593 (1974); C. Fuganti, D. Ghiringhelli, D. Giangrasso, P. Grasselli and A. Santopietro-Amisano, Chimica Ind., Milano *56*, 424 (1974); C. Fuganti, D. Ghiringhelli, D. Giangrasso and P. Grasselli, J. C. S. chem. Commun., p. 726 (1974); S. Sawada, H. Kumagai, H. Yamada and R. K. Hill, J. Am. chem. Soc. *97*, 4334 (1975); I. Y. Yang, Y. Z. Huang and E. E. Snell, Fed. Proc. *34*, 496 (1975); E. Schleicher, K. Mascaro, R. Potts, D. Mann and H. G. Floss, J. Am. chem. Soc. *98*, 1043 (1976).
7 K. R. Hanson and I. A. Rose, Accts Chem. Res. *8*, 1 (1975).
8 P. E. Hansen, J. Feeney and G. C. K. Roberts, J. Magn. Res. *17*, 249 (1975).