ABSOLUTE CONFIGURATION OF (3R,4S)-4-METHYL-3-HEXANOL—A PHEROMONE FROM THE HEAD OF THE ANT TETRAMORIUM IMPURUM FOERSTER

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Abstract—The isolation of 4-methyl-3-hexanol and 4-methyl-3-hexanone from the heads of all adult castes of T. impurum (previously wrongly reported as T. caespitum) is described. The important quantitative differences between the secretions of both sexes is confirmed, males producing much higher quantities of both compounds. The absolute configuration of the alcohol from the heads of males was found to be 3R,4S. In view of the sexual differences observed in T. impurum, the qualitative divergence in the secretions of T. impurum and T. caespitum is tentatively interpreted as a biochemical isolation mechanism between two sibling species.

Key Word Index: Ant, Tetramorium impurum, Tetramorium caespitum, pheromones, (3R,4S)-4-methyl-3-hexanol, 4-methyl-3-hexanone

INTRODUCTION

We recently reported the occurrence of 4-methyl-3-hexanol and 4-methyl-3-hexanone in the heads of Tetramorium caespitum collected in Belgium (Treignes). The amounts of both compounds were found to be larger in males than in those of alate females, whereas only the alcohol could be detected in small amounts in the heads of workers (PASTEELS et al., 1980).

Independently, LONGHURST et al. (1980) identified 3-octanone in the mandibular gland secretion of workers of Tetramorium caespitum collected in the U.K., near Furzeflack. In addition, SHIBAYASHI and KOMAE (1980) reported 2-hexenal together with monoterpenes and saturated hydrocarbons in extracts of whole Tetramorium caespitum workers from Japan, but none of the compounds found in the heads of the European samples.

These contradictory results prompted us to repeat our analyses on additional samples of Tetramorium from Belgium. In this paper, besides giving the correct species attribution of the Belgian Tetramorium, we assign the absolute configuration of the alcohol produced by the males, and report that 4-methyl-3-hexanone also occurs in the heads of workers.

MATERIALS AND METHODS

Source of material

Heads of males, alate females and workers from five different nests were collected separately in pentane. The samples varied from 30 to 100 heads, and the amount of solvent was adjusted to maintain the proportion at 200 heads/ml of pentane. Two colonies were found in Vierves and ‘La Carrière Michele’ respectively, only a few kilometres distant from Treignes where we had collected the specimens of our previous study. The three other colonies were found in Marchin, 60 km from Treignes. These three colonies were only a few metres distant from each other. The abdomens of males from each colony were sent to Dr. B. POLID (Mantua, Italy) for further identification. All colonies were collected in August.

A further sample of worker heads was collected in September from a colony without males, found at Sint Joris Weert (south of Leuven) 80 and 55 km distant, respectively, from Treignes and Marchin. Since only workers were found, precise identification of the species was not possible.

Preparation of the 4 stereoisomers of 4-methyl-3-hexanol

The synthesis of the (+)-erythro- and (–)-throo-4-methyl-3-hexanols was performed following the scheme described by ETTERZ et al. (1977), for the corresponding 4-methyl-3-heptanols.

Preparation of (S)-2-acetoxypropionyl chloride and esterification of the 4-methyl-3-hexanols

(S)-2-Acetoxypropionyl chloride was synthesized according to the method of BEAN et al. (1936). However, dimethyl formamide was used as a catalyst to prepare the acid chloride (BOUSHARD et al., 1959).

A typical esterification is as follows: 0.1 mg of the 4-methyl-3-hexanol was dissolved in a mixture of pentane (100 µl) and anhydrous pyridine (500 µl). (S)-2-Acetoxypropionyl chloride (50 µl) was added at room temperature (around 20°C) and the mixture was allowed to stand for 30 min. Isolation of the products was accomplished by pouring the reaction mixture into iced water (10 ml) and extracting twice with 5 ml of pentane. The combined organic extracts were washed successively with 5 ml of water (1 x), 1 ml of 1 M HCl (1 x) and with saturated NaCl solution (1 x), then dried with anhydrous magnesium sulphate, filtered and evaporated to dryness under a stream of N₂. For GC analysis, a minimum amount of pentane was added (10–50 µl).

GLC chromatography

Gas chromatographic analyses were performed on a Varian 560 gas chromatograph equipped with a flame ionization detector and a 25 m × 0.5 mm i.d. wall coated Carbowax 20 M capillary column, at a flow rate of 3 ml/min, with nitrogen as the carrier gas. Temperatures were: column, 120°C; injector, 175°C; detector, 150°C.
RESULTS

The males from all the five colonies were identified by Dr. B. POLDI as *Tetramorium impurum* Foerster, a little known species, closely related to *T. caespitum* from which it is barely distinguishable. This species has never been previously reported from Belgium.

The capillary GC analyses of pentane extracts of male heads of *T. impurum*, originating from the five different nests, show the presence of 4-methyl-3-hexanol and 4-methyl-3-hexanone, as previously described (PASTEELS et al., 1980) (Fig. 1). No trace of 3-octanol or 3-octanone could be detected. As before only the erythro isomer of the alcohol could be identified. However, different alcohol to ketone ratios were sometimes observed for different nests (from 3:1 to 8:1), whereas the previously reported ratio was 2:1.

Furthermore the heads of females and workers from all localities were shown to contain both compounds, but in very small amounts (Fig. 1). This contrasts with our earlier results where only 4-methyl-3-hexanol could be detected in the heads of workers.

To establish the absolute configuration of natural 4-methyl-3-hexanol, a synthetic racemic mixture of (+)-erythro- and (−)-threo-4-methyl-3-hexanols was esterified with optically active (S)-2-acetoxypropionyl chloride. This led to a mixture of four diastereoisomeric esters, easily resolved by capillary GC. The assignment of the four peaks to each diastereoisomer is based on the data of KIRMSE et al. (1977).

Esterification of a pentane extract of male heads of *T. impurum*, under the same conditions, led to only one diastereoisomer, which was identical with the ester derived from (3R,4S)-4-methyl-3-hexanol (retention time and co-injection in GC). The same ester has been obtained from male heads of the five different nests.

We have used the method on batches of about 100 male heads. However, the sensitivity of the method should allow the use of 10 heads or less (about 10 µg of alcohol).

DISCUSSION

The discovery in Belgium of five colonies of *T. impurum* was unexpected since *T. caespitum* was the
only species of the genus previously reported in this country (GASPAR, 1970–71; VAN BOVEN, 1977). It should be noted, however, that T. impurum is a little known species, not even listed by BERNARD (1968). Its confusion with T. caespitum is easy, and according to POLDI (private communication), only the male genitalia allow a clear distinction between the two species, although a series of males from one nest must be examined. Indeed, if one looks at Fig. 327 of KUTTER (1977), the male genitalia of both species appear rather variable.

The specimens studied by LONGHURST et al. (1980) were probably correctly identified as T. caespitum. POLDI (private communication) has identified T. caespitum specimens also originating from Dorset (Wareham). This would explain the divergence between their results and ours and suggest that the composition of the mandibular gland secretion could give an additional and unambiguous taxonomic character to separate the two species: 3-octanone being the major compound produced by T. caespitum, 4-methyl-3-hexanone and 4-methyl-3-hexanol those synthesized by T. impurum. All the colonies studied in this paper and in the previous one would then belong to the species T. impurum which, if unrecognized until now, appear to be a fairly frequent and largely distributed species in Belgium. The status of T. impurum and its distribution in Belgium will be discussed in more details elsewhere (POLDI and PASTEELS, in preparation).

Such a clear-cut qualitative difference between the mandibular gland secretions of two closely related species from the same genus is not frequent within the Myrmicines. For example, in the European species of Myrmica which have been thoroughly studied, the differences appear more in the proportions of the constituents than in their specific nature (CAMMAERTS et al., 1978). It is clear from our chromatograms that the males contain much higher quantities of both compounds than the female castes (Fig. 1), thus confirming our previous conclusions (PASTEELS et al., 1980). This suggests that the secretion might be used as a chemical signal during swarming. If this is also true for T. caespitum, the qualitative specific differences in the secretions would be part of the reproductive isolation system between the two sibling species and would represent another example of displacement characters between two sympatric species.

Obviously the recognition and distribution of the European Tetramorium species, for which many ill defined varieties have been proposed, badly need additional studies. We suggest that the chemical analysis of the mandibular gland secretion would be useful to confirm our previous conclusions.

The detection of both 4-methyl-3-hexanol and 4-methyl-3-hexanone in small amounts in the heads of workers contrasts with our previous results, where only the alcohol could be detected. This discrepancy is probably due to a better separation of the ketone from the solvent peak in capillary GC as compared with the filled columns used previously.

The natural occurrence of only one enantiomer is frequent for chiral insect pheromones as recently reviewed by SILVERSTEIN (1979) and has already been demonstrated in two Myrmicines, Atta texana and A. cephalotes which produces (S)-4-methyl-3-heptanone in their mandibular glands. Moreover this enantiomer is respectively 10 times and 20 times more active than the (R) enantiomer in these species (RILEY et al., 1974a, b). BENTHUYSEN and BLUM (1974) demonstrated that the same enantiomer is more active in another Myrmicine, Pogonomyrmex barbatus which also secretes 4-methyl-3-heptanone.

These results suggest that the 4S configuration is characteristic of 4-methyl-alkanones and -alkanols produced by Myrmicines, although the paucity of data still prevents any generalization. This is even more so for the R configuration at the C-3 centre. Indeed, although mandibular gland pheromones of several Myrmicines possess a chiral carbon atom at C-3 (BLUM and HERMANN, 1978, for a recent review), their absolute configuration has never been reported. The same 4S-configuration is also found in 4-methyl-3-heptanols from the aggregation pheromonal mixture of bark beetles. However, whereas Scolytus scolytus from the U.S.A. only produces the 3S, 4S- enantiomer (MORI, 1977), S. multistriatus from the U.K. produces a mixture of 3S, 4S- and 3R, 4S-heptanols (BLIGHT et al., 1979). This suggests that (S)-4-methyl-3-heptanones are intermediates in the biosynthesis of the corresponding heptanols by S. multistriatus. However similar conclusions cannot be made in the case of Tetramorium since the absolute configuration of the ketone is as yet unknown.

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REFERENCES


EINTERZ R., PONDER J. W. and LENOX R. S. (1977)
Synthesis of 4-methyl-3-heptanol and 4-methyl-3-heptanone—two easily synthesized insect pheromones. J. Chem. Educ. 54, 382.


Mori K. (1977) Absolute configuration of (−)-4-methylheptan-3-ol, a pheromone of the smaller European elm Bark beetle, as determined by the synthesis of its (3R, 4R)−(+)− and (3S,4R)−(+)−isomers. Tetrahedron. 33, 289–294.


