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BIOSYNTHESIS OF 6-METHYLHEPT-5-EN-2-ONE IN THE AUSTRALIAN MEAT ANT, *IRIDOMYRMEX PURPUREUS*

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ABSTRACT.—6-Methylhept-5-en-2-one, isolated from *Iridomyrmex purpureus* fed with *N,N*-dibenzylethylenediamine-di-DL-[2-^{14}C]mevalonate, has been shown to contain radiolabel. The distribution of label, as determined by chemical degradation, supports the hypothesis that the compound is a degraded terpenoid arising via the isoprenoid pathway.

6-Methylhept-5-en-2-one (methylheptenone) is a frequently reported natural product occurring in a wide variety of organisms, both flora and fauna. For example, it has been noted as a component in the essential oil from lemongrass, and it is produced by the wood rotting fungus *Endoconidiophora coerulescens* (1). It also occurs in several species of ants (2–4), and in the case of the common Australian ant, *Iridomymrex purpureus* F. Smith (Formicidae), it is the major volatile constituent (5,6).

Methylheptenone has also been of interest in relation to its biological functions. On one hand it has been shown to have insecticidal activity, in particular, a rapid knockdown effect (7), yet it occurs in members of the Formicidae where it has been shown to function as an alarm pheromone and as a defense secretion (7,8).

In terms of the biogenesis of methylheptenone in insects, Cavill and Hinterberger (9) have proposed it to be of isoprenoid origin and suggested that it could arise by a reverse aldol-type condensation from citral. Their pathway seemed particularly viable because the formation of citronellal, the iridodials and related compounds (2,3,7) and other substances that, like methylheptenone could be viewed as degraded terpenoids (10), and that also occur and in many cases co-occur in various species of these ants, could all be rationalized from citral as the common precursor.

Terpenes have been the subject of numerous biosynthetic studies, particularly using plants and fungi and more recently plant tissue culture. However, relatively little work has been done in insects. Of relevance to the present work, Happ and Meinwald (11) have demonstrated the incorporation of label into citral and citronellal, mandibular gland components isolated from *Acanthomyops clavinger* fed with the sodium salt of [1-^{14}C]acetic acid, [2-^{14}C]acetic acid, or [2-^{14}C]mevalonic lactone. Degradation work to ascertain the positions or distribution of label was, however, not reported.

In the present study we have investigated the biosynthesis of methylheptenone in *I. purpureus* with experiments designed to test the hypothesis noted above that the compound arises from the isoprenoid pathway.

RESULTS AND DISCUSSION

I. purpureus occurs widely throughout Australia and has been the subject of various chemical and biological studies (5,6). It is noteworthy that specimens of this species collected from a wide area of southeastern Australia possessed the same chemical components (6).

For the present work, ants were collected from various colonies. Once these had stabilized in the laboratory, of a variety of food sources offered, it was noted that they preferred small pieces of grape compared with honey solution or other alternatives.

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Moreover, H_2O placed in the cage appeared not to be utilized. Because direct injection of the radiolabeled precursors was deemed impractical because of the small size of the ants and their hard exoskeleton, radiolabel was simply placed on the fleshy part of small pieces of grape. When fed in this way an overall uptake of label of 7.5% was achieved. When the CH_2Cl_2 extract from these ants was examined by tlc, approximately 0.48% of label fed appeared to be incorporated into methylheptenone. The incorporation of label into methylheptenone was substantiated by standard radiodilution procedures in which the compound was isolated from the extract following addition of unlabeled material and the semicarbazone derivative of the reisolated material was prepared and recrystallized to constant specific activity.

Assuming an isoprenoid pathway to methylheptenone via citral, condensation of dimethylallyl pyrophosphate with isopentenyl pyrophosphate derived from C-2 labeled mevalonic acid is expected to lead to a labeling pattern as shown in Figure 1, where only C-3 and C-7 or C-8 of methylheptenone would contain label. While it was deemed impractical to degrade stepwise each carbon atom of the methylheptenone, degradation was carried out as illustrated in Figure 1.

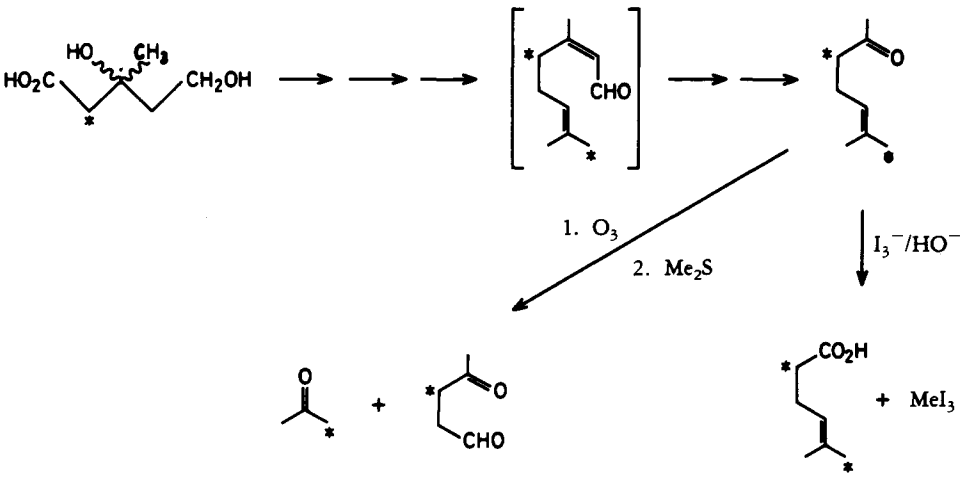

FIGURE 1. Proposed biosynthetic pathway to, and degradation scheme for methylheptenone.

Using the standard iodoform reaction, C-1 of methylheptenone was readily obtained as iodoform, and this could be recrystallized from EtOH. Initial counting of this material indicated the presence of some radiolabel. However, on subsequent recrystallizations the levels of radioactivity decreased progressively to approximately background level. The iodoform was also examined by analytical tlc and, in agreement with the above results, found to be unlabeled. The other product from the iodoform reaction, 4-methyl-4-hexenoic acid, proved difficult to isolate because of the residual reagents from the iodoform reaction and was not pursued further.

A further sample of labeled methylheptenone was subsequently ozonolyzed to generate Me_2CO and levulinaldehyde (4-oxopentanal). This provided a more informative pair of degradation products because both fragments, based on the position of label in the precursor, were anticipated to contain radiolabel.

While the Me_2CO component of the mixture was readily isolable via micro distillation, some difficulties were encountered with the aldehyde. Various methods of reductive workup of the ozonide were investigated including H_2O and Zn/HOAc. However, the best method proved to be with dimethyl sulfide (12), after which the aldehyde was readily isolated and characterized as the 2,4-dinitrophenylhydrazone. After recrystalli-

zation to constant specific activity the derivatives of both Me_2CO and the aldehyde were found to contain radiolabel (Table 1).

This distribution of label may be interpreted in several ways. One possibility is that the methylheptenone arises from condensation of mevalonate with a 3-carbon unit derived from mevalonate. This is, however, deemed unlikely because scrambling of label between C-1 and C-3 in the methylheptenone might reasonably be expected, and this is found not to be the case. Rather it is considered that the distribution of label supports the hypothesis that the compound is a degraded C_{10} terpenoid. The relative distribution of radiolabel within the methylheptenone also deserves comment. Approximately 88% of the label resides in the portion of the molecule (C-4,-5,-6,-7, and -8) that may be considered to be derived from isopentenyl pyrophosphate but only 12% in that (C-1,-2, and -3) from dimethylallyl pyrophosphate. This distribution suggests either the presence of a dimethylallyl pyrophosphate pool in the insect or a compartmentalization of precursors within the insect. Similar results have been obtained from experiments with higher plants (13–15).

TABLE 1. Distribution of Radiolabel in Methylheptenone and Degradation Products.

Compound	μCi/mmol	%
Semicarbazone of methylheptenone[a]	124	
Methylheptenone[a]	175	100
2,4-DNP of Me_2CO	21	12
2,4-DNP of levulinaldehyde	153	88

[a]Represent different degrees of dilution of the ant extract with cold methylheptenone.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot stage and are uncorrected. Analytical and preparative tlc utilized layers of Kieselgel GF (Merck; 0.25 and 0.5 mm, respectively). Plates were visualized under uv light or with iodine vapor. *N,N*-Dibenzylethylenediamine-di-DL-[2-^{14}C]mevalonate (51 mCi/mmol; specific activity 250 μCi/250μl) was from Amersham. To determine radioactivity profiles on the plates, plates were divided into bands and the silica from each band was scraped into counting vials, scintillant was added, and the mixture was counted. Radioactivity measurements were made using Brady's scintillant in a Beckman LS 2800 liquid scintillation counter with automatic background count and quench correction. Solid derivatives were each recrystallized to constant specific activity and radiochemical purities checked by analytical tlc. For the degradation reactions discussed below reference samples were prepared to allow direct comparisons to be made. Because the melting point found for the 2,4-DNP of levulinaldehyde (245–247°) did not agree with the results of Birkinshaw and Morgan (1) (mp 227–228°), the material was characterized fully. The nmr, ms, and ir data supported the proposed structure as did the microanalysis figures (found C 44.2%, H 3.3%, N 24.1%; $C_{17}H_{16}N_8O_8$ requires C 44.3%, H 3.5%, N 24.4%).

INSECT COLONIES AND FEEDING OF RADIOLABEL AND ISOLATION OF LABELED METHYLHEPTENONE.—Meat ants (*I. purpureus*) were collected from several colonies in close proximity in sandy areas along the Princes Highway north of Wollongong, New South Wales. Voucher specimens have been deposited in the National Insect Collection, CSIRO Division of Entomology, Canberra. When the nests were disturbed during the collection procedure, a strong smell of methylheptenone was noted. The ants were transported to the laboratory where the colonies were maintained separately from each other. The ants were maintained in an air-conditioned room, ventilated separately from the general laboratory, and offered H_2O and various foodstuffs. Small pieces of grape appeared to be preferred. After several weeks, at which point the colonies stabilized, the largest colony (ca. 500 ants) was fed twice weekly with small pieces of grape containing 25 μCi of mevalonic acid, over a 3-week period (total label fed: 125 μCi). Care was taken not to disturb the ants when material was placed into the cage. After completion of the feeding, ants were sacrificed by placing them in CH_2Cl_2. Anhydrous Na_2SO_4 was then added, and the mixture ground with a mortar and pestle prior to extraction in a Soxhlet apparatus for 12 h using the same solvent. Careful evap-

oration of the solvent gave a dark yellow oil (261 mg). The extract was diluted to 2 ml with $CHCl_3$ and subsampled for counting (total uptake of label as determined in the crude extract: 9.4 μCi, 7.5%). Unlabeled methylheptenone (250 mg) was added to a portion (108 mg) of the total radioactive ant extract and re-isolated (254 mg) by cc using petroleum ether/$CHCl_3$ solvent mixtures. The semicarbazone was prepared by the standard method and the derivative obtained as colorless plates which were recrystallized from aqueous EtOH to constant specific activity (124 μCi/mmol, mp 136–137°).

DEGRADATION OF METHYLHEPTENONE.—*Iodoform reaction.*—Labeled methylheptenone (44 mg) in dioxane was treated with aqueous 10% NaOH solution and KI_3 solution in the normal manner. The light yellow iodoform (50.7 mg) was recovered and dried (mp 123–124°). This material was recrystallized thrice from EtOH, and a portion was counted after each recrystallization. The iodoform was also examined by Si gel tlc [$CHCl_3$-petroleum ether (1:2)], and the radioactivity profile of the plate was determined. The material was found to be unlabelled.

Ozonolysis.—Labeled methylheptenone (99 mg, 175 μCi/mmol) in dry MeOH (100 ml) was cooled in an ice bath and then treated with excess ozone. The flask was subsequently flushed with N_2 after which dimethyl sulfide was added and the solution left for 3 h with stirring. The solution was partially distilled and the distillate (4 ml) treated with Brady's reagent. The resulting yellow precipitate was recrystallized from EtOH to constant specific activity (mp 125–126.5°, 21 μCi/mmol) and shown to be identical in all respects to the 2,4-DNP of Me_2CO. The remainder of the solvent from the ozonolysis reaction was distilled and the residue diluted with EtOH and treated with Brady's reagent. The resultant product was recrystallized from pyridine to constant specific activity as bright yellow needles of the 2,4-DNP of levulinaldehyde (mp 245–247°, 153 μCi/mmol).

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LITERATURE CITED

1. J.H. Birkinshaw and E.N. Morgan, *Biochem. J.*, **47**, 55 (1950).
2. G.W.K. Cavill, D.L. Ford, and H.D. Locksley, *Aust. J. Chem.*, **9**, 288 (1956).
3. G.W.K. Cavill and H. Hinterberger, *Aust. J. Chem.*, **13**, 514 (1960).
4. M.S. Blum, "Chemical Defences of Arthropods," Academic Press, New York, 1981, p. 139.
5. G.W.K. Cavill and D.L. Ford, *Chem. Ind. (London)*, 351 (1953).
6. G.W.K. Cavill, P.L. Robertson, J.J. Brophy, R.K. Duke, J. McDonald, and W.D. Plant, *Insect Biochem.*, **14**, 505 (1984).
7. G.W.K. Cavill, *J. Proc. R. Soc. N.S.W.*, **103**, 109 (1970) and references therein.
8. F.E. Regnier and J.H. Law, *J. Lipid Res.*, **9**, 541 (1968).
9. G.W.K. Cavill and H. Hinterberger, *Trans. Int. Congr. Entomol., 11th*, **3**, 284 (1960).
10. R. Trave and M. Pavan, *Chim. Ind.*, **38**, 1015 (1956).
11. G.M. Happ and J. Meinwald, *J. Am. Chem. Soc.*, **87**, 2507 (1965).
12. J.J. Pappas and W.P. Keaveney, *Tetrahedron Lett.*, **36**, 4273 (1966).
13. D.V. Banthorpe, J. Mannan, and K.W. Turnbull, *J. Chem. Soc. C*, 2689 (1970).
14. D.V. Banthorpe and D. Baxandale, *J. Chem. Soc. C*, 2694 (1970).
15. D.V. Banthorpe, B.V. Charlwood, and M.R. Young, *J. Chem. Soc., Perkin Trans. 1*, 1532 (1972).

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