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J. Nat. Prod., **1993**, 56 (6), 953-956• DOI:
10.1021/np50096a024 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 6, pp. 953-956, June 1993

BIOSYNTHETIC STUDIES ON ANT METABOLITES OF MELLEIN AND 2,4-DIHYDROXYACETOPHENONE FROM [1,2-$^{13}C_2$] ACETATE

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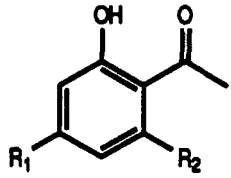
ABSTRACT.—The biosynthesis of mellein [**3**] which occurs in the Australian ponerine ant *Rhytidoponera chalybaea* was studied by feeding the ants with an aqueous solution of sodium [1,2-$^{13}C_2$]acetate. During this work, a further component not previously noted in this ant was also isolated and identified as 2,4-dihydroxyacetophenone [**4**]. Both mellein and acetophenone were examined by ^{13}C-nmr spectroscopy, and the ^{13}C-^{13}C couplings from the intact acetate units were detected. The results establish that in this ant both mellein and the acetophenone arise via the polyketide pathway.

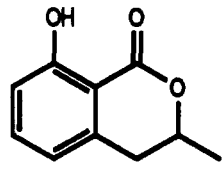
Various biosynthetic pathways are implicated in the production of different types of aromatic compounds in insects (1–3). Of these the polyketide route is the most common pathway. For example, 2-hydroxy-6-methylacetophenone [**1**] and 2,4,6-trihydroxyacetophenone [**2**] have been shown to arise via this pathway (4,5).

In the past decade, biosyntheses of many polyketide-derived metabolites have been reported using stable isotopes, particularly in the lower fungi and other microorganisms where high levels of incorporation are easily achieved (6). Some biosynthetic work has also been done in the insects, using both stable and radiolabelled precursors (7–9). The occurrence of mellein [**3**] in the Australian ponerine ant *Rhytidoponera chalybaea* Emery (Formicidae) (10,11), however, provided an opportunity to initiate a biosynthetic study of this compound in this insect, and in particular, to investigate the use of ^{13}C-labelled precursors in this situation. The use of doubly ^{13}C-labelled precursors offers the additional advantage that the connectivity in the molecule can be determined directly.

Mellein [**3**] has been found in many sources in nature (12), including fungi and insects. It has been shown to possess an impressive array of biological activities. For example, it is one of the constituents of the mandibular secretion of Carpenter ants (13) and the defensive secretion of termites (14). It occurs in the male hair pencils of the oriental fruit moth (15) and has been shown to have pheromonal properties in the castes of *Camponotus pennsylvanicus* (16). Mellein has been the subject of several previous biosynthetic investigations, carried out using a variety of labels, in fungi. For example, the in-



1 R_1=H, R_2=Me
2 R_1=R_2=OH
4 R_1=OH, R_2=H



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corporation of CD_3CO_2H and $^{13}CD_3CO_2H$ (17), and [1,2-$^{13}C_2$]acetate (18) into **3** in *Aspergillus melleus* demonstrated that it is biosynthesized from a common linear polyketide precursor.

In the present work, during the isolation of mellein another compound was noted and was identified as 2,4-dihydroxyacetophenone [**4**]. This was shown to arise via the polyketide pathway.

RESULTS AND DISCUSSION

The CH_2Cl_2 extract obtained from the bodies of *R. chalybaea* fed with [1,2-$^{13}C_2$]acetate was examined by tlc, and two uv-absorbing bands were noted. Subsequent preparative work led to the isolation of the anticipated **3**, while the second compound, which was also obtained crystalline, was identified on the basis of published data as **4**. It is noteworthy that this latter compound had not previously been reported from *R. chalybaea*; possibly it was unobserved in the earlier gc-ms studies in view of its retention times on the columns used (10,11).

The ^{13}C-nmr shift data and coupling constants for compound **3** as isolated from *R. chalybaea* are presented in Table 1. The results were generally in agreement with the earlier study in fungi and indicate that the biosynthetic pathway in ants is the same as that established in fungi (18).

The occurrence of **4** is of interest from two points of view. Although it has been isolated previously from natural sources, including plants (19–22) and sherry wine (23), this is the first report of its occurrence in insects. In considering the biosynthesis of **4**, while it can be predicted that it will arise from the acetate/malonate pathway, there are two possible foldings of the polyketide chain (Figure 1) which could yield this product. These alternatives can be differentiated on the basis of the ^{13}C-^{13}C coupling data. The ^{13}C-nmr spectrum of **4** isolated from *R. chalybaea* fed with [1,2-$^{13}C_2$]acetate is given in Table 2. The ^{13}C chemical shifts of **4** are assigned according to the literature report for unlabelled material (24). The results of the ^{13}C-^{13}C couplings indicate that **4** is derived from a tetraketide precursor with the folding mode as shown in Figure 2.

Two possible ways are suggested for the mode of action on a polyketide synthetase for aromatization in Figure 2. One of the routes is (i) the reduction of the carbonyl group at C-5 and then dehydration to form an unfunctionalized double bond. The other route is (ii) the

TABLE 1. ^{13}C Chemical Shifts and Couplings Observed for [1,2-$^{13}C_2$]Acetate-enriched Mellein [**3**].

Carbon	δ[a]	J[b]
C-9	20.8	39
C-3	76.1	39
C-4	34.6	40
C-4a	139.4	41
C-5	117.9	56
C-6	136.1	56
C-7	116.2	67
C-8	162.2	67
C-8a	108.3	66
C-1	169.9	68

[a]Chemical shift, δ, ppm, in $CDCl_3$ (125.77 Hz).

[b] ^{13}C-^{13}C coupling constant from [1,2-$^{13}C_2$]acetate, J, in Hz.

TABLE 2. ^{13}C Chemical Shift and Coupling Constants Observed for [1,2-$^{13}C_2$]Acetate-enriched 2,4-Dihydroxyacetophenone [**4**].

Carbon	δ[a]	J[b]
C-8	25.94	43
C-7	202.04	44
C-1	112.82	58
C-6	132.94	59
C-5	108.11	62
C-4	164.97	63
C-3	102.58	69
C-2	164.66	70

[a]δ, ppm, in $CDCl_3$+DMDO-d_6 (125.77 Hz).

[b] ^{13}C-^{13}C coupling constant from [1,2-$^{13}C_2$]acetate, J, in Hz.

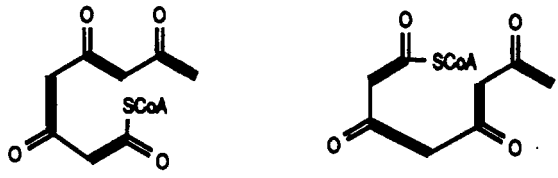

FIGURE 1. Alternative possible foldings for cyclization of the C-8 polyketide relative to the positions of the [1,2-$^{13}C_2$]acetate units.

reduction of carbonyl group at C-5 along with enolization at C-3. Both ways form a cis double bond so that three contiguous C-C bonds are held in a syn arrangement and the appropriate reactive sites (C-1 and C-6) are brought together for cyclization (25,26).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—[1,2-$^{13}C_2$]HOAc (90%) (0.5 g) was obtained from ICN Biomedicals. The acid was neutralized with aqueous NaOH and then diluted to a concentration of 2.0×10^{-2} M with deionized H_2O. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-500 (at 500 MHz and 125.77 MHz, respectively).

BIOLOGICAL MATERIALS.—Several groups of *R. chalybaea* were collected from Centennial Park, Randwick, New South Wales, Australia. Reference specimens were deposited in the Australian National Insect Collection, Division of Entomology, CSIRO, Canberra, ACT. The ants (ca. 4500) were collected from the nests, either individually with soft tweezers or by allowing them to attack an artist's paintbrush. The ants from different nests were maintained in separate cages in an air-conditioned room (19–20°). The colonies were allowed to stablize for 1 week before feeding the labelled acetate.

FEEDING PROCEDURE.—The ants were fed every second day over a 6-week period with [1,2-$^{13}C_2$]HOAc solution (7 ml/feeding). Each colony of ants was also given two mealworms twice per week.

EXTRACTION AND ISOLATION.—After completion of the feeding of acetate, the ants were sacrificed by placing them on dry ice 3 days after the last feeding of labelled material, and the heads were then dissected from the bodies. The bodies were ground with a mortar and pestle with anhydrous Na_2SO_4, and the mixture was exhaustively extracted with CH_2Cl_2 in a Soxhlet apparatus (20 h). Evaporation of the solvent gave a pale yellow oil (1.5 g).

The crude extract from the bodies of the ants was fractionated by centrifugal preparative tlc using Et_2O-petroleum ether (1:3) as eluent. Two

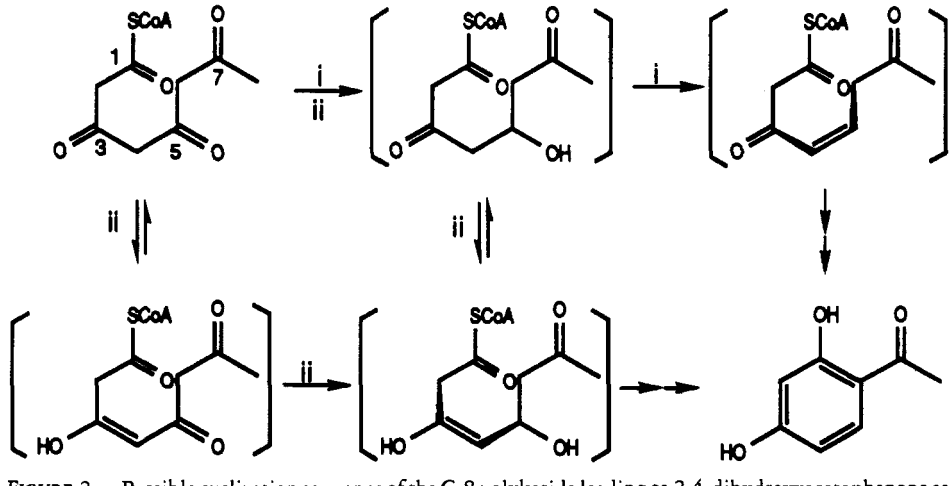

FIGURE 2. Possible cyclization sequence of the C-8 polyketide leading to 2,4-dihydroxyacetophenone as defined by the observed connectivity of the [1,2-$^{13}C_2$]acetate units.

uv-absorbing compounds were obtained, and each was purified by preparative tlc (silica) using Et_2O-petroleum ether (1:1) as eluent. The less polar compound, isolated as colorless needles (10 mg), was shown to be identical to mellein [**3**], mp 38–40° [lit. (27) mp 39°], ms and ^{13}C nmr. The more polar compound was isolated as colorless needles (14 mg) and shown to be identical in all respects to 2,4-dihydroxyacetophenone [**4**], mp 143–146° [lit. (28), mp 147°], ir, uv, and ms. ^1H nmr (500 MHz, $CDCl_3$+DMSO-d_6) δ 12.56 (1H, s), 10.28 (1H, s), 7.57 (1H, d, J=8.8 Hz), 6.30 (1H, dd, J=8.8 and 2.4 Hz), 6.20 (1H, d, J=2.4 Hz), 2.46 (3H, s).

ACKNOWLEDGMENTS

The Australian Research Grants Scheme is acknowledged for financial support.

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Received 30 July 1992