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Molecular Rearrangement of Longifolene by *Arthrobacter ilicis* T_2

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***Arthrobacter ilicis* T_2 brings about a unique type of cometabolic structural rearrangement of longifolene, a sesquiterpene, resulting in the formation of an acid. Infrared, nuclear magnetic resonance, mass spectrometry, and decoupling studies indicate that the acid product has a sativenelike structure, which is confirmed by conversion of the acid to its methyl ester and hydrocarbon.**

Several advantages (3, 8) of microbial enzymatic conversions have led to the exploitation of microorganisms as catalytic reagents in pollution control, elucidation of structural configurations of chemicals, and establishment of taxonomic characteristics and as analytical tools (2, 4, 11, 18, 19). An interesting and significant feature of these reactions is the wide array of intermediates and end products formed from a single compound by varied cometabolic (7, 17) or metabolic (1, 6, 9) transformation sequences.

During our studies of transformation of longifolene (Fig. 2, compound 1), organisms were isolated from soil by enrichment techniques. One of the isolated bacteria, *Pseudomonas alcaligenes* T_1, was found to selectively utilize the terpenic hydrocarbon impurities present in commercially available longifolene (Camphor & Allied Products, Bareilly U.P., India). With this culture, a microbial process for purification of longifolene was developed (10). A similar phenomenon was also shown by yet another isolate, T_2, identified as *Arthrobacter ilicis* (unpublished results). Interestingly, during growth on the impurities present in commercial longifolene, strain T_2 cometabolized the longifolene to a transformation product identified as an acid (Fig. 2, compound 5). This acid is also formed from pure longifolene during growth of the culture on other carbon sources such as mannitol. The pure longifolene used in this study was obtained by converting the longifolene to longifolol and then reducing this compound back to longifolene via longifolyl tosylate (16).

In this article, we discuss the isolation, identification, and characterization of the acid product and propose a mechanism for its formation from longifolene.

The culture of T_2 was grown on mineral medium (10) containing 0.2% (wt/vol) mannitol as the sole source of carbon. After 48 h of growth, pure 0.1% (wt/vol) longifolene was added to the culture broth, and the broth was incubated further. Culture aliquots were withdrawn 5, 12, 24, and 48 h after addition of longifolene and extracted with chloroform. Thin-layer chromatograms of these extracts run in a solvent system consisting of petroleum ether and ethyl acetate (90:10) showed identical patterns, with two spots with R_fs of 0.77 and 0.23 for longifolene (compound 1) and the product (compound 5), respectively. After 24 h of incubation, the culture broth was extracted in chloroform, the extract was concentrated under a vacuum, and the residue was separated into acidic and neutral extracts. The transformation product, which was obtained in the acid extract, was purified with activated charcoal and recrystallized with diethyl ether. A total of 15.9 mg of acid was obtained from 1 ml of longifolene added to 1 liter of medium. It was converted to its methyl ester by reaction with diazomethane and to its hydrocarbon by reduction with lithium aluminium hydride followed by tosylation and then reduction of the tosylate to hydrocarbon (21). The derivatives as well as the acid product were subjected to spectral analysis.

The characteristic features, as delineated by the infrared and nuclear magnetic resonance spectra of the product and methyl ester, are depicted in Table 1. The new acid (compound 5), with a molecular formula $C_{15}H_{22}O_2$ (M^+, 234), as shown on the basis of the mass spectrum, yielded the methyl ester $C_{16}H_{24}O_3$ (M^+, 248).

The proton nuclear magnetic resonance spectrum (360 MHz, $CDCl_3$) (Fig. 1 and Table 1) and decoupling studies of the acid product indicated that a partial structure (Fig. 2, structure A) is present in the acid. The presence of such a part structure is possible if this acid (compound 5) has the basic structural skeleton of sativene (5). In fact, the hydrocarbon sample obtained from the acid (compound 5) did show a peak in gas-liquid chromatography (silicon elastomer-30 and Carbowax column) which was confirmed to be sativene by coinjection with the authentic sample and by comparison of the chemical shifts with those reported for sativene (5, 12, 13). The mass spectrum obtained by gas chromatography-mass spectrometry was identical to that of sativene.

These results indicate that longifolene is cometabolized to an acid identified as sativic acid (compound 5), which has a

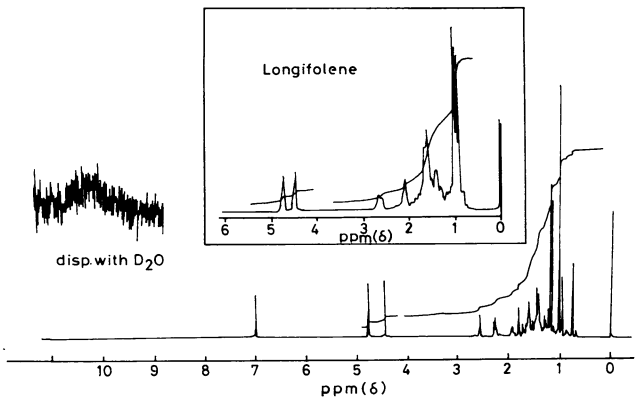

FIG. 1. Nuclear magnetic resonance spectrum of the acid product (compound 5). disp., disappears/exchanges with D_2O.

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TABLE 1. Spectral data depicting characteristic features of the acid product (compound 5)

Compound and infrared wave no.	^{1}H NMR[a] (ppm)	Structure
Acid product (M^+, 234)		
(1,650, 874)[b]	4.48 (1H, s)	>C=CH$_2$
	4.78 (1H, s)	
	2.6 (1H, s)	
(1,700)		>C=O
(2,600–3,100)	11.0 (1H, bs), exchangeable with D_2O	O ‖ —C—O—H
	1.0 (3H, s)	—C—CH$_3$
	1.2 (3H, d)	—CH—CH$_3$
Methyl ester (M^+, 248) (1,740)	3.62 (3H, s)	O ‖ —C—O—CH$_3$

[a] The multiplicities of proton signals are indicated as follows: s, singlet; d, doublet; bs, broad signal.
[b] Numbers in parentheses are wave numbers, expressed as centimeter^{-1}.

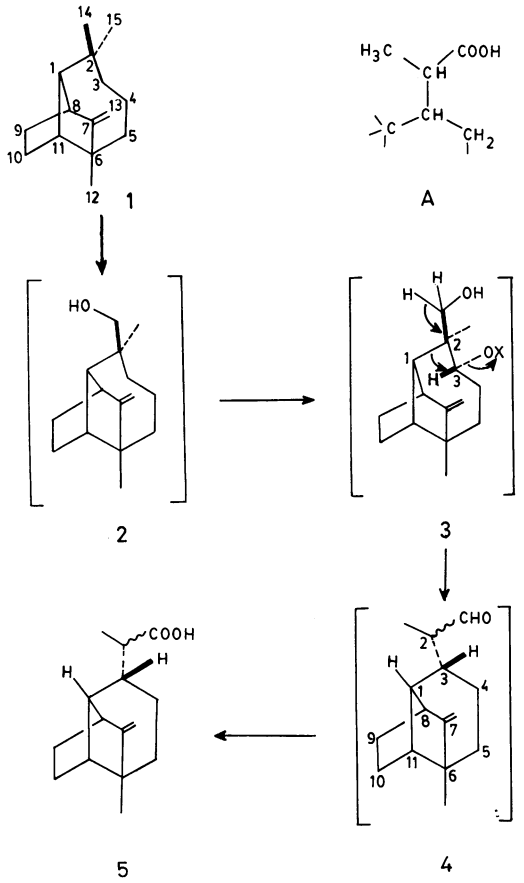

FIG. 2. Scheme of cometabolic transformation of longifolene (compound 1) by strain T_2 via functionalization of the C-14 methyl group (compound 2) followed by stereospecific hydroxylation at C-3 to yield a diol (compound 3), which is rearranged further to an unstable aldehyde (compound 4) and then converted to an acid (compound 5) having a sativene skeleton. Structure A is a partial structure of the acid product derived from nuclear magnetic resonance data.

sativene structural skeleton. The mechanism for the production of this acid from longifolene is hence proposed as shown in Fig. 2.

On the basis of the findings of Asakawa et al. (1), it is perhaps reasonable to assume that the C-14 methyl group in longifolene is functionalized first and then stereospecifically hydroxylated at C-3 (Fig. 2, compound 3) to yield a diol (20). Hydroxylations on saturated carbons on both rings and side chains are known to occur in steroids and are reported to be mediated by cytochrome P-450 systems (15).

Furthermore, inspection of the molecular models shows that a properly modified derivative (Fig. 2, compound 3; X ≠ H) can undergo the observed rearrangement, as the C-3-O bond, which is cleaved, and the C-1-C-2 bond, which migrates, are antiperiplanar. The resulting aldehyde (Fig. 2, compound 4) appears to be highly unstable and is oxidized to the acid (14) (Fig. 2, compound 5), a functionalized sativene molecule.

While Wagner-Meerwein- and Baeyer-Villiger-type rearrangements are reported to occur in microbial transformations, the novel biotransformation involving rearrangement of a 1,3-dihydroxy functionalized system or its equivalent has been observed in this study for the first time.

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