review

The biology of methyl ketones

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ABSTRACT Examples of the biological occurrence of methyl ketones are reviewed. The lack of significant accumulations of these compounds in the biosphere indicates that a recycling of these organic molecules is occurring. Evidence for biodegradation of acetone by mammals and longer methyl ketones by microorganisms via terminal methyl-group oxidation is discussed. A new mechanism for the subterminal oxidation of methyl ketones by microorganisms is proposed whereby the first intermediate produced is an acetate ester which subsequently is cleaved to acetate and a primary alcohol two carbons shorter than the original ketone substrate.

Methyl ketones can be produced by mammals and fungi by decarboxylation of β-keto acids. Some bacteria are able to form methyl ketones via the oxidation of aliphatic hydrocarbons at the methylene carbon α to the methyl group. Speculations on the biosynthesis of methyl ketones by insects and plants and a discussion of the possible biological roles of methyl ketones in diverse biological systems are presented.

SUPPLEMENTARY KEY WORDS ketones · acetone · plant oils · insect secretions · dairy products · abortive β-oxidation · Baeyer-Villiger oxidation · rancidity · pheromones · chemical communication

OCCURRENCE OF METHYL KETONES

Aliphatic methyl ketones are a class of organic chemical compounds whose cumulative biological history over many years has firmly established their natural origins. In 1858, Williams (1) began the documentation of methyl ketones in essential oils when he described undecan-2-one as the principal constituent of oil of rue from *Ruta graveolens*. Later, this ketone was found in the essential oil of lime leaves (2). Subsequently, many reports have confirmed a plant origin for methyl ketones. Nonan-2-one, the main constituent of Algerian oil of rue (3), occurs in smaller quantity in ordinary oil of rue from other sources (4, 5); it has been found in the essential oil of cloves from *Eugenia caryophyllata* (6). Heptan-2-one was detected in cinnamon oil (7) and in oil of cloves (6). Nonan-2-one, undecan-2-one, and tridecan-2-one were indentified in the essence of cocoanut oil (8, 9); tridecan-2-one was characterized as a crystalline constituent of the essential oil of matsubasa (*Shizandra nigra maxim*), a plant belonging to the magnolia family (10). Because of its aroma, matsubasa is used as a bath perfume. The essential oil from *Ruta montana* contains decan-2-one in addition to nonan-2-one and undecan-2-one (11). Undecan-2-one, undec-10-en-2-one, and the secondary alcohols, undecan-2-ol and undec-10-en-2-ol, were isolated from the ethereal oil of *Litsea odorifera* Val (12). Oil of rue from various sources contains three secondary alcohols, nonan-2-ol, decan-2-ol, and undecan-2-ol, in addition to C_9–C_{11} methyl ketones (13). Heptan-2-one and heptan-2-ol occur together in oil of cloves (14). These secondary alcohols are regarded as reduction products of their corresponding methyl ketones. In more recent studies undecan-2-one has been identified in cocoanut, palm kernel, palm, peanut, cottonseed, and sunflower seed oils (15), and in oil of hops (16). Acetone appears in the volatile products produced by certain apples (17). Acetone, nonan-2-one, and tridecan-2-one have been reported as additional constituents of hop oil (18).

Plants and their products are not the only natural sources of aliphatic methyl ketones. Cheeses, milk, butter, and other dairy products have been shown to

contain these compounds; these studies were carried out by chemists whose primary interest was to identify volatile flavors (19–24).

Methyl ketones also originate in insects, where they appear as components of odorous secretions. Cavill and Hinterberger (25) identified 4-methylhexan-2-one in the ant *Dolichoderus clarki*. 6-Methylhept-5-en-2-one is a component of the secretions in four ant genera, *Dolichoderus*, *Tapinoma*, *Iridomyrmex*, and *Lasius* (25–28). Heptan-2-one was identified in honeybees (29) and in the ants *Atta texana* (30), *Conomyrma pyramica*, and *Iridomyrmex pruinosus* (31, 32). Tridecan-2-one, pentadecan-2-one, and heptadecan-2-one have been shown to be present in the secretions of the ant *Acanthomyops claviger* (33), and the same three ketones are in *Lasius* (*Dendrolasius*) *fuliginosus* secretions (28). Tridecan-2-one is present in worker ants of *Lasius umbratus* and *Lasius bicornis* (34, 35), as well as in the slave species *Formica rufibarbis* (36). Butan-2-one and nonan-2-one were found among 20 constituents detected in the repugnant secretion taken directly from the scent storage sac of the green vegetable stink bug *Nezara viridula* (37).

Finally, mammals accumulate acetone, with acetoacetate and β-hydroxybutyrate, as "ketone or acetone bodies" in the blood under certain abnormal conditions associated with an excessive fat catabolism, such as from starvation or from experimental or actual diabetic disease. These compounds also occur when mammals are maintained on carbohydrate-free or very high fat diets.

BIODEGRADATION

Efficient biodegradation of products that are constantly being added to the biosphere by living and dead biological forms prevents accumulation of these products in quantities sufficient to eventually stifle all life. In this critically important process, the elements of which these products are composed are converted to accessible, reusable forms which are recycled in nature to sustain life. Microorganisms are the prime contractors for this task.

One is impressed by the apparent lack of extensive accumulation of aliphatic methyl ketones in the biosphere. Certainly this can be construed as evidence that these compounds undergo efficient biodegradation. Yet, because biochemical studies mainly have considered the anabolic rather than the catabolic aspect of their metabolism, utilization of methyl ketones in any biological system has received scant attention. Acetone is an exception.

Mammalian Systems

Information on the catabolism of acetone in mammalian systems has come mostly from tracer studies with intact rats and from in vitro experiments with rat tissue homogenates. It was concluded from in vivo studies that acetone is metabolized to acetate (38–41). A clearer picture of how this degradation might occur followed from the observation that methyl-labeled acetone gave rise to labeling in glycogen, in the β-carbon of serine, and in the labile methyl groups of choline and methionine (42). The hypothesis was that acetone is oxidized into separate two-carbon "acetate" and one-carbon "formate" moieties. No activity was introduced into liver serine from acetate-2-^{14}C, thus precluding the formation of "formate" from "acetate." Subsequent synthesis of serine, choline, and methionine must occur with "formate," or a one-carbon intermediate of its metabolism, serving as methylating agent. Evidence for another mechanism of acetone metabolism was offered by Sakami and Lafaye (43). From analysis of labeling patterns in liver glycogen of rats fed acetone-2-^{14}C, they postulated a direct conversion of acetone into a three-carbon intermediate of glycolysis. These mechanisms were substantiated by Rudney (44), who demonstrated that acetone and propanediol undergo analogous reactions in animal and plant tissues in that both compounds can be split into C_1 and C_2 units or can be converted to a C_3 intermediate of the glycolytic cycle. To support the latter reaction, labeled acetone was shown to be converted to propanediol, 1,2-propanediol-1-phosphate, and lactic acid. The actual sequence of reactions was not established by the data.

Still another mechanism for acetone metabolism was offered by in vitro labeling experiments which suggested a slow carboxylation of acetone to acetoacetate by rat liver homogenates (45). ^{14}C-labeled acetone predominantly labeled the acetone portion of acetoacetic acid, whereas the carbons of formate, carbonate, and CO_2 were incorporated into the carboxyl portion. Despite the relatively slow metabolism of acetone in tissues (39, 42), data support the thesis that carboxylation to acetoacetate plays a small role in acetone metabolism in vivo (42–44).

It is now generally believed that acetone is metabolized by mammals by two general pathways; one oxidizes it to acetate and formate, and the other converts it to lactic and pyruvic acids:

1. $CH_3COCH_3 \rightarrow CH_3COOH + HCOOH$

2. $CH_3COCH_3 \rightarrow CH_3CHOHCH_2OH \rightarrow CH_3CHOHCOOH \rightarrow CH_3COCOOH$

There is little evidence to indicate the relative importance of each of these two pathways. Although the results from liver glycogen studies suggest that both pathways are important in acetone catabolism (42–44), nothing is

known about the enzymic mechanisms involved. Recent studies suggest a slight predominance of the acetate route in the rat, whose capacity, however, to oxidize acetone is quite limited (46).

Microbial Systems

Information on the catabolism of acetone in microbial systems is meager, but it seems to be in accord with the results obtained in mammalian systems. *Bacillus pyocyaneus* grown on acetone as the sole carbon source produced acetic and formic acids as end products (47). More direct evidence for a C_1–C_2 split of acetone was obtained by Goepfert (48). During a series of studies on dehydrogenations carried out by *Fusarium lini* Bolley, Goepfert isolated formaldehyde from cultures grown on acetone, and acetol (1-hydroxypropan-2-one) and formaldehyde from cultures grown on propylene glycol. On the basis of isotopic dilution experiments, Levine and Krampitz (49) concluded that acetone was oxidized to acetol, thence to acetaldehyde and a C_1 product, by a soil diphtheroid. Simultaneous adaptation experiments with whole cells also indicated that propylene glycol was neither a precursor of acetone nor an intermediate in acetone oxidation, but was oxidized by a similar pathway involving acetol and acetaldehyde as intermediates.

Evidence has also been furnished for the primary carboxylation of acetone to acetoacetic acid. The photosynthetic bacterium *Rhodopseudomonas gelatinosa* was found to transform acetone aerobically in the light (50) and in the dark (51) to acetoacetate; the reaction was reversible in the dark under anaerobic conditions (52). These data, obtained with $^{14}CO_2$, indicate that the acetoacetate pathway is important for the photometabolism of acetone.

An indirect approach was employed in the foregoing studies and no C_3 products of acetone oxidation were isolated from microbial systems. However, Lukins (53) isolated and identified an acetol intermediate formed by *Mycobacterium smegmatis* strain 422 grown on acetone. Lukins and Foster (54) showed in studies with $^{18}O_2$ that molecular oxygen was incorporated into acetone during its conversion to acetol. The results were equivocal. Cells grown on acetone, acetol, or propan-2-ol in the presence of $^{18}O_2$ were enriched in ^{18}O, whereas acetol produced from acetone under the same conditions was not. Chemical exchange could not account for the essentially negative outcome of the experiments. These indirect results permit the conclusion that molecular oxygen is required for utilization of acetone, propan-2-ol (which presumably can be dehydrogenated to acetone), and acetol, but its incorporation occurs at a stage subsequent to acetol formation from either of the other two substrates. The stage at which this incorporation occurs is not established. Direct proof is lacking that molecular oxygen is involved in the primary attack on acetone or any other simple methyl ketone. Enzymatic studies of this oxidation reaction have not been reported.

All of the information pertaining to the catabolism of methyl ketones longer than acetone has come from studies of microbial systems. Hopkins and Chibnall (55) established that these compounds are assimilable substrates for microorganisms. *Aspergillus versicolor*, which grew on long-chain paraffins, also grew on related methyl ketones but not on the corresponding alcohols. Later assimilation studies (53, 54, 56) revealed that the mycobacteria have a conspicuous affinity for growing on a variety of methyl ketones. Further, respirometer experiments showed that nonproliferating cells of *M. smegmatis* strain 422 could oxidize methyl ketones that would not support growth of this organism. Resting cell suspensions of a gram-negative, rod-shaped bacterium, isolated by enrichment culturing on butan-2-one, accumulated periodate-reacting material in the culture filtrates. By analogy with acetol formation from acetone, the material was inferred to be 1-hydroxy-butan-2-one (54). This material was not isolated and identified nor was it shown to be a single four-carbon compound closely related to butan-2-one. Nonproliferating cells of *Brevibacterium* strain JOB5 also oxidized methyl ketones which would not serve as growth substrates (57). The effect of pentadecan-2-one as growth substrate on the fatty acid composition of a *Mycobacterium* and an *Achromobacter* species was determined (58). There was a marked increase in C_{13} and C_{14} fatty acids in cells of *Mycobacterium* not seen when the organism was grown on acetate. The fatty acid pattern of *Achromobacter* did not differ significantly from that obtained when the organism was grown on glucose or acetate. Species of yeasts were surveyed for their ability to utilize various ketones as growth substrates, and assimilation patterns were sought that might be helpful in the classification of these organisms (59). Several species of the genus *Candida* were able to utilize some of the methyl ketones tested. It was concluded that hexan-2-one and heptan-2-one were most readily utilized, but that shifting the position of the carbonyl groups, methyl substitution, cyclization, unsaturation, and the introduction of a second carbonyl group all rendered these methyl ketones unsuitable as growth substrates.

One obvious transformation of methyl ketones by microbial systems has been studied, their reduction to secondary alcohols. Methyl ketones can be formed in fungi (48) and in bacteria (60) by dehydrogenation of secondary alcohols. Presumably these reactions are reversible (8, 61–63). From metabolic studies with *Penicillium roqueforti* it was concluded that the secondary alcohols present in blue cheese are formed by the reduction of the corresponding methyl ketones (64).

Isopropanol is formed by *Lactobacillus brevis* var. *hofuensis* from acetone mediated by isopropanol dehydrogenase, an enzyme which differs from alcohol dehydrogenase (65). During a study on the production of undecan-2-one from lauric acid by spores of *Aspergillus niger*, it was found that undecan-2-ol was formed by reduction of the ketone under anaerobic conditions (63).

The first report of the isolation and characterization of an oxidative intermediate from the metabolism of any methyl ketone other than acetone was provided by Forney, Markovetz, and Kallio (66). A bacterium isolated by enrichment culturing on tridecan-2-one and identified as *Pseudomonas multivorans* oxidized the ketone with the accumulation of a primary alcohol, undecan-1-ol. This finding indicated that a mechanism other than methyl group oxidation was occurring, whereby the C_{13} ketone was split to the C_{11} alcohol plus a C_2 fragment. Subsequently, another oxidation product, identified as an acetate ester, undecyl acetate, was isolated and the following scheme was proposed for the oxidation of tridecan-2-one (67).

$$CH_3(CH_2)_9CH_2COCH_3 \rightarrow CH_3(CH_2)_9CH_2OCOCH_3 \rightarrow CH_3(CH_2)_9CH_2OH + CH_3COOH$$

In the utilization of tridecan-2-one it was determined that undecan-1-ol accumulates while acetate is utilized. It is only after substrate depletion that undecan-1-ol is oxidized. Evidence was also obtained that cell-free extracts from cells grown on the ketone carry out the reactions listed above (68) when supplemented with a reduced pyridine nucleotide in the presence of O_2. Enzymatic conversion of the ketone to the acetate ester may occur by insertion of oxygen into the carbon chain between carbons 2 and 3 of the ketone by a mechanism analogous to that of the chemical Baeyer-Villiger oxidation of carbonyl compounds by peracids (69, 70).

BIOSYNTHESIS

Although the foregoing enumeration of the natural occurrence of aliphatic methyl ketones is incomplete, it is apparent that these are ubiquitous compounds which have diverse biological origins and that this fact has been appreciated for many years. It is equally apparent that these natural products predominantly possess an odd-numbered carbon chain. Why this is so was first suggested by Dakin (71, 72). Acetone was thought to be formed from butyric acid in the animal body by a β-oxidation reaction that Dakin found he could artificially imitate with H_2O_2:

$$CH_3CH_2CH_2COOH \xrightarrow[\text{Heat}]{H_2O_2} CH_3COCH_2COOH \rightarrow CH_3COCH_3 + CO_2.$$

In this way laurate, caprate, and caprylate could be converted to undecan-2-one, nonan-2-one, and heptan-2-one, respectively. Because the principal naturally occurring methyl ketones have an odd number of carbon atoms, and because higher fatty acids with similar odd numbers of carbon atoms are rarely found in nature, Dakin reasoned that the natural methyl ketones might be synthesized from natural, even-numbered fatty acids in the organism by oxidation of these acids to unstable β-ketonic acids which readily lose CO_2. As a consequence, methyl ketones would have one carbon atom less than their progenitor acids. That fatty acids with even-numbered carbon chains actually were being found in natural oils which contained methyl ketones supported his hypothesis.

Since Dakin's time, the oxidative degradation of fatty acids has been well established as a biochemical capacity among living organisms. We now think that most naturally occurring methyl ketones are synthesized in the diverse biological systems in which they occur by decarboxylation of β-keto acids formed during the process of β-oxidation of fatty acids or by oxidation of aliphatic hydrocarbons. Evidence which supports these generalized biological concepts for the biosynthesis of natural methyl ketones will be reviewed.

1. Acetone

Mammals

Mammals produce acetone during fat catabolism by decarboxylation of acetoacetic acid, a product arising from β-oxidation of fatty acids or more indirectly from degradation of the ketogenic amino acids, leucine, isoleucine, lysine, phenylalanine, and tyrosine. Acetone is the only methyl ketone that has been detected in animal tissues.

Bacteria

Acetone is produced by the butyric acid bacteria as a product of butyl alcohol fermentation and, here again, the ketone is formed by decarboxylation of acetoacetate (73). Lactic acid streptococci produce acetone in skimmed milk (74), and this ketone has been isolated from cultures of *Pseudomonas methanica* oxidizing a gas mixture of propane–methane (75, 76). The formation of acetone in propane growth cultures of a number of different bacteria has been observed; this indicates that acetone synthesis from propane is not unique to *P. methanica* but is probably of widespread occurrence in organisms capable of utilizing this substrate for growth (75). A *Brevibacterium* species was found to oxidize propane-2-^{14}C to acetone, and isopropanol was proposed as the intermediate in this transformation (77). A novel mechanism for acetone biosynthesis by bacteria occurs

during the metabolism of 2-methyl-alanine by a soil pseudomonad whereby a decarboxylation-dependent transamination leads to the formation of acetone and CO_2 (78).

2. Higher Methyl Ketones

Fungi

In the biosynthesis of higher methyl ketones, the fungi will be considered first since the propensity of this group of organisms to form methyl ketones by oxidation of free fatty acids has been known for many years and the details of the process have received some attention. Stärkle (79) first implicated the fungi as causative agents of the rancidity of butter fat and incriminated methyl ketones as the components responsible for the odious smell of this decaying natural product. *Penicillium glaucum* was isolated from rancid fat, and it and several species of *Aspergillus* were shown to produce pentan-2-one and undecan-2-one from caproic and lauric acids. These and other methyl ketones could be produced when the organisms were grown in pure culture on individual fatty acids. Since then, the conversion of individual fatty acids to their corresponding methyl ketones by pure cultures of a variety of fungi has been confirmed by others, and production of each of the members of the homologous series of methyl ketones from acetone (derived from butyric acid) up to tridecan-2-one (derived from myristic acid) has been shown. In each case, the methyl ketone formed had one carbon atom less than the progenitor fatty acid and, in some cases, the expected ketones were obtained when pure triglycerides were provided as substrates (62, 80–84). It has also been reported that fungal spores, but not the hyphal cells, of *P. roqueforti* and other fungi convert octanoic acid to heptan-2-one (85–87), and this conversion has been extended to other fatty acids (88), but the contention that mycelia are inert in this process has been challenged (89).

The mechanisms by which fungi synthesize methyl ketones from fatty acids have been elucidated as an abortive β-oxidation sequence (84, 90–95):

$$\text{fatty acid} \rightarrow \alpha,\beta\text{-unsaturated acid} \rightarrow \beta\text{-hydroxy acid} \rightarrow \beta\text{-keto acid} \rightarrow \text{methyl ketone} + CO_2.$$

It is reasonable to assume that fungi can completely oxidize fatty acids to CO_2 and H_2O by a conventional process of β-oxidation even though all of the appropriate enzymes have not been detected. This assumption is supported by the fact that many fungi degrade fatty acids without any trace of ketone formation and, even when ketone is formed, the yields are not quantitative (86, 96, 97). In fact, the intermediate products expected of normal β-oxidation of various fatty acids were isolated from *Cunninghamella echinulata* Thaxter, a fungus which does not produce methyl ketones under any environmental conditions so far tested (98).

Plants

Many plants produce volatile essential oils in which higher methyl ketones often occur mixed with other organic constituents. Essential oils are secondary plant products that are synthesized and secreted by special plant organs, mostly by glandular hairs; they have definite aromas which give plants their odor. Plants also synthesize fats and surface lipids as primary products of their metabolism. The chemical constitutions of many plant products have been well documented (99–103).

Several generalities concerning plant products deserve some mention. Plant storage fats are comparable in structure and composition to those found in other higher organisms: they are triglycerides with even-numbered fatty acids. The surface lipids of plants usually contain hydrocarbons, waxes, primary alcohols, and fatty acids. The hydrocarbons generally are normal paraffins with odd-numbered chains of 21–35 carbon atoms and, in most cases, with one paraffin predominating. However, even-numbered *n*-paraffins, C_{24} to C_{36}, have been detected in wax (104), and other exceptions undoubtedly are known. Ketones with the same number of carbon atoms as the hydrocarbons occur, but these are internal, rather than methyl, ketones. Wax esters are usually made up of fatty acids and alcohols, each with an even number of carbon atoms, combined into chains ranging from C_{20} to C_{60}. Free alcohols and acids in the cuticle lipids also usually have an even number of carbon atoms and a chain length from C_{10} to C_{30}. Plants have been shown to possess a conventional β-oxidation pathway, the enzymes of which are localized in fragile organelles, called cytosomes (105) or glyoxysomes (106). Stepwise degradation of fatty acids also occurs by an α-oxidation pathway in germinating seed cotyledons (107) and pea leaves (108). Although a wealth of available substrates from which methyl ketones could originate are produced in plants, the reactions by which methyl ketones are synthesized have not been reported. These compounds could be synthesized either from fatty acids by a type of absorptive β-oxidation analogous to that known to occur in fungal cells, or by the direct oxidation of hydrocarbons, which will be discussed later.

Insects

Many insects secrete various scents in which higher methyl ketones often occur in combination with other organic constituents. These scents are synthesized in special ectodermal glands and have important specific

biological functions. Here again, the reactions leading to the synthesis of methyl ketones have not been reported. To link the formation of these compounds to fatty acid catabolism is more tenuous than with plants because a gap exists in our knowledge of fatty acid oxidation by insects. However, it is known that the metabolism of fatty substances is a very active process in insects, probably more so than in vertebrates. This aspect of insect metabolism, including the chemical composition of the various lipids, has been the subject of several reviews (109–111). Fat, glycogen, and protein are synthesized and stored chiefly in the "fat body," an organ in the body cavity of insects which may be regarded as the insect "liver." The reserve fat is usually in the form of triglycerides containing even-numbered fatty acids from C_4 to C_{20}. Insects also synthesize waxes, which actually are mixtures consisting of long-chain paraffins, acids, alcohols, and esters of the latter two classes. All insects secrete cuticular waxes or surface lipids that have a composition and a function similar to those of the waxy cuticle of plants. Some insects, mainly bees and scale insects, produce relatively large quantities of extracuticular waxes that are similar in chemical composition to their own cuticular waxes and to those of plants. The acids and alcohols contain all the even-numbered homologues from C_{24} to C_{34} and the paraffins contain the odd-numbered homologues from C_{25} to C_{31}. This rule is not invariable; gas–liquid chromatographic analyses reveal that odd-numbered acids and alcohols and even-numbered paraffins are present in beeswax, but only in small amounts (112). Extracuticular hydrocarbons also occur in the odorous glandular secretions where they are present as a shorter homologous series of odd-numbered, normal paraffins ranging from C_9 to C_{19}.

Although the complete β-oxidation scheme has not been worked out in detail in any one insect, it would be surprising not to find it. It is then proper to speculate that insects may synthesize higher methyl ketones by abortive β-oxidation of fatty acids. However, this explanation should not overshadow the possibility that these compounds are synthesized in the scent glands by other mechanisms, perhaps by oxidation of hydrocarbons, or even by symbiotic microorganisms inhabiting the glands.

Microorganisms

Microbial utilization of normal alkanes has been shown to occur primarily by oxidation at the terminal methyl group leading to the formation of the homologous alcohol, aldehyde, and fatty acid (56, 113, 114). However, alkane utilization has also been reported to yield methyl ketones. *Pseudomonas methanica*, an obligate methane bacterium, while growing in methane and in the presence of either propane or butane produced acetone and butan-2-one, respectively, by cooxidation of these gaseous hydrocarbons (75). The process is unique because the ketones are formed with no change in the carbon skeleton of their precursors. Butan-2-one, pentan-2-one, and hexan-2-one were produced from the respective alkanes by *Mycobacterium smegmatis*, and undecan-2-one was shown to have arisen from *n*-undecane in cultures of *M. rhodochrous* (54). Fredricks (115) did not detect methyl ketone formation from *n*-decane by *M. rhodochrous*. However, comparing this organism with *Pseudomonas aeruginosa* grown on *n*-decane under indentical conditions, it was found that 2-, 3-, 4- and/or decan-5-one, as well as the corresponding secondary alcohols, were produced. Decan-2-one was produced in highest concentration, indicating that, in a rather nonspecific attack, the methylene group α to the methyl group is oxidized preferentially to form the methyl ketone. An *Arthrobacter* species can transform *n*-decane, *n*-dodecane, *n*-tetradecane, and *n*-hexadecane into a series of ketonic products identified as the respective 2-, 3-, and 4-ketones produced in decreasing concentration from each substrate (116). The homologous alcohols were also produced. As the organism was incapable of growth on the hydrocarbons, the transformations occurred only in the presence of exogenous carbon and energy sources which provided for growth. Again, ketones seem to be synthesized nonspecifically, but in all cases methyl ketones were formed preferentially. Recently, it was demonstrated that *Pseudomonas aeruginosa* oxidized *n*-tridecane via a subterminal pathway involving tridecan-2-ol and tridecan-2-one (117). The ketone is then oxidized via the Baeyer-Villiger mechanism described above in the section Biodegradation.

Fungi produce methyl ketones by both abortive β-oxidation of fatty acids and oxidation of hydrocarbons. Various secondary alcohols and ketones were identified from cultures of a *Penicillium* species grown on *n*-tetradecane and tetradec-1-ene (118). Several other reports contain indirect evidence that microorganisms might be capable of transforming alk-1-enes as well as *n*-alkanes into methyl ketones. One study indicated that *P. aeruginosa* produces tetradecan-2-ol from tetradec-1-ene (119). *Candida lipolytica* produces secondary alcohols (2-ols) from long-chain *n*-alkanes, and also ω-unsaturated secondary alcohol(2-ol), respectively, from hexadec-1-ene and heptadec-1-ene (120, 121). Methyl ketones could result in these systems as oxidation products of their corresponding secondary alcohols, but none were reported. Biological interconversion of methyl ketones and secondary alcohols is known to occur readily in fungi (48, 63, 64) and in bacteria (60, 65). NAD-linked alcohol dehydrogenases, active on a variety of linear primary and

secondary alcohols, occur constitutively or can be induced readily in pseudomonads (122). It is notable that homologous secondary alcohols were present with their respective methyl ketones in the bacterial systems mentioned above (115, 116), and that this same association of compounds occurs in plant and insect products (12–14, 28, 36).

The normal transformation of *n*-alkanes into higher methyl ketones by bacteria must involve some type of subterminal or methylene-carbon oxidation. A mechanism has been proposed, but not established, for these transformations (76); this involves free radical formation at the 2-position, followed by addition of molecular oxygen to form a 2-hydroperoxide, followed by reduction to a secondary alcohol and then oxidation to a methyl ketone.

The biosynthesis of long-chain methyl ketones by bacteria during β-oxidation of fatty acids has not been reported to occur. In bacterial as in animal cells, complete oxidation of fatty acids proceeds, apparently without occurrence of the abnormal decarboxylation reaction which produces methyl ketones in fungal cells. This is understandable because it is now clear that all of the intermediates formed in the course of β-oxidation occur only as acyl SCoA derivatives, which eliminates the opportunity for decarboxylation of (free) β-keto acids into methyl ketones. Cells that do not produce higher methyl ketones may be incapable of deacylating β-ketoacyl CoA esters to form (free) fatty acids that could then be decarboxylated. But this does not rule out the possibility that methyl ketones might occur as transient intermediates during the biosynthesis of other metabolic products.

Under abnormal conditions bacteria can synthesize methyl ketones from fatty acids. Thijsse (123) found that acrylate inhibition of β-oxidation in an alkane-oxidizing *Pseudomonas* resulted in the accumulation of both free fatty acids and methyl ketones. The presence of acrylic acid during oxidation of hexanol, hexanoic acid, and hexane by the organism caused accumulation of pentan-2-one, whereas from heptane, hexan-2-one, and from octane, heptan-2-one and pentan-2-one accumulated. These methyl ketones undoubtedly were synthesized by decarboxylation of β-keto acids, as occurs in fungal systems, because they differed in carbon number from their precursor substrates. Support for this view comes from the demonstration that acrylyl CoA is an inhibitor of fatty acid synthesis in rat tissues (124). Evidently, acrylate competition for CoA deprives the cell of this coenzyme and β-oxidation can no longer proceed at a normal rate. In order to increase its supply of CoA during this period, the cell must deacylate β-ketoacyl SCoA esters; decarboxylations ensue, and methyl ketones are formed (89).

BIOLOGICAL ROLES

An adequate explanation of the biological roles that aliphatic methyl ketones play in their close association with diverse biological forms is difficult to formulate because information is lacking in most systems. Limited support can be claimed for some biological uses of these compounds, however, and others can be postulated on a purely theoretical basis.

The abundance of methyl ketones in soil and some food products may merely represent an accumulation of these compounds in metabolically inert pools, and these compounds must be regarded either as dead-end products of metabolism or as artifacts. Methyl ketones may appear in soils as aberrant degradation products produced by soil bacteria and fungi from fatty acids or paraffinic hydrocarbons present there. The occurrence of methyl ketones in certain cheeses can be explained on the basis of mold action on milk fats during the curing period. In spoiling plant and vegetable products, they appear as a result of the rancidification process, either abiologically by purely chemical reactions associated with the action of light, air, and water, or biologically by microbial action on the fatty materials present. Their occurrence in milk and other unspoiled dairy products could be the result of microbial action on milk fats, but it is more likely that they appear as artifacts during analysis of these products by virtue of their liberation from triglyceride precursors during saponification or heating in the presence of moisture (23, 125–128). Various ecological factors in soil and food would contribute to what appears to be an accumulation of ketones. The observation that they have not accumulated to the point of stifling life underscores one of their biological roles as primary sources of carbon and energy in the food chain of various microorganisms.

A more active natural role for higher methyl ketones stems from their presence in the volatile secretions of insects and in the ethereal oils of plants and flowers.

Insects

More is known about the chemistry and physiology of the defensive secretions of the arthropods, which include insects, crustaceans, arachnids, and myriapods, than about any other type. Several reviews dealing with defensive substances of arthropods have been published (129–131). The offensive odors of arthropods are attributed to secretions produced in various exocrine glands which are located on or in various parts of the arthropod's body (132). These secretions serve a defensive function by endowing the arthropod with an odor or taste that is repugnant to predators such as birds and small mammals, causing them acute discomfort, but rarely death. The components of these

defense secretions are complex mixtures of terpenes, quinones, and miscellaneous aliphatic and aromatic compounds.

Normal methyl ketones have been found only rarely in secretions used strictly for defense. A branched-chain methyl ketone, 4-methylhexan-2-one, occurs in the secretion of the ant *Dolichoderus clarki* (25), and 6-methylhept-5-en-2-one, a branched-chain, unsaturated methyl ketone, has been found in the secretions of six species of ants (25–27). The secretions are presumed to have a defensive function in these ants. Another example is the scent of the stink bug, *Nezara viridula*, which contains butan-2-one and nonan-2-one in its offensive odor (37). A final example may be the secretion which accumulates in the mandibular glands of foraging worker honeybees; this has recently been shown to contain heptan-2-one (29). However, although this secretion repels robber or other worker bees, it also may deter nurse bees from feeding larvae that have just been fed (133); thus, it cannot be said to function solely in defense. In fact, functions other than defense are being recognized for many of the glandular products of arthropods, especially the social insects such as termites, ants, and bees, whose instinctive behavior is released by stimulation of their chemical senses of smell and taste. Particularly important is the group of secreted substances called pheromones, which can range in complexity from single compounds to multicomponent systems, some with combined functions and some with different functions under different conditions. Pheromones are usually composed of mixtures of alkyl aldehydes, ketones, alcohols, acids, esters, and short-chain *n*-paraffins chemically related to the aliphatic compounds present in the insects' cuticular waxes. Most pheromones have not been completely characterized either chemically or physiologically. Regnier and Law (134) have published an excellent review on insect pheromones.

Methyl ketones have been identified most frequently in the alerting pheromones of ants, but this may only reflect the active scientific scrutiny this group of insects has received. Illustrative examples of these substances that contain methyl ketones follow; the list is not intended to be exhaustive.

Heptan-2-one was demonstrated as a volatile component of the mandibular glands of the ant *Atta texana* (30). In the field, low concentrations attract and alarm; high concentrations repel and alarm. This same ketone has been found in the anal glands of the ants *Conomyrma pyramica* and *Iridomyrmex pruinosus*,where it functions as an alerting and recruiting pheromone in low concentrations; it also probably functions for defense, since it causes alarm at high concentrations (31, 32). Regnier and Wilson (33) identified tridecan-2-one, pentadecan-2-one, and possibly traces of heptadecan-2-one in the Dufour's gland located in the anal region of the ant *Acanthomyops claviger*. Tridecan-2-one was the principal ketone constituent of this gland; a single gland contained 1.88 μg. The mandibular glands did not contain any ketones; instead, terpene-related compounds were present. A strong alarm function was measured for tridecan-2-one and it had great olfactory efficiency for short distances. Because of its low vapor pressure, tridecan-2-one cannot signal over long distances, but it might serve as a residual signal at very close range after most of the other volatile substances have dispersed. Various genera of ants were tested by artificial trail tests, and it was determined that the trail substances originate in the hind gut (135). It is possible that the methyl ketones found in *A. claviger*, particularly tridecan-2-one, also might be trail-marking pheromones. This function was not tested. It would seem that the higher methyl ketones are well suited for such a function because they possess very distinctive odors yet their vapor pressures are low enough to allow them to persist for appreciable periods of time before they evaporate.

Extracts from whole worker ants (*Lasius umbratus* and *L. bicornis*) were analyzed and found to contain tridecan-2-one, but no attempt was made to locate anatomically the substances found or to determine their functions. Tridecan-2-one, pentadecan-2-one, heptadecan-2-one, and 6-methylhept-5-en-2-one were identified in the ether extracts of whole worker ants of *L. fuliginosus* (28). When extracts of "abdomen-only" preparations of the ants were examined, only 6-methylhept-5-en-2-one was absent. Presumably, it resides only in the mandibular glands; the other three methyl ketones occur predominantly in the hind gut. The biological functions of the scents were not determined. By the artificial trail test it was proved directly that a trail substance exists in *L. fuliginosus* (136). Possibly, all of the saturated methyl ketones present in the hind gut of these ants may have a dual function as trail-marking and alerting pheromones.

As a final example, a study of the odor similarities and interactions of the slave-keeping ant *Formica sanguinea* and its slave workers, *F. fusca* and *F. rufibarbis*, was conducted (36). Scent secretions collected from Dufour's glands of these ants were analyzed by gas–liquid chromatography–mass spectrometry. All three species had very similar scent spectra based on the similarity of constituents and their relative concentrations. The slave species *F. rufibarbis* had a scent spectrum more similar to *F. sanguinea* than did *F. fusca* and, in addition, *F. rufibarbis* was the only species whose secretion contained methyl ketones; tridecan-2-one was identified. The secretion containing tridecan-2-one in *F. rufibarbis* may also have a recognition function to which the ketone contributes specificity. It was found that in spite of the odor similarities between *F. sanguinea* and its slaves,

F. fusca and *F. rufibarbis*, the latter species is more rarely found as a slave, even in areas where both slave species are equally abundant.

The foregoing observations can be interpreted in the following manner. The anatomical location of particular methyl ketones in ants may have some correlation with the biological functions of the secretions in which they occur. Cyclopentanoid, branched, and unsaturated methyl ketones that resemble monoterpenes predominate in secretions of the mandibular glands that serve as defensive substances. The structures of these ketones suggest that they are synthesized during terpene biosynthesis (129). On the other hand, saturated *n*-methyl ketones predominate in secretions of glands located in the hind gut that serve as alerting or alarm substances. Possibly, the methyl ketones present could enable these secretions to serve also as trail-marking or recognition substances, and these methyl ketones may be synthesized during biosynthesis or degradation of fatty acids or hydrocarbons.

In addition to whatever primary roles methyl ketones may play by virtue of their presence in certain insect secretions, they also may serve as precursors of other components of these scents even though they do not persist as components themselves. The formicine ant *Formica rufibarbis* has been shown to emit a secretion containing tridecan-2-one, *n*-tridecane, and undecyl acetate; also *n*-dodecane, decyl acetate, and dodecyl acetate, whereas the secretion from *F. sanguinea* contains *n*-tridecane, undecyl acetate, and undecan-1-ol; decyl acetate and decan-1-ol; dodecyl acetate and dodecan-1-ol (36). The chain length and structures of these compounds suggest plausible precursor–product relationships between them. A common biosynthetic pathway may exist in some insects by which acetate esters and alcohols are synthesized from methyl ketone precursors. Possibly all of these compounds ultimately stem from *n*-alkanes in a single biochemical sequence visualized as:

n-alkanes → methyl ketones → acetate esters →
primary alcohols (shorter by two carbons than their precursor alkanes or methyl ketones) + acetate.

This sequence has been shown to occur in bacteria (68, 117), but there is strong evidence that it occurs also in fungi (118), and we anticipate its occurrence in other biological systems.

Plants

Essential oils comprise one group of a vast array of rather unique substances or "secondary" products that plants contain in addition to the primary products of which they are composed. Secondary plant products occur sporadically, yet one or another of them may be specific for a particular family, subfamily, genus, or even species or subspecies of plant. Ethereal oils that contain methyl ketones are examples of this. The family Rutaceae is composed of members which characteristically develop oil glands producing aromatic oils such as oil of rue and various citrus fruit oils. Other examples of essential oils containing methyl ketones were listed in the section, Occurrence of Natural Methyl Ketones. Essential oils impart odors to plants, which function as olfactory stimuli for insects. The mode of action and effects of these plant odors upon recipient insects are often very similar to those of olfactory pheromones produced by insects themselves. This is because these plant secretions contain many chemical constituents that are the same as those in the odorous secretions of insects. Some of these plant odors repel certain insects while attracting others, and some are used by insects for host-plant recognition.

As with the odorous secretions of insects, the constituents and general functions are better known than are the functions of individual constituents of essential oils. Most of the work on chemical attractants and repellents found in plants has concerned the preparation of plant extracts which are then empirically tested for their effectiveness. For example, a compound "oryzanone," attractive to the rice stem borer, has been isolated from rice plants and provisionally identified as an aliphatic, unsaturated ketone (137). Some essential oils containing methyl ketones have been tested for their ability to attract or repel insects. As examples, cinnamon oil attracts *Euglossa viridissima*; clove oil attracts this species of insect and also the Natal fruit fly (138). Essential oils from eucalyptus and cloves gave satisfactory results in repelling several species of mosquitoes (139). One can assume that methyl ketones do contribute to the olfactory stimuli evoked in insects by the essential oil in which they are present. When more information is available, individual methyl ketones in plant essential oils may eventually be found to evoke subtle specific stimuli in insects which resemble those evoked by the same specific compounds synthesized by insects themselves.

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