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Metabolism of [U-^{14}C]Lauric Acid to Methyl Ketones by the Spores of *Penicillium roqueforti*

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Cultures of *Penicillium roqueforti* spores oxidized [U-^{14}C]lauric acid into carbonyl compounds including a series of *n*-methyl ketones. Spore concentrations of 6.3 × 10^8 spores/ml in the presence of 2 m*M* of D-glucose under optimum conditions of pH and temperature, *i.e.*, pH 6.5 and 30°, oxidized 16–20% of [U-^{14}C]lauric acid (5 m*M*) to carbonyl compounds. D-Glucose stimulated the formation of carbonyls and suppressed the complete oxidation of the lauric acid. A homologous series of methyl ketones C3 to C11 inclusive was formed. Some unidentified carbonyl compounds other than methyl ketones were also produced. Metabolic CO_2 increased the conversion of [U-^{14}C]lauric acid into labeled methyl ketones by the *Penicillium* spores.

Many fungi including *Penicillium roqueforti* metabolize fatty acids of short and medium chain lengths into methyl ketones with one less carbon atom (Gehrig and Knight, 1963; Hammer and Bryant, 1937; Lawrence, 1966; Lawrence and Hawke, 1968; Starkle, 1924). Gehrig and Knight (1963) attributed this ability to the spores of *P. roqueforti*; however, Rolinson (1954), Vinze and Ghosh (1962), and Lawrence and Hawke (1968) demonstrated the formation of methyl ketones from fatty acids by the mycelium of *P. roqueforti*.

Fatty acids may be toxic to *P. roqueforti*, and the degree of toxicity is dependent upon chain length, concentration of acid, and pH of the incubation medium (Franke *et al.*, 1962; Lawrence and Hawke, 1968). It has been suggested that the oxidation of fatty acids into methyl ketones may be a detoxifying mechanism employed by the mold. This tendency of *P. roqueforti* to form methyl ketones from fatty acids is exploited in manufacture of specific cheeses, *i.e.*, Roquefort, Blue, and Stilton (Anderson and Day, 1966; Niki *et al.*, 1966; Patton, 1950).

Lawrence and Hawke (1968) reported that *P. roqueforti* oxidizes lauric and palmitic acid to only 2-undecanone and 2-pentadecanone, respectively. Dartey and Kinsella (1973) found that the spores of *P. roqueforti* generated the homologous series of radioactively labeled methyl ketones, C3 to C15 inclusive, from [U-^{14}C]palmitate.

To determine the relationships of various fatty acids to the formation of specific methyl ketones, the metabolism of individual fatty acids by spore suspensions of *P. roqueforti* is being studied. In the present study some factors affecting metabolism of lauric acid and its ability to serve as precursor of methyl ketones when incubated with *P. roqueforti* spores were studied.

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MATERIALS AND METHODS

Solvents and Chemicals. Practical grade *n*-hexane (Eastman Kodak Co., Rochester, N. Y.) and analytical grade chloroform (Mallinckrodt Chemical Works, St. Louis, Mo.) were purified as reported previously (Dartey and Kinsella, 1971). Nitromethane and benzene, both spectral grade (Eastman), polyethylene glycol 400 (Fisher Scientific Co., Rochester, N. Y.), analytical grade acetone (Mallinckrodt), [U-^{14}C]lauric acid (radiochemical purity 98%, Schwarz/Mann, Division of Becton Dickinson Co., Orangeburg, N. Y.), and 2,4-dinitrophenylhydrazine (DNPH) (Eastman) were used as purchased.

Celite (Johns-Manville, analytical Filter-aid) and Sea-Sorb 43 (Fisher Scientific Co.) were treated as reported previously (Dartey and Kinsella, 1971). Analytical grade magnesium oxide (Mallinckrodt) and Kieselguhr G (E. Merck, Darmstadt, Brinkmann Instruments, Inc., Westbury, N. Y.) were used as purchased.

A stock solution of sodium laurate (100 m*M*) was made in 0.1 *M* phosphate buffer, pH 6.5, for each experiment. Appropriate quantities of [U-^{14}C]laurate were added to each incubation flask to attain a known specific activity as indicated in the tables.

The spores of *P. roqueforti* were grown, harvested, and stored under conditions described (Dartey and Kinsella, 1973).

The effects of D-glucose, temperature, and pH on the metabolism of lauric acid were determined as outlined in

Table I. The Effect of D-Glucose on Evolution of $^{14}CO_2$ from the Metabolism of [U-^{14}C]Lauric Acid by the Spores of *P. roqueforti*

Substrate composition	Incubation period, hr: 2	4	6	10
	Radioactivity in $^{14}CO_2$, cpm			
Control at pH 6.5, 30°	9040	16,040	18,160	23,400
Control + 2.0 mmol of D-glucose at pH 6.5, 30°	580	830	1370	2050
Control + 2.0 mmol of D-glucose at pH 6.0, 30°	200	350	130	110
Control + 2.0 mmol of D-glucose at pH 6.5, 37°	260	470	330	300
Control + 2.0 mmol of D-glucose at pH 7.0, 30°	420	530	680	370

The control contained 100 μmol of [U-^{14}C]lauric acid (6.8×10^5 cpm) and 1.26×10^{10} spores of *P. roqueforti* in 20 ml of 0.1 *M* phosphate buffer.

the previous paper (Dartey and Kinsella, 1973). Known spore concentrations in 0.1 *M* phosphate buffer were aseptically placed in culture flasks (125 ml) fitted with a center well. Measured amounts of [U-^{14}C]laurate were added and, in the experiments where carbon dioxide was being removed, 1.0 ml of 20% KOH solution was placed in the center well. The details of each experiment and experimental conditions are included in the appropriate tables. Duplicate incubations were carried out in a constant temperature reciprocating water bath.

Experiments were terminated by the addition of DNPH reagent (2 g of DNPH/l. of 2 *N* HCl; Lawrence, 1965) to form DNPH derivatives of carbonyl compounds. The tightly closed flask was swirled gently at 10-min intervals for 1 hr. After 3 hr, duplicate samples (0.1 ml) of the KOH solutions containing radioactive CO_2 were transferred into scintillation vials and 4 ml of absolute alcohol was added. Ten milliliters of scintillation fluid was added and the mixture was shaken vigorously. The radioactivity in the $^{14}CO_2$ (KOH solution) was determined in Packard Tri-Carb liquid scintillation spectrometer (Model 314E). Quenching by the alcohol and the KOH solution was corrected in calculation of the results.

The contents of the incubation flask were transferred into a 250-ml separatory funnel and thrice extracted with a total volume of 120 ml of *n*-hexane. The concentration of the total carbonyl DNPH derivatives in the *n*-hexane was computed from the optical density measurements using the molar extinction coefficient $E = 22{,}500$ (Schwartz *et al.*, 1968).

Column chromatography (Schwartz *et al.*, 1968) was used to remove the neutral lipids from the total carbonyl DNPH derivative mixture. The chloroform–nitromethane eluent was evaporated and the residue containing the monocarbonyl DNPH derivatives was dissolved in 1.0 ml of $CHCl_3$ and 4 ml of *n*-hexane. Radioactivity was determined on aliquots of the sample. The concentration of the total monocarbonyl DNP fraction was determined from optical density (Schwartz *et al.*, 1968).

The methyl ketones were separated from the total carbonyl DNPH derivatives by thin-layer chromatography (Dartey and Kinsella, 1973). The methyl ketone band was eluted from the chromatographic material with chloroform, and the concentration of methyl ketones was determined by the method of Schwartz *et al.* (1968). Using aliquots, the total radioactivity in this fraction was determined in a liquid scintillation spectrometer.

The individual methyl ketones DNPH derivatives were separated by reverse-phase thin-layer chromatography (Dartey and Kinsella, 1973). The individual methyl ketones (C3 through C15) were each transferred to scintillation vials and their radioactivity was determined. From these data the total radioactivity in each compound was calculated.

RESULTS

The spores of *P. roqueforti* oxidized [U-^{14}C]laurate to carbon dioxide. D-Glucose suppressed the rate of catabolism of [U-^{14}C]lauric acid into $^{14}CO_2$ (Table I). Both pH and temperature influenced the rate of liberation of the $^{14}CO_2$ from the [^{14}C]lauric acid. The lowest rate of carbon dioxide evolution from lauric acid occurred at pH 6 and 30°.

Temperature and pH of incubation affected the production of carbonyl compounds from [U-^{14}C]lauric acid by the spores of *P. roqueforti* (Table II). In the present experiments maximum yield of carbonyl compounds was obtained at pH 6.5 and 30° and these were composed predominantly of monocarbonyls (methyl ketones). Variation of pH and/or temperature depressed monocarbonyl production by spores. This was particularly noticeable at 37°. The relative concentration and distribution of radioactivity generally paralleled the quantities of monocarbonyls

Table II. The Effects of pH and Temperature on the Rate of Metabolism of [U-^{14}C]Lauric Acid into Carbonyl Compounds by the Spores of *P. roqueforti*

Substrate composition	Temperature	Incubation period, hr	Concentration of total carbonyl DNPH derivatives, μmol	Monocarbonyl DNPH derivatives: Concentration, μmol	Radioactivity, cpm
Control pH 6.0	30°	2	0.13	0.03	2000
		4	0.29	0.10	3600
		6	2.22	0.08	3600
		10	2.25	0.10	5200
Control pH 6.5	30°	2	0.44	0.42	6380
		4	1.30	1.26	39,500
		6	3.48	3.34	54,130
		10	8.99	7.74	115,680
Control pH 6.5	37°	2	0.35	0.05	3800
		4	0.48	0.12	6800
		6	0.26	0.09	5800
		10	0.08	0.03	4200
Control pH 7.0	30°	2	0.23	0.20	3000
		4	0.42	0.30	5600
		6	2.70	0.38	8600
		10	2.70	0.87	10,600

The control contained 100 μmol of [U-^{14}C]lauric acid (6.8×10^5 cpm), 2.0 mmol of D-glucose, and 1.26×10^{10} spores of *P. roqueforti* in 20 ml of 0.1 *M* phosphate buffer, pH 6.5.

Table III. The Effects of Evolved Carbon Dioxide on the Formation of Carbonyls from [U-^{14}C]Lauric Acid by the Spores of *P. roqueforti*

Incubation period, hr	Concentration of total carbonyl DNP derivatives, μmol		Total monocarbonyl DNPH derivatives			
			Concentration, μmol		Radioactivity, cpm	
	+KOH	No KOH	+KOH	No KOH	+KOH	No KOH
2	1.23	0.54	1.12	0.36	8490	69,600
4	1.22	1.32	1.13	1.30	20,000	81,800
6	2.24	3.49	2.15	3.48	25,360	106,810
10	3.96	8.21	3.83	8.00	52,940	134,990

Each incubation mixture contained 100 μmol of [U-^{14}C]lauric acid (6.8×10^5 cpm), 2.0 mmol of D-glucose, and 1.26×10^{10} spores of *P. roqueforti* in 20 ml of 0.1 *M* phosphate buffer, pH 6.5, at 30°.

Table IV. The Effects of D-Glucose on the Metabolism of [U-^{14}C]Lauric Acid into Carbonyl Compounds by the Spores of *P. roqueforti*

Substrate composition	Incubation period, hr	Concentration of total carbonyl DNPH derivatives, μmol	Monocarbonyl DNPH derivatives		Methyl ketone DNPH derivatives	
			Concentration, μmol	Radioactivity, cpm	Concentration, μmol	Radioactivity, cpm
	2	1.23	1.12	8490	0.33	9590
Control	4	1.22	1.13	20,010	1.25	11,240
	6	2.24	2.15	25,360	2.24	20,540
	10	3.96	3.83	52,940	3.63	46,140
Control + 2.0	2	0.44	0.42	6380	0.40	7790
mmol of	4	1.30	1.26	39,500	1.06	16,940
D-glucose	6	3.48	3.34	54,130	3.20	21,910
	10	8.99	7.74	115,680	7.02	69,630

The control contained 100 μmol of [U-^{14}C]lauric acid (6.8×10^5 cpm) and 1.26×10^{10} spores of *P. roqueforti* in 20 ml of 0.1 *M* phosphate buffer, pH 6.5, at 30°.

Table V. Distribution of Radioactivity in Individual Methyl Ketone DNPH Derivatives[a] Separated by Reverse-Phase Thin-Layer Chromatography

Substrate composition	Incubation period, hr	Radioactivity, cpm				
		Methyl ketone DNPH derivatives (carbon chain length)				
		C3	C5	C7	C9	C11
	2	1110	1090	1630	1470	4290
Control	4	960	1430	1620	1500	5730
	6	940	1030	1380	1600	15,590
	10	1770	1630	1370	1400	39,970
	2	880	1330	1440	530	3610
Control + 2.0	4	780	1050	570	1480	13,060
mmol of	6	940	1180	160	600	19,030
D-glucose	10	1000	1240	720	1900	62,770

[a] From Table IV.

produced by the spores (Table II). Under all experimental conditions the radioactivity increased with time, but it was markedly influenced by pH and temperature. At pH 6.5 and 30°, approximately 16.5% of the [U-^{14}C]lauric acid was converted to labeled monocarbonyls.

Removal of metabolic CO_2 apparently inhibited the formation of carbonyl compounds (Table III). Generally no significant changes occurred between the relative concentration of total monocarbonyl compounds whether in the presence or absence of metabolic CO_2. The radioactivity in the carbonyl DNPH derivatives also increased with incubation time.

D-Glucose (100 m*M*) stimulated the rate of formation of carbonyl compounds from lauric acid by the spores of *P. roqueforti* (Table IV). The initial rate of formation of carbonyl compounds in the presence of D-glucose was slower than the control; however, the rate was almost double that of the control after 10 hr of incubation. Methyl ketones accounted for most of the carbonyls synthesized from the lauric acid by the spores of *P. roqueforti* in both the control and the D-glucose-enriched media.

The distribution of radioactivity in the individual methyl ketones following the incubation of [U-^{14}C]lauric acid with the spores of *P. roqueforti* (Table V) showed that the homologous series of methyl ketones, C3 to C11, was generated. The preponderance of the radioactivity was in the 2-undecanone and this was the only ketone whose radioactivity increased with time. The distribution of radioactivity in the other ketones was irregular and showed no increase with duration of incubation.

DISCUSSION

The stimulatory effects of glucose on the production of methyl ketones from lauric acid are consistent with earlier findings. Lawrence (1965) reported the enhancement of 2-heptanone formation from octanoic acid and we found that glucose stimulated the generation of carbonyls from palmitic acid (Dartey and Kinsella, 1973). The concomitant reduction of radioactive CO_2 production from the [U-^{14}C]lauric acid would indicate that, in the presence of available glucose, the spores of *P. roqueforti* do not de-

grade lauric acid completely to CO_2 and apparently utilize glucose as an energy source. However, in the absence of glucose, the lauric acid was oxidized by the spores. Earlier Gehrig and Knight (1958) showed that the spores of *P. roqueforti* oxidize fatty acids *via* the classical β oxidation pathway.

The generation of carbonyls from lauric acid by the spores was very sensitive to pH and temperature of incubation, as reported by Lawrence (1966) and Dartey and Kinsella (1973). Gehrig and Knight (1963) found that ketone production from octanoic acid was suppressed at 37°. The stimulation of ketone production from laurate by metabolic CO_2 contrasted with its effects on palmitate, where it depressed carbonyl production (Dartey and Kinsella, 1973). Lawrence (1966) stated that CO_2 accentuated ketone formation and this would be consistent with the conditions prevailing in cheesemaking, where carbon dioxide concentration builds up during maturation.

Methyl ketones were the principal products of lauric acid oxidation by the spores incubated at 30°, pH 6.5. While 2-undecanone was the predominant ketone formed, shorter chain ketones were also produced from lauric acid. Lawrence and Hawke (1968) reported that only heptanone was formed from octanoic acid. The use of uniformly labeled radioactive fatty acids has enabled us to demonstrate that several methyl ketones can be formed *via* β oxidation of lauric and myristic acid (Dartey and Kinsella, 1973). Thus, the long-chain fatty acids of milk fat may be a significant source of the methyl ketones occurring in mold ripened cheese, as reported by Anderson and Day (1966), Dartey and Kinsella (1971), and Patton (1950).

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Volatile Retention during Freeze Drying of Aqueous Suspensions of Cellulose and Starch

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This paper studies the retention of ^{14}C-labeled 2-propanol in freeze-dried starch or cellulose suspensions. Among the variables affecting the retention level are concentration of solids and initial concentration of the alcohol in the suspension. The observed retentions can be explained by inclusion within the polymer chains, the predominant mechanism of retention, and adsorption. Cellulose gave a much lower retention than starch, probably because the low mobility of the chains in the highly crystalline cellulose reduces the capacity for retention of the alcohol through inclusion.

In the past few years, significant progress has been made in studies on the mechanism of volatile retention in freeze-dried foods. Most of these studies have been based on model systems, mainly carbohydrate solutions (Flink and Karel, 1970a,b; King, 1970; Rulkens and Thijssen, 1972; Thijssen and Rulkens, 1968) and water-soluble polymers (Chirife and Karel, 1973b; Chirife *et al.*, 1973). It is to be expected that studies on model systems, based on individual food components, could eventually lead to a better understanding of volatile retention in more complex food systems.

In this study we present results which characterize the retention of 2-propanol in model systems based on cellulose and starch, polysaccharides widely found in fruits and vegetables. The observed retentions are analyzed in terms of possible interactions between the polymeric substrates with volatile.

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EXPERIMENTAL SECTION

Model Systems Preparation. The model systems consisted of either cellulose powder (Whatman CC 41, mean particle size passing 200 B.S.S.) or starch (Merck, Soluble Starch), ^{14}C-labeled 2-propanol, and water. They were prepared by suspending the desired amount of cellulose or starch in water and adding 2-propanol; 0.1% (w/w) of carboxymethylcellulose (CMC) was added to facilitate the handling of the suspensions.

Five-milliliter aliquots of the suspensions were pipetted into 50-ml Erlenmeyer flasks and frozen immediately in liquid nitrogen to maintain the solids in the suspended state. The resultant sample thickness was about 4 mm. The samples were then freeze-dried for 48 hr at ambient temperature and at a chamber pressure of less than 100 μm in a Virtis freeze drier (model 10-MRTR).

Reagent grade 2-propanol was mixed with ^{14}C-labeled 2-propanol to give the desired specific radioactivity. The