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BACTERIAL METABOLISM OF 2-METHYLALANINE[1]

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ABSTRACT

AASLESTAD, H. G. (Louisiana State University, Baton Rouge), AND A. D. LARSON. Bacterial metabolism of 2-methylalanine. J. Bacteriol. **88**: 1296-1303. 1964.—A bacterium isolated from soil was found to oxidize 2-methylalanine to 65% of the theoretical value by an adaptive enzyme system. Manometric experiments with whole cells indicated that acetone but not isopropylamine was an intermediate. Cellular extracts produced carbon dioxide from 2-methylalanine when pyruvate was added to the reaction mixture. Dialysis stimulated the 2-methylalanine metabolizing system, providing pyridoxal phosphate and pyruvate were supplied. A study of the stoichiometry of this pyridoxal phosphate-dependent reaction indicated that acetone and carbon dioxide were principal products. The formation of carbon dioxide from 2-methylalanine seemed dependent upon transfer of the amino group to pyridoxal phosphate. Other 2-methyl amino acids were found to be decarboxylated by this system, thus indicating a lack of absolute specificity.

In recent years, considerable interest in the effect of 2-methyl or α-methyl amino acids on different biological systems has developed. The inhibitory effects of 2-methyl amino acids on amino acid decarboxylating and transaminating enzyme systems and their mechanism of action were reviewed briefly by Umbreit (1955) and more extensively by Clark (1963). Various methyl-substituted amino acids, notably 2-methylalanine, were employed in the study of amino acid transport in mammalian tissues (Christensen et al., 1952; Christensen, Aspen, and Rice, 1956) as model substrates which escape detectable metabolic alteration. It was presumed that 2-methyl amino acids fail to be degraded because of the absence of a hydrogen atom on the α carbon and subsequent impairment of the most commonly used metabolic pathways of amino acids (e.g., transamination, oxidative deamination, and certain α-β eliminations). The metabolic disposition of amino acids having a tertiary α carbon atom is, therefore, of interest.

Few studies on the metabolic fate of 2-methyl amino acids have been made. Weissbach, Lovenberg, and Udenfriend (1960) demonstrated that a mammalian amino acid decarboxylase acted on 2-methyltryptophan, 2-methyl-5-hydroxytryptophan, and 2-methyldihydroxyphenylalanine. Wilson and Snell (1962) reported that 2-methylserine and 2-hydroxymethylserine were metabolized to alanine and serine, respectively, by a soil pseudomonad. Their work represents the enzymatic equivalent of the nonenzymatic pyridooal-catalyzed cleavage of 2-methylserine studied by Longenecker, Ikawa, and Snell (1954).

Kalyankar and Snell (1962), employing a nonenzymatic system, showed that methyl-substituted amino acids, in particular 2-methylalanine, underwent two closely related but independent reactions when heated with pyridoxal in an acidic aqueous solution in the absence of metal ions:

(1) $(CH_3)_2—C(NH_2)—COOH \xrightarrow{\text{pyridoxal}} (CH_3)_2—CH—NH_2 + CO_2$

(2) $(CH_3)_2—C(NH_2)—COOH + \text{pyridoxal} \rightarrow (CH_3)_2—C{=}O + CO_2 + \text{pyridoxamine}$

Reaction 1 is analogous to the decarboxylation of amino acids by pyridoxal phosphate enzymes; reaction 2 was described as a decarboxylation-dependent transamination.

This report presents data in support of the presence of an enzymatic system in a soil bacterium which catalyzes reaction 2.

MATERIALS AND METHODS

The organism used was obtained from soil by elective culturing, with 2-methylalanine as a sole source of carbon in the basal salts medium of

[1] A preliminary report of these results was presented at the Annual Meeting of the American Society for Microbiology, Washington, D.C., 3-7 May 1964.

Stanier (1947). The bacterium was isolated on solid medium containing 0.2% 2-methylalanine, and was routinely subcultured on the same medium plus 0.005% each of sodium acetate and yeast extract in basal salts at neutral pH. Cells for whole-cell studies were grown on the medium solidified with 2.0% agar in stainless-steel trays at 30 C. For the preparation of cellular extracts, bacteria from 10- to 30-liter volumes were harvested from a New Brunswick continuous-culture apparatus. Immediately after harvest, the cells were washed twice and were finally suspended in 0.067 M phosphate buffer (pH 7.0).

To prepare cellular extracts, approximately 10 g (wet weight) of bacteria plus 30 ml of phosphate buffer were chilled in an ice bath and were subjected to sonic treatment by use of a 20-kc Branson sonifier. Ten 1-min periods of sonic treatment, each followed by 3 or 4 min of cooling, were found to yield adequate breakage. Cellular debris was removed by centrifugation for 15 min at 27,000 $\times$ g in a Servall RC-2 centrifuge. Protein content of cellular extracts was determined by the biuret method of Gornall, Bardawill, and David (1949).

Oxygen consumption and carbon dioxide evolution were measured manometrically with a Warburg respirator by the procedures outlined by Umbreit, Burris, and Stauffer (1957). The density of the bacterial suspensions added to the Warburg vessels was standardized by relating the turbidity of the cell suspensions to a dry-weight standard curve. The cellular extracts ranged from 10 to 28 mg of protein per vessel. Phosphate buffer was used throughout, and all substrates were adjusted to neutrality. All biochemicals were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

The disappearance of 2-methylalanine and the production of alanine were measured by the ninhydrin method of Housewright and Thorne (1950) after separation by thin-layer chromatography on cellulose. Pyruvic acid was determined by the Katsuki et al. (1961) modification of the Friedmann-Haugen method; acetone was measured by the salicylaldehyde method of Behre (1940). Microdiffusion according to the method of Conway (1957) and titration with standardized HCl permitted the quantitation of ammonia.

RESULTS AND DISCUSSION

Organism. A number of cultures capable of growing at the expense of 2-methylalanine were isolated from soil. Although none of the isolates grew rapidly, the organism used was chosen because of its relatively abundant growth on the isolation medium. The bacterium is an aerobic, gram-negative, nonmotile rod, 0.8 to 1.1 by 1.6 to 2.3 μ.

The following cultural and nutritional characteristics were determined at 25 C. Growth on nutrient agar slants was filiform, smooth, and butyrous. In nutrient broth, growth was heavy throughout the tube, without formation of a pellicle or sediment. No pigment was evident in either of the above media. When the organism was grown in litmus milk, a slight alkaline reaction developed after 1 week. Growth in gelatin was scant after 2 weeks of incubation; gelatin was not hydrolyzed. A brown, nondiffusible pigment was produced when the organism was cultured on a potato core.

The organism did not utilize any of the following carbohydrates, as judged in phenol red broth base with Durham tubes added: glucose, galactose, fructose, maltose, sucrose, and lactose. However, it could be shown with a Warburg apparatus that all of the above carbohydrates except lactose were oxidized.

Indole and hydrogen sulfide were not produced when the organism was grown in SIM Medium. Nitrate was not reduced to nitrite in nitrate broth. Acetylmethylcarbinol was not produced and the methyl red test was negative after growth in MR-VP Medium. The organism failed to hydrolyze fat but did hydrolyze starch.

On the basis of these observations, the organism was placed in the genus *Pseudomonas* (Breed, Murray, and Smith, 1957). Assignment to this genus was made after careful consideration of the possibility of placing the organism in one of the genera in the order Eubacteriales. Although the organism lacked both motility and pigmentation, which are characteristic of pseudomonads, its fundamental physiology, especially its action on carbohydrates, appeared to be similar to members of the genus *Pseudomonas*. The characteristics of the organism did not fit any of the described species of *Pseudomonas* closely enough to permit speciation.

Published reports indicated that methyl-substituted amino acids were resistant to metabolic attack in mammalian systems (Leighty and Corley, 1937; Christensen et al., 1952), and Christensen and Jones (1962) demonstrated that

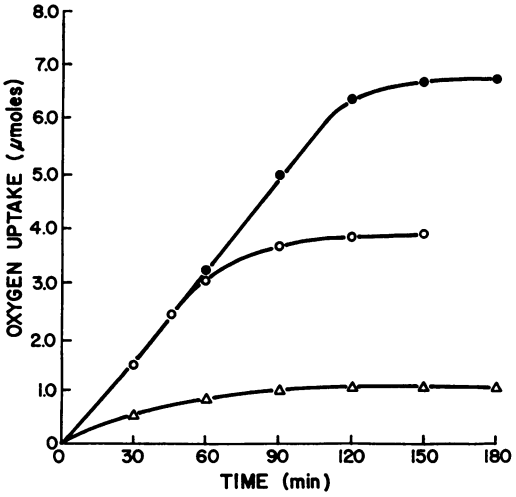

FIG. 1. *Oxidation of 2-methylalanine. Each Warburg vessel contained 15.0 mg (dry weight) of cells; 0.5 ml of 0.067 M phosphate buffer (pH 7.0); 1 μmole (○), 2 μmoles (●), or no (△) 2-methylalanine; 0.2 ml of KOH in the center well; and distilled water to a final volume of 3.0 ml.*

TABLE 1. *Oxidation of 2-methylalanine and possible intermediates in the dissimilation of 2-methylalanine by whole cells grown on different media**

Substrate	Growth media†		
	1	2	3
2-Methylalanine	18.3‡	17.6	2.3
Isopropylamine	1.3	2.7	2.0
Acetone	23.2	23.4	1.9
Isobutyrate	5.0	4.0	4.3
2-Hydroxyisobutyrate	—	2.3	1.9
Methacrylate	—	3.7	9.1
2-Methylserine	7.2	5.9	1.9
Endogenous	1.2	2.0	1.8

* Conditions identical to those described in Fig. 1, except that the substrate concentration was 12.5 μmoles.

† Growth media contained basal salts plus (1) 0.2% 2-methylalanine, (2) 0.2% 2-methylalanine plus 0.005% acetate, (3) 0.2% acetate.

‡ Figures indicate oxygen uptake expressed as micromoles per 60 min.

2-methylalanine-*1*-C^{14} was not measurably degraded in the mouse. However, they did show that a small quantity of labeled carbon dioxide was produced from 2-methylalanine-*1*-C^{14} by the intestinal flora of the mouse. No definitive study of the bacteria implicated in this decarboxylation was made. Various bacteria (Stephenson, 1949) are unable to grow at the expense of 2-methylalanine, and the stock cultures of our laboratory uniformly failed to utilize this compound for growth.

Oxidation of 2-methylalanine. When suspensions of washed whole cells were incubated with limiting quantities of 2-methylalanine in a Warburg apparatus, oxygen uptake was found to be 60 to 65% of the theoretical value. These data (Fig. 1) indicate the ability of the organism to oxidize the amino acid. Oxidative assimilation probably accounts for the discrepancy between experimental and theoretical oxygen utilization. Addition of 2,4-dinitrophenol at a concentration of 10^{-5} M increased the percentage of theoretical oxidation to approximately 75%. The optimal pH for the oxidation of 2-methylalanine by whole cells was found to be between 7.2 and 7.5. It was also found that carbon dioxide was evolved by whole cells when incubated under an air atmosphere in a Warburg apparatus. No carbon dioxide was produced under a nitrogen atmosphere.

The ability of the organism to oxidize 2-methylalanine is adaptive. When cultivated on a medium containing glucose or peptone, or both, the ability of the bacterium to oxidize 2-methylalanine was lost or greatly depressed; this effect was noticed even if the inducer was incorporated into such media. However, it was observed that cells grown on media containing glucose or isobutyric acid as a sole source of carbon could be adapted to 2-methylalanine. This was accomplished by shaking a heavy suspension of glucose- or isobutyrate-grown cells in the presence of 2-methylalanine for an appropriate period of time. Table 1 illustrates the adaptive nature of 2-methylalanine dissimilation in this organism. When the organism was grown on a medium containing 2-methylalanine as a sole source of carbon, very active cells could be obtained; however, growth was sparse. Inclusion of a trace amount of sodium acetate markedly stimulated growth and produced cells active toward 2-methylalanine. Acetate-grown cells oxidized 2-methylalanine very slowly.

Table 1 also presents data concerning the ability of the organism to oxidize possible intermediates in the dissimilation of 2-methylalanine. These compounds were chosen as possible intermediates after a consideration of the feasible pathways by which the 2-methylalanine molecule

might be attacked. These were considered to be (i) a decarboxylation to isopropylamine and subsequent oxidation (White and Larson, *unpublished data*); (ii) a hydrolytic, reductive, or desaturative deamination to 2-hydroxyisobutyric acid, isobutyric acid, or methacrylic acid, respectively [organic acids possibly metabolized in a manner similar to valine intermediates (Robinson et al., 1957)]; (iii) an attack on one of the methyl groups to produce the corresponding alcohol, 2-methylserine. The third possibility could occur by the direct incorporation of oxygen by enzymatic mechanisms, as reviewed by Hayaishi (1962).

The rate at which acetone was oxidized by the cells grown on 2-methylalanine suggested that this compound was an intermediate. However, since isopropylamine formation theoretically should precede acetone if 2-methylalanine had been initially decarboxylated, the inability of the organism to oxidize isopropylamine was confusing. It appeared unlikely that a reductive or desaturative deamination was operative, since isobutyric acid and methacrylic acid were oxidized to an equal or greater extent by nonadapted cells than by adapted cells. The fact that 2-hydroxyisobutyric acid was very slowly oxidized by adapted cells seemed to exclude the compound as an intermediate. 2-Methylserine could be an intermediate if oxidative attack were to occur on a methyl group of 2-methylalanine; however, the organism's activity toward 2-methylserine could be explained by constitutive hydroxymethyltransferase enzymes (Wilson and Snell, 1962). Although a pathway through 2-methylserine could not be discounted at this point, these data (Table 1) strongly indicated that acetone was an intermediate in the catabolism of this amino acid, in spite of the inability of whole cells to oxidize isopropylamine.

Preparation of cellular extracts. Cell permeability barriers could explain the inability of the organism to oxidize isopropylamine; therefore, cellular extracts were prepared. Isopropylamine was not oxidized by extracts prepared by a variety of methods. Likewise, initial attempts with the use of cellular extracts failed to demonstrate carbon dioxide production from 2-methylalanine under aerobic or anaerobic conditions. Only very slow oxidation of 2-methylalanine could be shown with cellular extracts.

Kalyankar and Snell (1962) suggested that a nonenzymatic model reaction (a decarboxylation-dependent transamination) between pyridoxal and 2-methylalanine could produce acetone, carbon dioxide, and pyridoxamine without isopropylamine as an intermediate. They also pointed out that this nonenzymatic reaction was strongly inhibited by di- and trivalent metal ions, the same ions that catalyze all previously studied model reactions between pyridoxal and amino acids. The observed inability of whole cells and cellular extracts of the organism to metabolize isopropylamine could be explained if a similar reaction occurred.

Cellular extracts obtained from bacteria ruptured by sonic treatment were dialyzed at 4 C against 5×10^{-5} M phosphate buffer (pH 7.0) for 10 to 12 hr. Production of carbon dioxide from 2-methylalanine under an atmosphere of nitrogen was used as an index of activity. Data obtained from two experiments with dialyzed and undialyzed enzyme preparations, in the presence of catalytic amounts of pyridoxal phosphate and stoichiometric amounts of pyruvic acid, are shown in Table 2. Pyruvate was added to the reaction mixture to permit the regeneration of pyridoxal phosphate from pyridoxamine phosphate, which was expected to be produced.

The data in Table 2 indicate that an active 2-methylalanine decarboxylating system can be demonstrated, providing a keto acid is present. It is also evident that pyridoxal phosphate is the

TABLE 2. *Carbon dioxide production by cellular extracts*

Expt	System*		
	Complete	Minus pyridoxal phosphate	Minus pyruvate
1. Undialyzed	2.7†	2.0	—
Dialyzed	5.8	1.0	—
2. Undialyzed	0.2	—	0.0
Dialyzed	2.0	—	0.1

* Complete reaction mixture contained 12.5 μmoles of 2-methylalanine, 12.5 μmoles of pyruvate, 150 μg of pyridoxal phosphate, 0.5 ml of 0.067 M phosphate buffer (pH 7.0), 1.0 ml of cellular extract, and distilled water to a final volume of 3.0 ml. The reaction was stopped by the addition of 0.2 ml of 20% trichloroacetic acid and carbon dioxide measured manometrically.

† Figures indicate amount of carbon dioxide expressed in microliters per milligram of protein per 60 min.

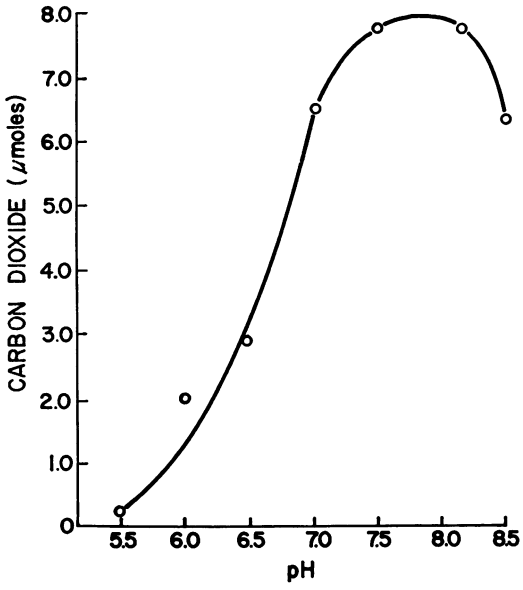

FIG. 2. *Effect of pH on the decarboxylation of 2-methylalanine by cellular extracts. The reaction mixture was identical to that described in Table 2, except that 1.0 ml of 0.2 M phosphate buffer of the desired pH was used. The experiment was stopped after 120 min.*

coenzyme, since carbon dioxide produced in the dialyzed preparation minus pyridoxal phosphate is one-half that of the undialyzed preparation. Further evidence for the role of pyridoxal phosphate as coenzyme is shown by the production of almost six times as much carbon dioxide by the complete dialyzed system as compared with the dialyzed system without added pyridoxal phosphate. Apparently, dialysis removes an inhibitory factor, possibly a metal ion, since carbon dioxide production is greater in the complete dialyzed system than in the complete undialyzed system. The optimal pH (Fig. 2) for the decarboxylation of 2-methylalanine by the cellular extract was found to be 7.8. The carbon dioxide produced by the cellular extract at the optimal pH represents decarboxylation of 62% of the added substrate.

Paper chromatography of the contents of Warburg vessels in which decarboxylation of 2-methylalanine had been demonstrated revealed two principal products. The production of alanine was demonstrated with the use of numerous solvent systems suitable for amino acids; no isopropylamine was detected. Acetone and residual pyruvate were chromatographically detected by use of methods specific for keto compounds.

Stoichiometry of the reaction. To delineate more carefully the dissimilation of 2-methylalanine, experiments were performed to determine the stoichiometry of the reaction. The reaction was carried out in a Warburg apparatus under a nitrogen atmosphere, thus permitting direct manometric measurement of carbon dioxide. The reaction mixture consisted of dialyzed cellular extract, phosphate buffer, and equimolar amounts of 2-methylalanine and pyruvic acid plus a catalytic amount of pyridoxal phosphate. The reaction was terminated by acidification with metaphosphoric acid, and the contents were freed from protein by centrifugation. The supernatant fluid was analyzed for the disappearance of the starting constituents and the production of acetone, alanine, and ammonia (Table 3). The data shown were corrected for endogenous activity by substracting appropriate values obtained from controls consisting of the complete system plus inactivated cellular extract (boiled for 5 min) and the complete system minus 2-methylalanine. The results from the complete system offer a reasonable balance, in view of the fact that a crude cellular extract was employed. Although more 2-methylalanine disappeared than could be accounted for, a difference of about 0.9 μmoles, it may be seen that values of 2.0 μmoles of carbon dioxide, 1.6 μmoles of acetone, and 2.2 μmoles of amino nitrogen (alanine plus ammonia) roughly balance. Isopropylamine was not detected. The results obtained when pyruvate or pyridoxal phosphate were omitted indicate the dependence of the enzyme on these compounds.

Specificity of the 2-methylalanine-metabolizing enzyme system. The specificity of the enzyme system was determined by utilizing different methyl-substituted amino acids as substrates. The rate at which whole cells grown on 2-methylalanine oxidized various 2-methyl amino acids is shown in Fig. 3. Isovaline (2-methyl-2-aminobutyric acid), a homologue of 2-methylalanine, is oxidized at a greater rate than is the amino acid used as an inducer. It is obvious that the chemical character of the amino acid residue or side chain is of considerable importance, since the organism's ability to oxidize the different 2-methyl amino acids decreased as the character of the residue changed from that of a hydrocarbon (isovaline, 2-methylalanine), to a hydroxymethyl (2-methyl-

TABLE 3. *Stoichiometry of the reaction*

System*	Change in reactants and products†					
	2-Methyl-alanine	Pyruvate	Carbon dioxide	Acetone	Amino nitrogen	
					Alanine	Ammonia
1a. Complete	−3.1	−4.3	+3.5	+1.6	+3.0	+2.8
1b. Complete with boiled enzyme	−0.2	−0.1	+0.5	0.0	+1.2	+2.1
1c. Complete minus 2-methylalanine	—	−1.1	+1.5	0.0	+1.3	+2.1
1d. Complete corrected for endogenous	−2.9	−3.2	+2.0	+1.6	+1.7	+0.7
2. Complete minus pyridoxal phosphate‡	−1.2	−1.3	+0.6	+0.5	+0.1	+0.3
3. Complete minus pyruvate‡	−1.4	—	+0.4	+0.7	+0.3	+0.2

* Complete reaction mixture contained 20.0 μmoles of 2-methylalanine, 20.0 μmoles of pyruvate, 150 μg of pyridoxal phosphate, 0.5 ml of 0.2 M phosphate buffer (pH 7.8), 1.0 ml of cellular extract, and distilled water to a final volume of 3.0 ml. The reaction was carried out under a nitrogen atmosphere, and was terminated after 120 min by the addition of 0.2 ml of 20% metaphosphoric acid. Carbon dioxide was measured manometrically. For other analytical methods, see Materials and Methods.

† Expressed in micromoles.

‡ These data were corrected by appropriate controls.

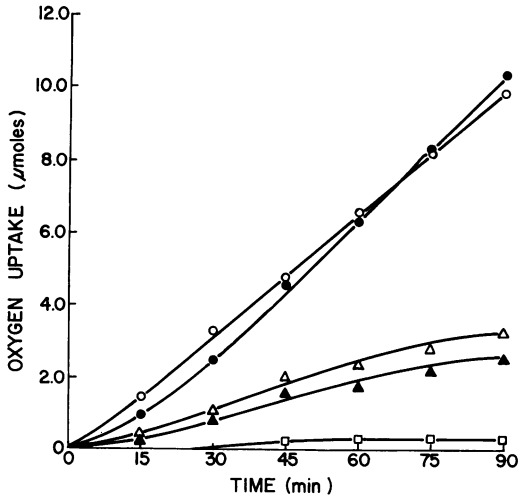

FIG. 3. *Oxidation of 2-methyl amino acids by whole cells. Each Warburg vessel contained 25.0 mg (dry weight) of cells, 0.5 ml of 0.067 M phosphate buffer (pH 7.5), 12.5 μmoles of substrate, 0.2 ml of KOH in the center well, and distilled water to a total volume of 3.0 ml. Symbols: 2-methylalanine (○), isovaline (●), 2-methylserine (△), 2-methylmethionine (▲), 2-methylglutamic (□). All data minus endogenous activity, which was 1.6 μmoles after 90 min.*

TABLE 4. *Comparison of the decarboxylase activity of cellular extracts and the oxidative activity of whole cells toward 2-methyl amino acids*

Substrate	Carbon dioxide produced*	Oxygen uptake†
2-Methylalanine	2.0	9.9
Isovaline	7.0	10.4
2-Methylserine	0.9	3.2
2-Methylmethionine	0.5	2.5
2-Methylglutamate	0.0	0.3
2-Methyldihydroxyphenyl-alanine	0.0	—‡

* Expressed as micromoles per 120 min. Conditions identical to those described in Table 3.

† Expressed as micromoles per 90 min. Conditions identical to those described in Fig. 3.

‡ Auto-oxidized.

serine), to a thioether (2-methylmethionine), to an acid (2-methylglutamic acid). In an experiment in which limiting amounts of isovaline were used, whole cells were found to oxidize the substrate to 32% of the theoretical value. Since DL-isovaline was used in this experiment, it would appear that one isomer of the isovaline was oxidized nearly to completion.

The data shown in Fig. 3 are apparently not related to cellular permeability characteristics, because data pertaining to the decarboxylase activity of cellular extracts on the different 2-methyl amino acids show the same relative rates (Table 4). The rate of carbon dioxide production from isovaline is three times that of 2-methylalanine, which strongly indicates that isovaline is a

more suitable substrate. The fact that carbon dioxide was produced by cellular extracts from four of the six 2-methyl amino acids tested as substrates may indicate a lack of absolute specificity.

The mechanism of the pyridoxal-catalyzed cleavage of amino acids to carbon dioxide and an amine, proposed by Metzler, Ikawa, and Snell (1954), ascribed no essential role to the α hydrogen of the amino acid. The retention of the α hydrogen during enzymatic decarboxylation in deuterium oxide (Mandeles, Koppelman, and Hanke, 1954) is significant in this regard. Weissbach et al. (1960) demonstrated that a partially purified amino acid decarboxylase from guinea pig kidney was capable of degrading certain aromatic 2-methyl amino acids. Likewise, Wilson and Snell (1962) reported that a soil pseudomonad possessed enzymes capable of degrading 2-methylserine and 2-hydroxymethylserine. These nonenzymatic and enzymatic systems amply support the credibility of enzymes active on methyl-substituted amino acids.

The pyridoxal-catalyzed cleavage of 2-methylalanine to carbon dioxide, acetone, and pyridoxamine (reaction 2 of Kalyankar and Snell, 1962) may be a nonenzymatic precedent for the mechanism of 2-methylalanine catabolism suggested in this paper. The two reactions are similar in that both require pyridoxal or its phosphorylated derivative. Also, the observation that dialysis stimulated the activity of the cellular extract may be related to the inhibition of the nonenzymatic reaction by di- and trivalent metal ions.

We visualize enzymatic mechanism as proceeding by Schiff's base formation between 2-methylalanine and the coenzyme, pyridoxal phosphate. The amino acid may then be decarboxylated, and the resulting substrate-coenzyme complex could be enzymatically directed into one of two possible stabilized Schiff's base structures. One of these (structure III of Kalyankar and Snell, 1962) upon hydrolysis would form an amine, in this case isopropylamine, and the aldehyde form of the coenzyme, whereas the other Schiff's base (structure IV of Kalyankar and Snell, 1962) would yield a keto compound, acetone, and the amine form of the coenzyme after hydrolysis. Enzymatic control favoring the latter reaction could explain the results of this study. The stimulatory effect of a keto acid, presumably required to regenerate the aldehyde form of the coenzyme by transamination, supports this view. Since no free isopropylamine was detected in 2-methylalanine–metabolizing systems, and since this compound was not oxidized by whole cells or cellular extracts, the enzymatic formation of isopropylamine and the regeneration of pyridoxal phosphate appear doubtful. It would be of interest to determine whether isopropylamine could be transaminated by this cellular extract.

Experiments are underway to determine whether the decarboxylation and transamination of 2-methylalanine are carried out by one or a number of enzymes. In this regard, the work of Novogradsky, Nishimura, and Meister (1963) is of interest. They reported that a highly purified aspartic acid β-decarboxylase obtained from *Alcaligenes faecalis* acts both as a general amino acid transaminase and as an aspartic acid decarboxylase. Preliminary studies in this laboratory indicate that a fraction of the cellular extract containing 2-methylalanine–decarboxylating activity may be separated from a pyridoxal phosphate-regenerating system by ammonium sulfate precipitation.

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