of [G-^{3}H]vaccenate into the methylheptadecanes of *N. muscorum* support a direct decarboxylation mechanism in blue-green algae as opposed to a head-to-head condensation of shorter chain fatty acids. Their incorporations were low, however, and one could not exclude degradation to acetate or some other simple precursor prior to incorporation.

Although direct decarboxylation of a saturated fatty acid to form a hydrocarbon would appear to be a very simple reaction, its mechanism may not be at all simple. Most decarboxylations appear to involve assistance of a neighboring functional group which can serve as an electron sink.

The reaction catalyzed by the *A. variabilis* extract reported in this paper represents the last stages of biosynthesis of the branched methylheptadecanes. The identification of an endogenous methyl group acceptor or of a methylated intermediate in this reaction should clarify the biosynthetic pathway, and might also provide clues to the mechanism of *n*-heptadecane biosynthesis in this organism.

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Studies on the Mutual Influences of Substrates on Bovine α-Keto Acid Metabolism†

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With the Technical Assistance of Martin T. Glynn

ABSTRACT: The metabolic interrelationships among the branched-chain α-keto acids, α-ketoisocaproate, α-keto-β-methylvalerate, α-ketoisovalerate, and effects of these metabolites upon the metabolism of pyruvate and of α-ketoglutarate in bovine liver mitochondria have been investigated. The apparent Michaelis constants, determined for mitochondrial dehydrogenases, are of the order of 4×10^{-4} M, for the branched-chain α-keto acids, while those for pyruvate and α-ketoglutarate are slightly lower. The degree and type of influence exerted among the various α-keto acids was examined directly by following the enzymic liberation of $^{14}CO_2$ from carboxyl-labeled substrate in the presence of a second unlabeled α-keto acid. A mutually inhibitory pattern among α-ketoisocaproate, α-ketoisovalerate, and α-keto-β-methylvalerate was noted. In addition, each of the branched-chain keto acids exerted a marked inhibition of pyruvate and α-ketoglutarate dehydrogenase activity. Inhibition was of the competitive type in all instances except for α-ketoglutarate dehydrogenase activity, which is "mixed." The apparent inhibitor constants were in a range such that a significant mutual influence among the branched-chain α-keto acids could exist *in vivo*, at normal cellular concentrations of these metabolites. Moreover, a significant effect of α-ketoisocaproate on pyruvate dehydrogenase was noticed. The net effect of the inhibitory action of the branched-chain α-keto acids on each other and upon pyruvate dehydrogenase in the normal animal is likely a homeostatic balance, which allows for catabolism of excess branched-chain amino acids, while concurrently influencing the flow of pyruvate to acetyl coenzyme A. These studies indicate that interrelationships among the various α-keto acid metabolisms could constitute a significant physiological regulatory mechanism in the normal animal. These relationships, extrapolated to the branched-chain ketoaciduric condition, are consistent with observed symptoms of the disease.

Since the discovery of branched-chain ketoaciduria (BCKA)[1] in humans, a number of investigators have proposed possible modes of action whereby the elevated levels of the branched-chain amino and keto acids may produce the observed physiological and neurological symptoms. Silberman *et al.* (1961) have suggested that increased keto acid concentration in the developing brain may decrease synthesis of

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[1] Abbreviations used are: BCKA, branched-chain-ketoaciduria; KIC, α-ketoisocaproate; KIV, α-ketoisovalerate; KMV, α-keto-β-methylvalerate; KB, α-ketobutyrate; KV, α-ketovalerate; KC, α-ketocaproate; NAD^-, nicotinamide-adenine dinucleotide (oxidized form); CoA, reduced coenzyme A.

cerebronic acids necessary for myelin formation. Others (Howell and Lee, 1963; Tashian, 1961; Dreyfus and Prensky, 1967) have observed that high levels of branched-chain α-keto acids (above 1.0 mM) slightly inhibit glutamic acid decarboxylase in brain and liver of rat and that KIC decreased oxygen consumption by brain slices.

An alternate mechanism of toxic effects of the branched-chain amino and keto acids in BCKA is through inhibition by these compounds at the level of pyruvate dehydrogenase in the glycolytic pathway, or at α-ketoglutarate dehydrogenase of the tricarboxylic acid cycle. Recently Kanzaki *et al.* (1969) have found that the activity of α-ketoglutarate dehydrogenase purified from pig heart muscle is inhibited by KIC, KMV, and KIV. Pyruvate dehydrogenase isolated from the same tissue was inhibited by KMV and KIV. Other investigators have studied the inhibition of pyruvate dehydrogenase by the branched-chain α-keto acids from a semiquantitative viewpoint, and have reported varying degrees of inhibition by KIC in rat brain and liver homogenates (Bowden, 1965; Dreyfus and Prensky, 1967) and in rat liver slice preparations (Bowden *et al.*, 1970).

These preliminary observations indicate a necessity for further investigation into the mode and the extent of the effects of branched-chain α-keto acids, at the concentrations obtained during normal metabolism and in manifestations of BCKA, upon these very important segments of intermediary metabolism. Since the glycolytic pathway and the tricarboxylic acid cycle are of fundamental importance to the provision of energy equivalents in the form of the reduced nucleotide coenzymes, a disruption of function at either site could conceivably be of serious consequence to the general oxidation state of the cell and to the production of energy necessary for anabolic processes. Decreased pyruvate dehydrogenase activity would also result in lowered production of acetyl coenzyme A for the tricarboxylic acid cycle and for fatty acid and steroid synthesis. The potential reality of such mechanisms at play in the abnormal system allows the possibility that the same mechanisms may be operative as regulatory processes in the normal animal, among both the branched-chain α-keto acids and the metabolism of pyruvate and α-ketoglutarate. From the viewpoint of regulatory interrelationships among the keto acids, the metabolism of branched-chain amino acids and keto acids in normal tissue has received less attention than has the pathology of the diseased tissue of the individual with BCKA.

The aim of the present investigation is to characterize the utilization of, and the *in vitro* metabolic interrelationships among, the branched-chain α-keto acids in preparations of bovine liver tissue; and secondly, to ascertain whether the influences of branched-chain α-keto acids upon the metabolism of pyruvate and α-ketoglutarate could constitute a significant physiological regulatory phenomenon.

Materials

The chemicals used in these studies were obtained from the following sources: β-nicotinamide–adenine dinucleotide (NAD^+); trilithium coenzyme A; sodium salts of α-ketoisocaproate, α-ketoisovalerate, α-keto-β-methylvalerate, α-ketobutyrate, α-ketoglutarate, α-ketovalerate, α-ketocaproate, malate, pyruvate, L-amino acid oxidase; D-amino acid oxidase (Sigma Chemical Co., St. Louis, Mo.); L-leucine-*1*-^{14}C, DL-valine-*1*-^{14}C, sodium pyruvate-*1*-^{14}C, sodium α-ketoglutarate-*1*-^{14}C (New England Nuclear, Boston, Mass.); L-isoleucine-*U*-^{14}C (Cal-Atomic Division, Calbiochem Co., Los Angeles, Calif.).

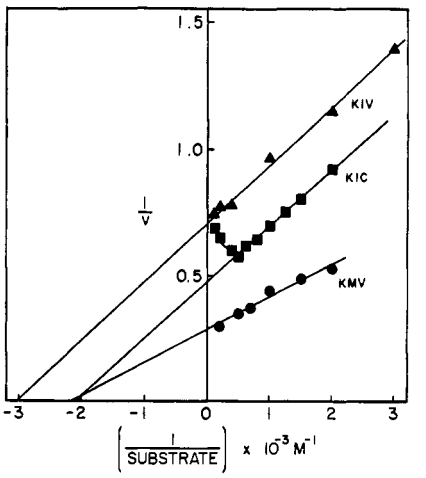

FIGURE 1: Lineweaver–Burk plots of the effect of substrate concentration of the branched-chain α-keto acid dehydrogenase activity of bovine liver mitochondria. Enzyme activity was measured with the $^{14}CO_2$ assay as described in the Methods section. Each reaction mixture contained, in a final volume of 1.0 ml, the following (in micromoles): mannitol, 150; potassium phosphate buffer, pH 7.2, 33; NAD^+, 1.0; Li_3CoA, 0.6; $MgCl_2$, 1.0; $CaCl_2$, 1.0; Na_2CO_3, 1.0; 0.1 ml of a washed preparation of bovine liver mitochondria (about 2.5 mg of protein), and the indicated amounts of carboxyl-labeled KIV, KIC, or KMV. Reactions were allowed to proceed for 20 min at 30°. v_i, nanomoles of CO_2 produced per minute per milligram of protein.

Methods

Preparation of ^{14}C-labeled α-keto acid substrates, assay of α-keto acid dehydrogenase activity, preparation of bovine liver mitochondria, and determination of protein were accomplished as described previously (Johnson and Connelly, 1972).

Results

Quantitative studies of the influence of substrate concentration upon the rate of oxidation of the α-keto acids were essential as a prerequisite to the investigation of mutual metabolic interactions among these compounds. Apparent K_m values were determined for KIV, KMV, KIC, α-ketoglutarate, and pyruvate. Substrate effects were investigated over at least a tenfold variation of concentration for each keto acid. The relationship between the reciprocal of initial reaction velocity (v_i) and the inverse of the branched-chain α-keto acid concentrations is shown in the Lineweaver–Burk plots of Figure 1. These plots are representative of several studies and serve to indicate the character of the inverse plot. Most noticeable is the substrate inhibition by KIC, not seen with KIV, or KMV. Experimental results obtained, but not shown, with α-ketoglutarate give linear double-reciprocal plots for substrate concentrations between 0.05 and 1.0 mM and a significant deviation from linearity below 0.04 mM. The latter phenomenon may be due to the existence of the presumed permeability barrier to α-ketoglutarate or the effects of a translocase at the inner membrane. This phenomenon was also observed when washed preparations of rat liver mitochondria served as the source of enzyme, but was not evident

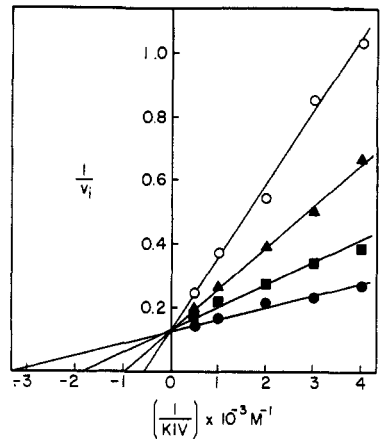

FIGURE 2: Lineweaver–Burk plots of the inhibition of KIV dehydrogenase activity by unlabeled KIC. All experimental conditions are as in Figure 1. (●) No inhibitor, (■) 0.25 mM KIC, (▲) 0.50 mM KIC, and (○) 0.75 mM KIC.

with the purified α-ketoglutarate dehydrogenase from pig heart muscle.

The apparent K_m values[2] obtained from the average of several determinations are summarized in Table I. Substrate inhibition by KIC concentrations in excess of 2.0 mM was noted, while no inhibition of the dehydrogenase activity by concentrations of KIV to 10 mM was found. None of the other α-keto acids exhibited substrate inhibition within the ranges of concentration utilized for these studies.

A thorough investigation of the relative inhibitory interactions among several α-keto acids, including the branched-chain acids KIV, KIC, and KMV, was conducted with two major objectives. These were first, to determine whether these interrelationships could constitute a possible physiological regulatory mechanism among the keto acids under conditions of "normal" α-keto acid levels in mammalian tissues, and second, to ascertain to what extent the *in vitro* metabolism of α-ketoglutarate and pyruvate is affected by the levels of KIV, KIC, and KMV reported to occur in untreated branched-chain ketoaciduria.

Reciprocal plots in Figure 2 illustrate that the oxidative decarboxylation of KIV is inhibited competitively by KIC. Similar results (not shown), also indicating competitive inhibition, were obtained for all other cases of branched-chain α-keto acid influence on the production of $^{14}CO_2$ from carboxyl-labeled substrates. Thus, each of the branched-chain α-keto acids is able to compete for the oxidative process in the mitochondrial system.

The rate of *in vitro* oxidative decarboxylation of pyruvate by bovine liver mitochondria was decreased significantly (Table II) in the presence of the branched-chain keto acids. KIC acts as a competitive inhibitor of pyruvate oxidation, while similar but less significant competitive effects were noted for KMV and KIV. In contrast to the character of these inhibitions the catabolism of α-ketoglutarate was inhibited by KIV in an apparent mixed type of inhibition. KIC and KMV were slightly less effective as inhibitors (see Table II), based on apparent K_i values. These findings are similar

TABLE I: K_m Values for Several α-Keto Acid Dehydrogenases of Bovine Liver Mitochondria.

α-Keto Acid Substrate[a]	K_m (moles l.$^{-1}$)
α-Ketoisovalerate	$3.3 \pm 0.4 \times 10^{-4}$ (5)[b]
α-Ketoisocaproate	$5.2 \pm 0.7 \times 10^{-4}$ (4)
α-Keto-β-methylvalerate	$4.7 \pm 0.8 \times 10^{-4}$ (4)
Pyruvate	$2.4 \pm 0.2 \times 10^{-4}$ (5)
α-Ketoglutarate	$0.81 \pm 0.09 \times 10^{-4}$ (4)

[a] Carboxyl-labeled α-keto-^{14}C acids were used as substrates for the measurement of the enzyme activities by the $^{14}CO_2$ evolution assay as described in the Methods section. Each reaction mixture (1 ml) contained the following in μmoles: mannitol, 150; potassium phosphate buffer (pH 7.2), 33; NAD, 1.0; Li_3CoA, 0.6; $MgCl_2$, 1.0; $CaCl_2$, 1.0; Na_2CO_3, 1.0; 0.05 or 0.1 ml of a washed preparation of bovine liver mitochondria, and appropriate amounts of carboxyl-labeled α-keto acids. Determinations were conducted at 30°. [b] K_m values tabulated are the mean ± 1 std dev for the number of determinations indicated in parentheses.

to those previously obtained for the purified α-ketoglutarate dehydrogenase complex from pig heart mitochondria (Kanzaki *et al.*, 1969).

The influence of α-ketobutyrate (KB), an α-keto acid of considerable importance in the catabolic pathways of methionine and threonine, on the metabolism of other α-keto acids was also studied, since this normal metabolite has been reported to accumulate in the plasma of branched-chain ketoaciduric patients (Dawson and Hird, 1968). Recent investigation has shown that KB is oxidized to propionyl-CoA by the pyruvate dehydrogenase complex (Kanzaki, *et al.*, 1969) at a substantial rate. The oxidation of KIC and of pyruvate was affected deleteriously by low levels of KB *in vitro*. Inhibition was competitive in both cases. Influences by ketovalerate, straight-chain analog of KIV, and α-ketocaproate, straight-chain analog of KIC and KMV, were inhibitory and of the mixed type. The results from the inhibition studies, summarized in Table II, indicate that the metabolism of each of these keto acids is markedly influenced by the relative levels of the various α-keto acids in the normal liver cell (see Table III). From these data it is evident that one consequence of increased KIC (for example) concentrations will be the effective and detrimental influence upon the conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase.

Discussion

The major thrust of initial studies of the metabolism of the branched-chain amino acids centered upon the characterization of the overall degradative pathways of leucine, isoleucine, and valine (Coon *et al.*, 1955); elucidation of the site of the catabolism of these compounds in the genetic disease branched-chain ketoaciduria (Dancis *et al.*, 1963; Dancis and Levitz, 1966; Dancis, 1957), and development of therapeutic dietary treatment designed to control the symptoms of the disease (Westall, 1963; Snyderman *et al.*, 1964). Recently, however, there has been a growing awareness of the complexity and multiplicity of the regulatory mechanisms which

[2] True Michaelis constants can not be easily determined for these reactions by the methods of Florini and Vestling (1957), since at least three substrates (NAD^+, CoA, and α-keto acid), and perhaps additional cofactors, participate in the tightly coupled reaction sequences of the α-keto acid dehydrogenase complexes.

TABLE II: K_i Values of Various α-Keto Acids on Bovine Liver Mitochondria α-Keto Acid Dehydrogenase Activities.[a]

Inhibitor	K_i (M)				
	α-Keto-β-methyl-valerate Dehydrogenase	α-Ketoisocaproate Dehydrogenase	α-Ketoisovalerate Dehydrogenase	Pyruvate Dehydrogenase	α-Ketoglutarate Dehydrogenase
1-^{12}C-Labeled KMV		8.6×10^{-4}	2.5×10^{-4}	2.3×10^{-3}	1.4×10^{-3}
1-^{12}C-Labeled KIC	6.9×10^{-4}		1.3×10^{-4}	5.4×10^{-4}	2.4×10^{-3}
1-^{12}C-Labeled KIV	2.2×10^{-4}	1.95×10^{-4}		1.4×10^{-3}	8.2×10^{-4}
1-^{12}C-Labeled KV			0.5×10^{-4}		
1-^{12}C-Labeled KC		2.2×10^{-4}	4.8×10^{-4}		
1-^{12}C-Labeled KB		1.1×10^{-3}		4.1×10^{-4}	

[a] α-Keto acid dehydrogenase activities were measured by quantitation of $^{14}CO_2$ produced from carboxyl-labeled α-keto acids. Reaction conditions are as in Table I. Each K_i value tabulated represents the average of at least three determinations.

TABLE III: Some Amino Acid and α-Keto Acid Concentrations in Normal and Branched-Chain Ketoaciduric Tissues.

Amino Acid	Normal Concn (mM)	BCKA Concn (mM)	α-Keto Acid	Approx[a] Normal (mM)	Approx BCKA (mM)	Tissues	Ref
Leucine	0.1–0.5		α-Ketoisocaproate	0.1–0.5		Rat liver	Anderson *et al.* (1968); Ness (1971)
Leucine	0.1–0.4	4–6	α-Ketoisocaproate	0.1–0.4	2–4	Human plasma	Snyderman *et al.* (1964)
Isoleucine	0.08–0.2		α-Keto-β-methyl-valerate	0.08–0.2		Rat liver	Ness (1971)
Isoleucine	0.07–0.25	1.0–1.5	α-Keto-β-methyl-valerate	0.07–0.25	1.0–1.5	Human plasma	Snyderman *et al.* (1964)
Valine	0.1–0.4		α-Ketoisovalerate	0.1–0.4		Rat liver	Ness (1971)
Valine	0.24	1.85	α-Ketoisovalerate	0.24	1.85	Human plasma	Snyderman *et al.* (1964)
			Pyruvate	0.2		Rat liver (fed)	*b*
			Pyruvate	0.03		Rat liver (24-hr fast)	*b*

[a] Based on a K_{eq} of approximately one for the transamination reaction (Meister, 1957). [b] Personal communication from P. D. Ray (personal communication, 1971).

may function to maintain a fine balance among the cellular levels of the branched-chain amino and keto acids in the normal mammalian system (Harper *et al.*, 1954; Wohlheuter and Harper, 1970).

One major consideration in this regard is the mode and extent of mutual interferences among the transaminase and α-keto acid dehydrogenase reactions. Incidentally, it might be speculated that the oxidative decarboxylation of the branched-chain α-keto acids, the second step in the catabolism of the corresponding amino acids, is a logical site of metabolic regulation, owing to the complex nature of the multienzyme units which catalyze this conversion, and to the essentially irreversible diversion of carbon away from the mainstream amino acid and protein metabolism.

It is well established that a substantial elevation of the plasma levels of the branched-chain amino acids occurs in animals fed a diet high in protein (Anderson *et al.*, 1968). Under conditions of increased leucine, isoleucine, and valine the concentrations of KIC, KMV, and KIV exist in concentrations which are able to inhibit dehydrogenase activity. Mutual inhibitory interactions among the branched-chain α-keto acids would thus enable the organism to maintain cellular homeostasis by permitting the preferential oxidation of a particular keto acid present in excess amounts. This phenomenon could attain a significant physiological role in the event of a disparity in the concentration of these metabolites in the tissues (*e.g.*, high dietary intake of milk protein which has a high leucine content, Snyderman, 1967).

Possibly the most important consequence of elevated branched-chain amino and α-keto acid concentrations in the cell is the potential modulating effect of these acids upon the overall utilization of carbohydrate. Each of the branched-chain α-keto acids is able to inhibit the oxidation of pyruvate and α-ketoglutarate. When the level of KIC in plasma and liver increases subsequent to high dietary consumption of protein, its K_i is sufficiently low as to allow a marked reduction in the rate of oxidation of pyruvate to acetyl-CoA. Pyruvate concentrations in rat liver are reported to range

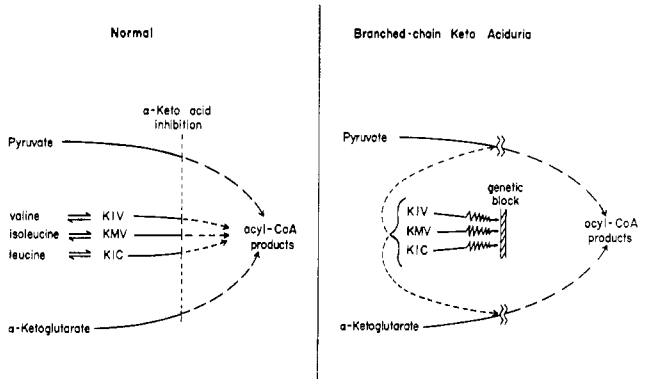

FIGURE 3: Summary of some metabolic interactions of α-keto acids *in vitro*.

from 0.034 mM for 24-hr fasted animals to approximately 0.2 mM in the fed rat (P. D. Ray, 1971, personal communication). Assuming that these results can be extrapolated to other mammals, the following situations would obtain. In mammalian liver, KIC concentrations of about 0.1 mM (lower end of the normal range, see Table II) would decrease the pyruvate dehydrogenase activity only slightly (5–10%)[3] in the fed animal. However, if the level of KIC reaches a somewhat elevated concentration, such as is known to occur after consumption on increased amounts of protein, inhibition is considerably greater (about 35%), a nearly fourfold potentiation in the inhibition of pyruvate utilization. By comparison, KMV concentrations in the normal range of 0.08–0.2 mM (see Table III) would result in merely a 2–5% inhibition of pyruvate dehydrogenase activity in the fed animal. In contrast, the oxidation of α-ketoglutarate would be less influenced even by the conditions resulting from high-protein diets.

A second, indirect effect of elevated KIC, KMV, and KIV lies in the fact that the catabolism of these compounds leads ultimately to succinyl-CoA and acetyl-CoA, each of which has been reported to inhibit the enyzmatic functions of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase (Erfle and Sauer, 1968). The combined effects of direct and indirect inhibition by branched-chain α-keto acids on the metabolism of pyruvate and α-ketoglutarate could result in a condition in which the branched-chain α-keto acids would promote their own catabolism, since oxidation of excess branched-chain compounds would be favored, until normal cellular concentrations are reattained.

Although the exact cause of the neurological pathology commonly associated with branched-chain ketoaciduria is not known, a correlation between the neurologic symptoms and elevated leucine and KIC has been established (Snyderman *et al.*, 1964). The results of the mutual inhibition studies (Table II) disclose that KIC among the three branched-chain keto acids is the most potent inhibitor of pyruvate oxidation. Concentrations of KIC have been reported to exceed 2.5 mM in plasma, and 0.8 mM in brain tissue of BCKA patients (Dancis *et al.*, 1960; Snyderman *et al.*, 1964; Dreyfus and Prensky, 1967). It is evident from the previous discussion that the metabolism of pyruvate dehydrogenase would be severely impaired by KIC at these concentrations; the effects of KMV and KIV probably being minimal by comparison. The observed accumulation of KB in tissues of persons afflicted with branched-chain ketoaciduria may also result from inhibition of the pyruvate dehydrogenase complex, if in fact this enzyme is the primary decarboxylase of KB.

Figure 3 displays the general interrelationships among the various α-keto acids in normal and BCKA metabolism. In the normal the net overall result would be a homeostatic balancing of keto and amino acids with little net change in the production of acyl-CoA products since even marked inhibition of pyruvate would allow continued production of acyl-CoA intermediates from the branched-chain keto acids. The abnormal BCKA case would be very different. Pyruvate dehydrogenase activity would be severely limited by cellular levels of KIC at or above 1 mM, with lower alternative production of acyl-CoA products. This would be consistent with the concensus that KIC is a primary cause of the adverse neurological effects in the disease. Thus, in the normal, and certainly more so in the abnormal mammal, fluctuations in the levels of branched-chain α-keto acids would contribute significantly to the regulation of the levels of these metabolites, to the status of the rates of pyruvate and α-ketoglutarate metabolism and to the resulting metabolic consequences.

In summary, these studies show that the branched-chain α-keto acids can be mutually inhibitory at normal cellular levels and that KIV, KMV, and especially KIC are potential regulatory influences on pyruvate and α-ketoglutarate metabolism. The extreme case of this, seen in branched-chain ketoaciduria, may underlie the more critical symptoms of this genetic disease.

[3] Since the inhibition of pyruvate oxidation by KIC is of the competitive type, eq 1 is applicable for the calculation of the inhibitor constants, K_i. Use of this constant and the $K_{m,app}$ allowed for calculation of the per cent inhibition of the dehydrogenase activity with eq 2, as proposed by Nordlie *et al.* (1968), at any suitable levels of substrate and inhibitor.

$$v_i = \frac{V_{max}\,[\text{pyruvate}]}{\left(1 + \frac{[\text{KIC}]}{K_i}\right) K_m + [\text{pyruvate}]} \quad (1)$$

$$\%\ \text{inhibition} = \frac{(v - v_i) \times 100}{v} \quad (2)$$

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Aromatic Side-Chain Cotton Effects in Cyclic Hexapeptides†

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ABSTRACT: We report circular dichroism curves of the cyclic hexapeptides *cyclo*-(Gly$_2$-Tyr)$_2$, *cyclo*-Gly-His-Gly-Ala-Tyr-Gly, and *cyclo*-Gly-His-Gly-Tyr-Ala-Gly. Published nuclear magnetic resonance (nmr) data indicate that these compounds adopt conformations having all-trans peptide bonds and two β loops stabilized by trans-annular hydrogen bonds. The circular dichroism of the cyclic peptide in the 210–289-nm region is approximately separated into contributions of the peptide bonds and those of the side-chain chromophores. When a tyrosyl residue is the first corner residue of a β loop we estimate that it contributes a rotational strength of -0.242×10^{-40} cgs esu at 277 nm and 2.94×10^{-40} cgs esu at 227 nm. We further conclude that the circular dichroism of *cyclo*-Gly-His-Gly-Tyr-Ala-Gly contains contributions typical of a tyrosyl residue in an extended conformation. We also propose a simple coupled oscillator theory to calculate Cotton effects due to tyrosyl chromophores at 277 and 227 nm. We give calculated results for several backbone conformations which have been proposed on the basis of nmr data. We find satisfactory agreement with experiment for a tyrosyl residue as the first corner residue of a β loop ($\phi = -80°$, $\psi = 120°$) if we take the side-chain conformational angles $\chi_1 = 300°$ and $\chi_2 = 60°$. Our results on the circular dichroism of cyclic peptides may be generalized to the structure *cyclo*-(Gly-L-residue-D-residue)$_2$ and we use our method to reinterpret some circular dichroism data from other laboratories.

A cyclic hexapeptide is the smallest cyclic peptide which may have all-trans peptide bonds. A number of cyclic hexapeptides adopt a characteristic conformation having two β loops and two internal amide hydrogens in a conformation originally proposed by Schwyzer (1959). The internal amide protons may be detected by temperature dependence of nuclear magnetic resonance (nmr) (Kopple *et al.*, 1969a). In some, but not all cases, the internal amide proton may form trans-annular hydrogen bonds (Kopple *et al.*, 1972).

Further information on the conformation of cyclic hexapeptides can be gained from nmr by measuring the amide NH to CαH coupling constant. This coupling constant is related to the dihedral angle about the N–C bond (Ramachandran *et al.*, 1971; Bystrov *et al.*, 1969). Since this dihedral angle is closely related to the peptide conformation angle, ϕ_{CN}, such information is quite useful in building models of cyclic hexapeptides. Temperature dependence of chemical shift and coupling constants may be combined with conformational energy maps to propose complete backbone conformations of cyclic hexapeptides. Some generalizations about the influence of various side chains on the backbone conformation have been made recently (Kopple *et al.*, 1972).

The optical activity curves of cyclic peptides, although often similar in appearance to those of linear polypeptides, require an interpretation quite different from those of linear polypeptide structures such as α helix, β-pleated sheet, and random coil (Bush, 1971). An interesting illustration of this fact is offered by gramicidin S whose circular dichroism (CD) curve resembles that of an α helix (Urry *et al.*, 1969). In fact, a conformation, quite unlike an α helix, has been proposed based on nmr data (Stern *et al.*, 1968). This conformation, which is now widely accepted as correct, has two β loops. Yet optical activity calculations based on the model of Stern *et al.* (1968) yield an optical rotatory dispersion (ORD) curve which is consistent with experiment (Pysh, 1970). In the present study, we will approach the optical activity of cyclic peptides not from the standpoint of linear peptides but rather from the basis of cyclic peptide models whose conformation is known from nmr data.

In an earlier study, we showed differences in CD between

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