Abelson[4] found that pyruvate, valine and α-ketoisovalerate as well as acetate all lowered the specific activity of leucine synthesized by *E. coli* growing on uniformly C^{14}-labeled glucose and suggested that ketovaline might condense with acetate to yield leucine. The present results confirm and extend this suggestion by indicating that carbons 1 and 2 of leucine are derived directly from acetate or a close relative thereof. In speculating on a mechanism for this transformation, we have proposed as a working hypothesis[3] a series of reactions analogous to those of the citric acid cycle. In the citric acid cycle, oxalacetic acid condenses with acetate to yield citrate, ultimately yielding α-ketoglutarate, the next higher homologous α-keto acid. In a study of the biosynthesis of lysine[6] it was found that a similar mechanism would account for the formation of α-aminoadipic acid, its six-carbon precursor, *viz.*, a condensation of α-ketoglutarate with acetate to yield a homolog of citric acid and, ultimately, α-ketoadipic acid.

In the present instance, acetate may be envisioned to condense with α-ketoisovaleric acid, ultimately yielding α-ketoisocaproic acid. By analogy with the citrate-yielding reaction, acetate is presumed to be in the form of its coenzyme A ester. Figure 1 shows the proposed sequence of reactions.

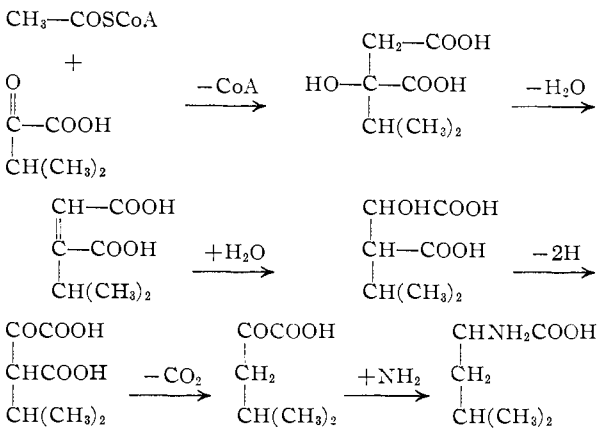

Fig. 1.—Postulated mechanism of leucine biosynthesis.

These considerations lead us to the belief that the citric acid cycle may be one example of a general process employed in nature for the production of α-keto acids. Virtanen[16] has recently discovered α-aminopimelic acid in a microörganism, and it is suggested that this may arise by a similar sequence of reactions from α-ketoadipic acid leading to α-ketopimelic acid. It is recognized, however, that these mechanisms, though plausible, have no experimental groundwork as yet, and the proof of their occurrence will require the study of the biological activities of the intermediates. Such studies are now under way.[17]

Addendum.—After preparation of this paper there appeared a report by Reiss and Bloch[18] on the distribution of acetate carbons and glucose carbon 1 in leucine synthesized by *S. cerevisieae.* These investigators made substantially the same observations as here reported and came to essentially the same conclusion concerning the participation of acetate and the isobutyl moiety of valine in the formation of leucine. The only discrepancy between the two sets of data was the very high level of activity in leucine carbon 3 from glucose carbon 1, observed by Reiss and Bloch. However, these authors point out that the activity of this carbon was calculated by difference and therefore may not be entirely reliable.

(16) I. A. Virtanen and A. M. Berg, *Acta Chem. Scand.*, **8**, 1985 (1954).

(17) Professor Masatoro Yamasita is now preparing intermediates of this postulated series of reactions in order to ascertain their biological activity and with the aim of testing their effects in meeting the growth requirements of leucine-requiring mutants.

(18) O. Reiss and K. Bloch, *J. Biol. Chem.*, **216**, 703 (1955).

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[CONTRIBUTION FROM THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE]

Studies on Diastereoisomeric α-Amino Acids and Corresponding α-Hydroxy Acids VI. Rotatory Dispersion of Copper Complexes

BY NOBUO IZUMIYA, MILTON WINITZ, SANFORD M. BIRNBAUM AND JESSE P. GREENSTEIN

RECEIVED NOVEMBER 2, 1955

The optical rotatory dispersion of the copper complexes of six diasymmetric L-amino acids, as well as their L-allo-stereomers, was studied at 578, 546, 435, 405 and 365 mμ. Calculations of the contribution (partial molar rotation) of the α- and ω-asymmetric centers to the observed molar rotation were effected and the empirical relation between the rotatory characteristics of the copper complexes of α-amino acids and wave length proposed by Pfeiffer and Henseleit tested. It was found that this relation, while inapplicable to the total molar rotation of diastereomeric amino acids, nonetheless becomes entirely applicable when based upon the partial rotation of the α-center of asymmetry.

Empirical rules for the determination of the optical configuration of the α-asymmetric center of α-amino acids have been derived from rotatory dispersion methods,[1-3] as well as from rotational shifts induced by *p*H changes.[4-6] The foregoing methods were intimately concerned with either the ionization, or the absorption at different wave

(1) P. Karrer and W. Kaase, *Helv. Chim. Acta*, **2**, 436 (1919).

(2) E. Waser, *ibid.*, **6**, 206 (1923).

(3) J. W. Patterson and W. R. Brode, *Arch. Biochem.*, **2**, 247 (1943).

(4) L. Pasteur, *Ann. chim. phys.*, **31**, 81 (1951); E. P. Cook, *Ber.*, **30**, 294 (1897); J. K. Wood, *J. Chem. Soc.*, **105**, 1988 (1914).

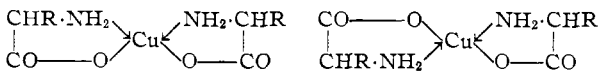
(5) G. W. Clough, *ibid.*, **107**, 1509 (1915).

(6) O. Lutz and V. Jirgensons, *Ber.*, **63**, 448 (1930); **64**, 1221 (1931); **65**, 784 (1932).

lengths, of the amino and carboxyl groups associated with the α-asymmetric center of the amino acid under investigation, and such studies were almost exclusively confined to those α-amino acids which contained only a single center of asymmetry. The empirical concepts developed for monoasymmetric amino acids were recently made applicable to amino acids with two centers of optical asymmetry in a manner analogous to that employed by Hudson[7] for the sugar group. Such extension, reported in earlier papers of this series,[8,9] was based on the contribution (partial molar rotation) of the α- and ω-asymmetric centers to the total molar rotations of several diasymmetric amino acids at wave lengths ranging between 365 and 589 mμ. Further extension of these latter studies to rotatory dispersion measurements of the copper complexes of diastereomeric amino acids is the intent of the present communication.

That the shape of the rotatory dispersion curve exhibited by the copper complex of an amino acid can be related to its optical configuration was demonstrated by Pfeiffer and Christeleit[10] in 1937. In this rather unique report, it was noted that an intense absorption in the visible region of the spectrum, with concomitant high molecular rotation values, appeared to be characteristic of these compounds. The behavior of the L-amino acids as a group was strikingly similar, not only with respect to the general shape of the dispersion curve, but also with regard to the 520 to 540 mμ range wherein a maximal rotation was elicited. The maximum at 480 mμ which occurs for L-proline could be conceivably attributable to tetracyclic ring formation. Dispersion curves for the D-amino acids were exactly opposite to those of their enantiomorphs, a phenomenon related to their configuration.

Formation of the copper complex of an amino acid involves the chelation of one molecule of copper with two of the amino acid to give a ring system which, theoretically, may be either planar or tetrahedral. If the former is valid, then the *cis* and *trans* isomerides should exist, as is shown in what follows



The comparable coplanar platinum and palladium complexes of glycine have been isolated and separated.[11] However, if the ring system formed results in a complex which is tetrahedral, then an asymmetrical, and thereby resolvable, bicyclic system similar to the spiranes would result. Since no isomerides of this latter type have yet been isolated, the assumption should tentatively be made that the optical activity of such copper complexes resides entirely within the asymmetric carbon atoms of the amino acids under investigation.

(7) C. S. Hudson, THIS JOURNAL, **31**, 66 (1909).

(8) M. Winitz, S. M. Birnbaum and J. P. Greenstein, *ibid.*, **77**, 716 (1955).

(9) M. C. Otey, J. P. Greenstein, M. Winitz and S. M. Birnbaum, *ibid.*, **77**, 3112 (1955).

(10) P. Pfeiffer and W. Christeleit, *Z. physiol. Chem.*, **245**, 197 (1937).

(11) F. W. Pinkard, E. Sharratt, W. Wardlow and E. G. Dox, *J. Chem. Soc.*, 1012 (1934).

Total Molar Rotations of Copper Complexes of Diasymmetric Amino Acids.—The rotatory dispersion measurements of the amino acid copper complexes here reported were effected with the diasymmetric L- and L-*allo*-forms of threonine, phenylserine, hydroxyproline, O-methylthreonine, isoleucine and aminotricarballylic acid, all of which were made available by resolution procedures developed in this Laboratory.[12] Conversion to the copper complex of the particular amino acid studied was performed by the addition of exactly one equivalent of amino acid to one equivalent of cupric acetate to give a final concentration of 0.05 mM. of complex per 10 ml. of solution. The instrument employed was a Rudolph photoelectric polarimeter equipped with glass filters suitable for isolation of the 578, 546, 435, 405 and 365 mμ lines of the mercury arc spectrum. Actual rotations were observed, within the temperature range of 25 to 27°, *via* the aid of a photomultiplier tube connected to a photometer and were determined by the method of symmetrical angles. Calibration of the instrument was effected by means of two quartz control plates, with a maximum error of 0.22% at any of these wave lengths. The molar rotation values observed for the aforementioned diasymmetric amino acids, as well as the monoasymmetric L-glutamine, L-glutamic acid, L-asparagine and L-aspartic acid, are indicated in Table I.

TABLE I

TOTAL MOLECULAR ROTATIONS OF COPPER COMPLEXES OF DIASYMMETRIC AMINO ACIDS AT VARIOUS WAVE LENGTHS[a]

	Molecular rotation[d] in degrees at wave length of				
L-Amino acid	578 mμ	546 mμ	435 mμ	405 mμ	365 mμ
Isoleucine	+ 21	+ 42	− 17	− 26	−102
Alloisoleucine	− 7	+ 10	− 59	− 90	−127
O-Methylthreonine	− 51	− 12	−127	−180	−266
O-Methylallothreonine	+185	+176	+104	+ 97	+113
Threonine	− 22	− 49	−157	−214	−328
Allothreonine	+117	+150	+ 92	+ 58	+131
Hydroxyproline	−170	−212	−224	−285	−361
Allohydroxyproline	+ 46	+245	+237	+214	+274
Phenylserine	− 70	− 88	−238	−303	−445
Allophenylserine	+292	+391	+301	+306	+323
Aminotricarballylic acid[b]	− 93	− 89	−144	−165	−200
Alloaminotricarballylic acid[c]	− 70	− 72	−101	−119	−161
Aspartic acid	− 37	− 39	− 37	− 46	− 68
Asparagine	− 22	− 4	0	− 12	− 24
Glutamic acid	− 43	− 49	− 70	− 88	−104
Glutamine	− 22	+ 13	+ 16	− 2	− 18

[a] Optical rotations determined with a photoelectric polarimeter at temperatures between 25 and 27°. [b] Refers to B(−) form. [c] Refers to A(+) form. [d] Molar rotations based on molecular weight of amino acid used.

Examination of Table I reveals that the molar rotation values of the monoasymmetric dicarboxylic acids, L-glutamic and L-aspartic acids, and their corresponding ω-amides, L-glutamine and L-asparagine, respectively, are in complete agreement with the behavior expected for L-amino acids as inter-

(12) J. P. Greenstein, *Advances in Protein Chem.*, **9**, 121 (1954).

TABLE II

PARTIAL MOLAR ROTATIONS OF THE α- AND ω-ASYMMETRIC CENTERS OF DIASYMMETRIC AMINO ACIDS AS THEIR COPPER COMPLEXES

L-Amino acid	Partial molar rotation in degrees at wave length of— 578 mμ		546 mμ		435 mμ		405 mμ		365 mμ	
	α	ω	α	ω	α	ω	α	ω	α	ω
Isoleucine	+ 7	+ 14	+ 26	+ 16	− 38	+ 21	− 58	+ 32	−115	+ 13
Alloisoleucine	+ 7	− 14	+ 26	− 16	− 38	− 21	− 58	− 32	−115	− 13
O-Methylthreonine	+ 67	−118	+ 82	− 94	− 12	−116	− 42	−139	− 77	−190
O-Methylallothreonine	+ 67	+118	+ 82	+ 94	− 12	+116	− 42	+139	− 77	+190
Threonine	+ 48	− 70	+ 51	−100	− 33	−125	− 78	−136	− 99	−230
Allothreonine	+ 48	+ 70	+ 51	+100	− 33	+125	− 78	+136	− 99	+230
Hydroxyproline	− 62	−108	+ 17	−229	+ 7	−231	− 36	−250	− 44	−318
Allohydroxyproline	− 62	+108	+ 17	+229	+ 7	+231	− 36	+250	− 44	+318
Phenylserine	+111	−181	+152	−240	+ 32	−270	+ 2	−305	− 61	−384
Allophenylserine	+111	+181	+152	+240	+ 32	+270	+ 2	+305	− 61	+384
Aminotricarballylic acid	− 82	− 12	− 81	− 9	−123	− 22	−142	− 23	−181	− 20
Alloaminotricarballylic acid	− 82	+ 12	− 81	+ 9	−123	+ 22	−142	+ 23	−181	+ 20

preted from the results of Pfeiffer and Christeleit.[10] Thus, a plot of molar rotation *versus* wave length (not shown) indicates that each of these amino acids either attains or approaches a maximal rotation value between 435 and 546 mμ, whereas a minimal value is approached below 435 mμ. However, further examination of the table reveals no such predictable behavior for the diasymmetric L-amino acids. Although L-isoleucine, L-alloisoleucine, L-O-methylthreonine, L-threonine, L-hydroxyproline and L-phenylserine are in apparent agreement with the expected behavior, the L-*allo*-forms of the latter four amino acids are not. Note should here be taken, however, that the total molar rotation of a diasymmetric amino acid is equal to the sum of the partial molar rotations of its α- and ω-asymmetric centers.[8] Since the formation of the copper complex of such amino acids is primarily involved with functional groups attached to the α-asymmetric center, the introduction of a second center of asymmetry into the molecule may potentially mask any characteristic relation of optical configuration to optical rotation based on the α-asymmetric center alone. Interpretations which may therefore be confined solely to the α-configuration of such molecules arise from the calculations derived in the section directly below.

Partial Molar Rotations of Copper Complexes.—The total molar rotation of the copper complexes of those α-amino acids with two centers of asymmetry may, for the present purposes, be regarded as a function of the sum of the partial molar rotations of each asymmetric center and represented by the equations

$$[M]_L = \alpha_L + \omega_L, \text{ and} \quad (1)$$

$$[M]_{L\text{-allo}} = \alpha_{L\text{-allo}} + \omega_{L\text{-allo}} \quad (2)$$

where $[M]_L$ and $[M]_{L\text{-allo}}$ are the total molar rotations of the copper complexes of the L- and L-allo-forms of the amino acid, and α_L, $\alpha_{L\text{-allo}}$, ω_L and $\omega_{L\text{-allo}}$ are the partial molar rotations of the α- and ω-centers of asymmetry. Addition of equations 1 and 2 gives

$$[M]_L + [M]_{L\text{-allo}} = \alpha_L + \omega_L + \alpha_{L\text{-allo}} + \omega_{L\text{-allo}} \quad (3)$$

However, the α-asymmetric carbon atoms of the L- and L-allo-forms of a given amino acid are of like configuration and may, for the present purposes, be considered of like rotational magnitude, *i.e.*, $\alpha_L = \alpha_{L\text{-allo}}$, whereas the ω-centers are of the opposite configuration and, therefore, of opposite sign, *i.e.*, $\omega_L = -\omega_{L\text{-allo}}$. Appropriate substitution and transposition in equation 3 therefore results in the final equations

$$2\alpha_L = 2\alpha_{L\text{-allo}} = [M]_L + [M]_{L\text{-allo}}, \text{ or} \quad (4)$$

$$\alpha_L = \alpha_{L\text{-allo}} = 0.5\,([M]_L + [M]_{L\text{-allo}}) \quad (5)$$

Also

$$\omega_L = -\omega_{L\text{-allo}} = 0.5\,([M_L] - [M_{L\text{-allo}})] \quad (6)$$

With the aid of equations 5 and 6 formulated above, the partial molar rotation values of the α- and ω-asymmetric centers were calculated for each of the diasymmetric amino acids investigated. These values, presented in Table II, reveal, that with the exception of the diastereomeric L-forms of isoleucine and aminotricarballylic acid, the contribution of the ω-asymmetric carbon atom to the total molar rotation of all diasymmetric amino acids studied is in every instance markedly greater than the corresponding contribution of the α-asymmetric center. Such greater magnitude of the secondary center of asymmetry could therefore tend to mask completely the contribution of this latter center and thereby serve to obscure the relation between α-configuration and optical rotation. The L-allo-forms of O-methylthreonine, threonine, hydroxyproline and phenylserine are illustrative of this point. However, if consideration is confined solely to the contribution of the α-asymmetric carbon atom of each amino acid, then the relation between the rotatory dispersion of the copper complexes and configuration are in complete concordance with the original interpretations of Pfeiffer and Christeleit.[10] Thus, as is apparent in Table II, the optical rotation of the α-center, in every instance investigated, either attains or approaches a maximum value at some point between 435 and 546 mμ and approaches a minimum value below 435 mμ. In general, then, it may be stated that although the empirical rule after Pfeiffer and Christeleit[10] need not necessarily apply to the total molar rotation of amino acids (as their copper complexes) with more than one center of asymmetry, the rule nonetheless becomes entirely applicable when employed exclusively in connection with the rotatory dispersion exhibited by the α-asymmetric center of such amino acids.

With the single exception of the isomeric forms

of α-aminotricarballylic acid, the ω-configuration of each of the diastereomeric amino acids presented in Table II has been satisfactorily established. Since it was previously noted[8] that the change in the partial rotation of the ω-asymmetric center, from water to acid, was in the same direction for several amino acids of identical ω-configuration, it becomes of interest here to explore briefly whether a comparable consistent behavior with regard to the partial rotation of this center is indicated in the present rotatory dispersion studies. Thus, at all of the wave lengths herein investigated, the direction of the contribution of the ω-center of the L-antipodes of alloisoleucine,[13] O-methylthreonine, threonine,[14] hydroxyproline[15] and phenylserine,[16] all of which have an ω-D-configuration, is negative in every instance. It is of additional interest to note that in the case of the latter three diasymmetric amino acids, all of which contain a hydroxyl group on the ω-asymmetric carbon atom, there is an apparently consistent increase in the magnitude of rotation with decreasing wave length. In the absence of more abundant data, however, it would be both superfluous and indiscreet to speculate whether such consistencies can be correlated with optical configuration or are merely fortuitous.

Reliability of Optical Rotation for Configurational Determination.—That the use of optical or rotatory dispersion data for the configurational determination of the α-asymmetric center of a diasymmetric amino acid requires more data, and is somewhat more complex, than the utilization of these same methods for the determination of the configuration of an amino acid with a single center of asymmetry, becomes readily apparent. In any event, since the use of such data is contingent upon different magnitudes, and sometimes varying shades of optical rotation, these rotational values must, of necessity, be derived from amino acids of the highest degree of optical and chemical purity. With the sole exception of the stereomers of aminotricarballylic acid which could not be so measured, routine purity determinations with amino acid oxidases, developed in this Laboratory,[17] demonstrated that the optical rotation values, on which the foregoing calculations were based, were determined with amino acids with a minimal optical purity of 99.9%. By a somewhat indirect method whereby the four stereomers of aminotricarballylic acid were converted to the corresponding isocitric acid lactones[18] and then subjected to the action of the highly specific isocitric dehydrogenase-triphosphopyridine nucleotide system,[19] no detectable optical contamination was indicated.

Dependent upon the magnitude and the direction of the contribution to the total molar rotation of the ω-center of asymmetry, the use of optical data can be expected to give the α-configuration of at least one and sometimes both amino acids of a diastereomeric pair. In the absence of other data, calculations based on the four theoretical *l,d*-allo, *l,l*-allo, *d,l*-allo, and *d,d*-allo combinations can be effected, in a manner shown previously,[8] in order to permit a proper choice of the L,L-allo diastereomeric pair.

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(14) C. E. Meyer and W. C. Rose, *J. Biol. Chem.*, **115**, 721 (1936).
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(16) W. S. Fones, *Arch. Biochem. Biophys.*, **36**, 486 (1952).
(17) A. Meister, L. Levintow, R. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **192**, 535 (1951).
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[CONTRIBUTION FROM THE U. S. PLANT, SOIL AND NUTRITION LABORATORY, AGRICULTURAL RESEARCH SERVICE]

The Identification of (+)S-Methyl-L-cysteine Sulfoxide in Plants

BY CLAYTON J. MORRIS AND JOHN F. THOMPSON

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An unusual amino acid has been isolated from turnip roots using ion-exchange methods. This compound has been shown to be (+)S-methyl-L-cysteine sulfoxide by comparison with synthetic material. It has been shown that this compound did not arise as an artifact during the isolation procedure. Quantitative data on the amount of S-methylcysteine sulfoxide in turnips and related plants are presented.

A brief report[1] has been made of the isolation of an amino acid from turnip roots and its identification as (+)S-methyl-L-cysteine sulfoxide. A complete account of this work is presented here along with further evidence of its occurrence in other crucifers.

Detection, Isolation and Identification of (+)S-Methyl-L-cysteine Sulfoxide.—In studying the amino acids of the non-protein fraction of turnip roots (*Brassica rapa*) by chromatography on paper using butyl alcohol–acetic acid and phenol,[2] a prominent ninhydrin-reactive substance was noted next to glutamine. This material was striking because of the relatively large amount present and the unusual brownish-blue color it produced with ninhydrin. This compound gave R_F values of 0.30 in collidine, of 0.62 in phenol and 0.09 in butyl alcohol–acetic acid, was unstable to acid hydrolysis, and behaved like an α-amino acid on passage

(1) C. J. Morris and J. F. Thompson, *Chemistry & Industry*, 951 (1955).
(2) R. J. Block, E. L. Durrum and G. Zweig, "A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press, New York, N. Y., 1955, pp. 77–80.