Levin, A. S., Fudenberg, H. H., Hopper, J. E., Wilson, S. K., and Nisonoff, A. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 169.
Mann, K. G., Fass, D. N., and Fish, W. W. (1973), *Adv. Chem. Ser. No. 125,* 310.
Mann, K. G., Heldebrant, C. M., and Fass, D. N. (1971), *J. Biol. Chem. 246,* 5994.
Manning, D. D. (1974), *J. Immunol. 113,* 455.
Martin, L. N., and Leslie, G. A. (1974), *J. Immunol. 113,* 120.
Moore, C. V. (1967), in Textbook of Medicine, Beeson, P. B., and McDermott, W., Ed., Philadelphia, Pa., W. B. Saunders Co., p 1087.
Panyim, S., and Chalkley, R. (1969), *Arch. Biochem. Biophys. 130,* 337.
Pierce, C. W., Solliday, S. M., and Asofsky, R. (1972), *J. Exp. Med. 135,* 675.
Pink, J. R. L., Wang, A. C., and Fudenberg, H. H. (1971), *Annu. Rev. Med. 22,* 145.
Rudders, R. A., Yakulis, V., and Heller, P. (1973), *Am. J. Med. 55,* 215.
Sanders, J. H., Fahey, J. L., Finegold, I., Ein, D., Reisfeld, R., and Berard, C. (1969), *Am. J. Med. 47,* 43.
Seon, B. K., Yagi, Y., and Pressman, D. (1973a), *J. Immunol. 110,* 345.
Seon, B. K., Yagi, Y., and Pressman, D. (1973b), *J. Immunol. 111,* 1285.
Silverman, A. Y., Yagi, Y., Pressman, D., Ellison, R. R., and Tormey, D. D. (1973), *J. Immunol. 110,* 350.
Sledge, C., Fair, D. S., Black, B., Krueger, R. G., and Hood, L. (1975), *Proc. Natl. Acad. Sci. U.S.A.*, submitted for publication.
Smithies, O., Gigson, D., Fanning, E. M., Goodfliesh, R. M., Gilman, J. G., and Ballantyne, D. L. (1971), *Biochemistry 10,* 4912.
Stears, E., Jr., Craven, G. R., Anfinsen, C. B., and Bethune, J. L. (1965), *J. Biol. Chem. 240,* 2478.
Wang, A. C., Fudenberg, H. H., Goldrosen, M. H., and Freedman, M. H. (1972), *Immunochemistry 9,* 473.
Wang, A. C., Gergely, J., and Fudenberg, H. H. (1973), *Biochemistry 12,* 528.
Wang, A. C., Wang, I. Y. F., McCormick,J. N., and Fudenberg, H. H. (1969), *Immunochemistry 6,* 451.
Wang, A. C., Wilson, S. K., Hopper, J. E., Fudenberg, H. H., and Nisonoff, A. (1970), *Proc. Natl. Acad. Sci. U.S.A. 66,* 337.
Warner, N. L. (1972), *Contemp. Top. Immunobiol. 1,* 87.
Warner, N. L. (1974), *Adv. Immunol. 19,* 67.
Waterson, R. M., and Konigsberg, W. H. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 376.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406.
Wolfenstein-Todel, C., Franklin, E. C., and Rudders, R. A. (1974), *J. Immunol. 112,* 871.
Wu, T. T., and Kabat, E. A. (1970), *J. Exp. Med. 132,* 211.
Yagi, Y., and Pressman, D. (1973), *J. Immunol. 110,* 335.

Biosynthesis of the Polyoxins, Nucleoside Peptide Antibiotics: Glutamate as an Origin of 2-Amino-2-deoxy-L-xylonic Acid (Polyoxamic acid)†

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ABSTRACT: The biosynthetic origin of the carbon skeleton of 2-amino-2-deoxy-L-xylonic acid (polyoxamic acid) is described. This aminoaldonic acid is the N terminus of the nucleoside peptide antibiotics, the polyoxins, produced by *Streptomyces cacaoi* var. *asoensis*. In vivo experiments concerning incorporation and distribution of radioactivity from a number of ^{14}C-labeled compounds have clearly shown that the carbon skeleton of glutamate is a precursor for this aminoaldonic acid and sugars are incorporated only after their conversion into glutamate through the glycolytic and the tricarboxylic acid cycle pathways. Experiments utilizing [^{14}C]acetate and succinate have also indicated multiple passages through the Krebs cycle are operating before their incorporation into polyoxamic acid via glutamate. The distribution of ^{14}C between C-1 and C-5 of polyoxamic acid from the [5-^{14}C]glutamate experiment has indicated that 40% of glutamate incorporated into polyoxamic acid has been incorporated only after the reversible conversion into α-ketoglutarate followed by the passage through the Krebs cycle. Lack of incorporation of ^{3}H in the [1-^{14}C;2-^{3}H]- and [5-^{14}C;2-^{3}H]glutamate experiments is discussed in terms of a reaction(s) between glutamate and polyoxamic acid.

In our last paper (Isono et al., 1975), we reported a new metabolic role for L-isoleucine as a precursor of 3-ethylidene-L-azetidine-2-carboxylic acid (polyoximic acid). This amino acid is a C terminal amino acid of the nucleoside peptide antibiotics, the polyoxins (Chart I) (Isono et al., 1969), produced by *Streptomyces cacaoi* var. *asoensis* (Isono et al., 1965). Another unusual amino acid, 2-amino-2-deoxy-L-xylonic acid (polyoxamic acid) (I) or its 3-deoxy analogue (II) constitutes the N terminus of the biologically active polyoxins. This amino acid bears a carbamoyl group on the C-5 hydroxy-oxygen (Isono et al., 1969) (Chart I). Chemical replacement of this amino acid with a variety of

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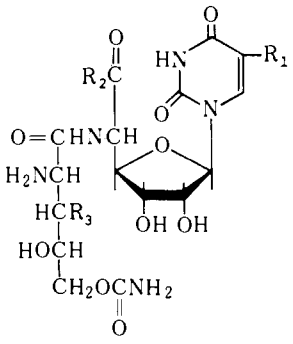
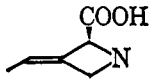
Chart I. The Structure of the Polyoxins.



R_1 = H, CH_3, CH_2OH, OR COOH

R_2 = HO OR 

R_3 = OH OR H

amino acids has been studied, which shows the importance of this amino acid for the polyoxin activity (Isono et al., 1971).

Because I is a rare example of a 2-amino-2-deoxyaldonic acid occurring in nature, it was of interest to study the biosynthesis of this compound. Further, since I is an aldonic acid as well as an α-L-amino acid, it was particularly interesting to determine if I is formed by sugar or amino acid metabolism. This paper proves on the basis of in vivo experiments utilizing ^{14}C-labeled sugars, mono- and dicarboxylic acids, and amino acids, that the carbon skeleton of I is biosynthesized from that of glutamate. A considerable part of the glutamate is metabolized via the Krebs cycle before incorporation into I.

Materials and Methods

D-[1-^{14}C]Glucose, D-[6-^{14}C]glucose, D-[3,4-^{14}C]glucose, D-[1-^{14}C]ribose, DL-[3-^{14}C]serine, DL-[5-^{14}C]glutamic acid, [1-^{14}C]acetic acid sodium salt, [2-^{14}C]acetic acid sodium salt, [1,4-^{14}C]succinic acid, and [2,3-^{14}C]succinic acid were purchased from the New England Nuclear Corporation; [3-^{14}C]pyruvic acid sodium salt, DL-[1-^{14}C]glutamic acid, and DL-[2-^{3}H]glutamic acid were purchased from the Radiochemical Centre, Amersham; D-[1,3-^{14}C]glycerol was from ICN Corporation. Radioactivity measurements were made in a Packard TriCarb Model 3330 liquid scintillation spectrometer using Bray's dioxane scintillation solution (Bray, 1960) with the addition of a 2% 0.6 *N* toluene solution of hyamine (NCS tissue solubilizer, Amersham/Searle). Preparative paper chromatography was done by the descending method using Whatman 3 MM paper. Microcrystalline Avicel was employed for cellulose chromatography, and Whatman DE52 was used for DEAE-cellulose chromatography. The maintenance and fermentation of *S. cacaoi* and the radioisotope tracer technique were the same as described in our last paper (Isono et al., 1975). The polyoxin complex was purified from the culture filtrates as described before (Isono et al., 1967).

Hydrolysis of the Polyoxin Complex and Isolation of Polyoxamic Acid (I). The polyoxin complex (1 g) was hydrolyzed with 20 ml of 0.5 *N* sodium hydroxide for 2 hr on a steam bath. The hydrolysate was passed through a column of Dowex 50W-X8 (H^+) (100–200 mesh, 20 ml). After washing with water, I was eluted with 750 ml of 0.6 *N* ammonium hydroxide. The eluate was then concentrated to a small volume and passed through a column of DE52 (100 ml, equilibrated with 0.2 *M* triethylamine–carbonate buffer (pH 7.2)). The first fraction containing I was further purified either by Avicell cellulose chromatography using 1-butanol–acetic acid–water (4:1:1.5) as solvent or by preparative paper chromatography using 1-butanol–acetic acid–water (4:1:2) as solvent. On the average, 30 mg of crystals of I was obtained from a small volume of aqueous ethanol.

In the [1-^{14}C;2-^{3}H]- and [5-^{14}C;2-^{3}H]glutamate experiments, acid hydrolysis instead of alkali hydrolysis was utilized to avoid the loss of tritium from C-2 by racemization. The polyoxin complex (1 g) was refluxed in 5 ml of 3 *N* hydrochloric acid for 1 hr. The hydrolysate was passed through a column of Amberlite IR-45 (OH^-, 20 ml), then through Dowex 50W-X8 (H^+, 20 ml), from which amino acids were eluted by 0.5 *N* ammonium hydroxide, and then concentrated and passed through a carbon column (20 g). The eluate was concentrated and separated by paper chromatography (solvent, 75% aqueous phenol) yielding 10 mg of I. To prove no ^{3}H is lost from C-2 by racemization, nonradioactive polyoxin complex (1 g) was hydrolyzed under the same conditions as above except using a mixture of 2.5 ml of 6 *N* hydrochloric acid and 2.5 ml of tritium oxide (specific activity 6.6 mμCi/μmol). After the same work-up as described above, 10 mg of crystals of I with a specific activity of 0.021 mμCi/μmol was obtained. From this experiment, the rate of racemization was calculated to be very low (0.64%).

Ninhydrin Oxidation of Polyoxamic Acid (I). Ten to thirty micromoles of crystals of I (500–2000 dpm) was dissolved in 2.5 ml of water. To this solution, an equal volume of 5% ninhydrin solution in 2 *M* citrate buffer (pH 2.5) was added, and the resulting solution was heated on a steam bath for 45 min. Nitrogen was passed through the solution and then through 1 ml of an ice-cold hyamine solution to collect the carbon dioxide evolved. The total radioactivity of the hyamine solution was then determined. When authentic I (18.7 mg) was also oxidized under conditions identical with those above, 77% of the theoretical amount of carbon dioxide was recovered and weighed as barium carbonate. When DL-[1-^{14}C]leucine was oxidized under these same conditions, 97.0% of the theoretical amount of radioactivity was recovered in the hyamine solution.

Periodate Oxidation of Polyoxamic Acid (I). Polyoxamic acid (I, 50–80 μmol; carrier I was added when needed) was dissolved in 2 ml of 0.1 *M* potassium phosphate buffer (pH 8.0). To this solution, 5 ml of 0.1 *M* sodium periodate was added. The resulting solution was left to stand for 2 hr at room temperature. Nitrogen was bubbled through the solution into 1 ml of an ice-cold solution of hyamine to collect the carbon dioxide evolved. To the residual solution, 5 ml of 0.1 *M* sodium arsenate was added to destroy the excess periodate. After 10 min, the solution was adjusted to pH 4.0 with 1 *N* acetic acid. To this solution, 3 ml of a mixture of 2 *N* sodium acetate and 1 *N* hydrochloric acid (1:1) was added, followed by 3 ml of 0.4% aqueous dimedone solution. The solution stood several hours at room temperature, then overnight in a refrigerator. Crystals were collected by filtration and washed with water. After a recrystallization from aqueous ethanol, the crystals having a constant specific activity were obtained. When authentic I (10.5 mg) was oxidized under identical conditions, 116% of the theoretical amount of carbon dioxide was recovered and weighed as barium carbonate, and 71% of the theoretical amount of

formaldimedone was obtained.

Isolation and Ninhydrin Oxidation of the Cell Protein Glutamate. A sterile solution of DL-[2-^{3}H;5-^{14}C]glutamate was added to the culture medium (five flasks, each 60 ml of the medium) 27 hr after inoculation. At this time, the cells were in a logarithmic growth phase. Twenty-one hours later, the cells were collected by centrifugation (3000*g*) and washed with 0.5% sodium chloride, and then with water. The washed cells were homogenized with a French press. To 200 ml of this ice-cold homogenate, 400 ml of cold 15% trichloroacetic acid was added with stirring. The precipitates formed were collected by centrifugation (3000*g*) and washed with 10% trichloroacetic acid, then with 95% ethanol, and finally with ethanol–ether (1:1, v/v). The precipitate was suspended in 5% trichloroacetic acid (100 ml) and heated on a steam bath for 15 min. The insoluble fraction was collected by centrifugation and washed with ethanol. It was then extracted with 200 ml of 1 *N* ammonium hydroxide (50°, 30 min). The extract was concentrated to a small volume and lyophilized yielding 3 g of protein. The protein (1.5 g) thus obtained was hydrolyzed with 13 ml of 6 *N* hydrochloric acid (110–120°, 20 hr). The hydrolysate was filtered and concentrated in vacuo to dryness. Concentration was repeated several times with addition of water. The residue was dissolved in a few milliliters of water and passed through a column of Dowex 50W (H^+) (60 ml). Amino acids were eluted from the column with 0.6 *N* ammonium hydroxide (500 ml). The eluate was concentrated to a small volume and the resulting solution was passed through a column of Amberlite CG-4B (OH^-) (80 ml). Elution was made by a linear gradient with water–0.8 *N* acetic acid as the solvent. Fractions containing glutamic acid were combined and concentrated to dryness. The residue was further purified by cellulose chromatography using 1-butanol–acetic acid–water (4:1:1, v/v) as solvent. Glutamic acid was crystallized from aqueous ethanol; yield, 15 mg, mp 214°. Mixture melting point with an authentic sample was not depressed.

Procedure of ninhydrin oxidation was the same as that used for I as described above.

Results

Incorporation and Distribution of Labeled Compounds into Polyoxamic Acid. L-Xylonate (Kanfer et al., 1960) and L-xylulose (Ashwell et al., 1959) have the same configuration as I and both are related to sugar metabolism. Therefore, we considered first the possibility that I is related directly to the sugar metabolism and feeding experiments were started with ^{14}C-labeled sugars and related compounds. As shown in Table I, [1-^{14}C]glucose, [6-^{14}C]glucose, [1-^{14}C]ribose, and [1,3-^{14}C]glycerol were incorporated into I, however, [3,4-^{14}C]glucose was not. Each of these compounds produced almost the same distribution of radioactivity as in I; i.e., 20–26% in C-1, 69–76% in C-2,3,4, and 4–6% in C-5. These results together with the absence of incorporation from [3,4-^{14}C]glucose suggested that the biosynthesis of I was not related directly to sugar metabolism. Next we considered the possibility that some metabolite originating from the Krebs cycle might be a precursor of I, because on entering the glycolytic pathway, all the labeled sugars tested produced [2-^{14}C]acetate except [3,4-^{14}C]glucose. In the latter case, all the ^{14}C was lost as carbon dioxide producing an unlabeled acetate. This assumption was confirmed by [3-^{14}C]pyruvate and [3-^{14}C]serine experiments. The incorporation and the distribution of radioactivity in I from these experiments were similar to that in the sugar experiments.

Among the amino acids originating from the Krebs cycle, we first considered glutamate as the candidate precursor for I, since this amino acid possesses a C-5 straight chain and an ω-functional group. [1-^{14}C]Glutamate was indeed found to be incorporated into I. Although the incorporation efficiency was relatively low, all the radioactivity from this experiment was found at C-1 suggesting that glutamate was a direct precursor for I. [5-^{14}C]Glutamate was incorporated into I at a similarly low rate, but the radioactivity was distributed in both C-1 (25%) and C-5 (75%). This distribution can be explained as follows. Imagine there are 125 units of radioactivity in the original [5-^{14}C]glutamate. Sixty percent (or 75 units) of the [5-^{14}C]glutamate is directly converted to [5-^{14}C]I with 75 units of ^{14}C. The remaining 40% (or 50 units) of [5-^{14}C]glutamate is converted by transamination into [5-^{14}C]-α-ketoglutarate and passes through the Krebs cycle loosing half of its ^{14}C as CO_2 and producing [1-^{14}C]-α-ketoglutarate, which is then converted to [1-^{14}C]glutamate with 25 units of ^{14}C and finally converted again to [1-^{14}C]I with 25 units of ^{14}C. Thus, 25 units of ^{14}C are incorporated into I at C-1 and 75 units of ^{14}C are incorporated into I at C-5. It is worth noting that by determination of the distribution of ^{14}C in I from the [5-^{14}C]glutamate experiment, it has become possible to monitor a dynamic equilibrium between glutamate and the Krebs cycle in intact cells of *Streptomyces cacaoi*. Relatively low incorporation efficiency of glutamate can be explained by the fact that glutamate is one of the key amino acids in amino acid metabolism and is also an important amino group donor in a number of transamination reactions.

To confirm the role of the Krebs cycle in the biosynthesis of I, some member carboxylic acids of the Krebs cycle were also tested, namely, [1-^{14}C]acetate, [2-^{14}C]acetate, [1,4-^{14}C]succinate, and [2,3-^{14}C]succinate. Table I shows all these acids were incorporated into I. Distribution of ^{14}C in I from [2-^{14}C]acetate was found to be essentially the same as those from the [1-^{14}C]glucose, [6-^{14}C]glucose, [1-^{14}C]ribose, and [1,3-^{14}C]glycerol experiments. Distribution of ^{14}C over all the five carbons from this experiment can be explained by multiple recycling through the Krebs cycle and operation of malic enzyme. The distribution pattern from the [1-^{14}C]acetate experiment was similar to the [5-^{14}C]glutamate experiment. Radioactivity from [1,4-^{14}C]succinate resided almost exclusively in C-1 of I. On the other hand, radioactivity from [2,3-^{14}C]succinate was distributed over all the carbons. All these results can be reasonably explained by recycling through the Krebs cycle supplemented by the operation of malic enzyme.

Incorporation of Double-Labeled Glutamate into I. To determine if the α-amino acid structure of glutamate is incorporated intact into I, an experiment was performed utilizing [1-^{14}C;2-^{3}H]- and [5-^{14}C;2-^{3}H]glutamate. However, complete loss of ^{3}H was observed in both cases (Table II). An equilibrium between α-ketoglutarate can explain partial loss of ^{3}H as shown in the cell protein glutamate experiment (next section), but may not be able to explain the total loss of ^{3}H. The reaction mechanism involved in the later step(s) between glutamate and I may be responsible for the loss of the remaining ^{3}H.

Incorporation and Distribution of DL-[2-^{3}*H;5-*^{14}*C]Glutamate into Cell Protein Glutamate.* To confirm the randomization of glutamate by recycling through the Krebs cycle in growing *S. cacaoi* cells, it has become desirable to

Table I: Incorporation and Distribution of Labeled Compounds in Polyoxamic Acid (I).

Compounds Added [a]	Total μCi Added [b]	Sp Act. of I (mμCi/μmol)	Incorp. [c] (%)	Dilution [d] (×10³)	% ^{14}C Distribution [e] C-1 Ninhydrin	C-1 Periodate	C-2,3,4	C-5
D-[1-^{14}C]Glucose	43	0.071	1.4	*f*		22	74	4
D-[6-^{14}C]Glucose	97	0.22	1.9	*f*		20	76	4
D-[3,4-^{14}C]Glucose	7	0.000	0.00	*f*				
D-[1-^{14}C]Ribose	12	0.007	0.48	*f*		26	69	5
D-[1,3-^{14}C]Glycerol	23	0.011	0.40	*f*		24	71	5
[3-^{14}C]Pyruvate	12	0.025	2.9	40	12	16	82	4
DL-[3-^{14}C]Serine	31	0.070	4.4	14	12	15	81	5
[1-^{14}C]Acetate	44	0.11	2.8	9.1	25	22	21	55
[2-^{14}C]Acetate	49	0.22	5.6	4.5	17	18	78	4
[1,4-^{14}C]Succinate	26	0.010	0.84	100	90	88	8	3
[2,3-^{14}C]Succinate	12	0.041	3.7	25	18	17	74	8
DL-[1-^{14}C]Glutamate	47	0.010	0.28	100	100	100	0	0
DL-[5-^{14}C]Glutamate	48	0.026	0.80	39	26	25	0	75

[a] Specific activity of all the carboxylic acids and amino acids added was 1 Ci/mol. [b] Twenty-five culture flasks containing 60 ml each of the organic medium were used for each experiment. A sterile solution of radioisotopes was added after 45-hr fermentation (Isono et al., 1975). [c] Since the degradation yield of I from the polyoxin complex was low and not consistent, the incorporation value was expressed on the basis of the total radioactivity of I present in the polyoxin complex. Total micromoles of the polyoxin complex was calculated from the total OD value (ϵ = 8000 at 262 nm). Calculation is as follows. Total μCi = specific activity of I (mμCi/μmol) (total OD of the polyoxin complex/8000). [d] Specific activity of the compound added/specific activity of I isolated. [e] Ninhydrin and periodate oxidation were performed as described under Materials and Methods. C-1 was obtained from the total disintegration per minute from the carbon dioxide evolved. C-5 was obtained from the specific activity of formaldimedone. C-2,3,4 was obtained by difference. [f] Dilution is not given since the medium contained much glucose and starch.

Table II: Incorporation of Double-Labeled Glutamate into Polyoxamic Acid (I).

Type of Glutamate	Glutamate Added Sp Act. (μCi/μmol) ^{3}H	^{14}C	^{3}H/^{14}C	I Isolated Sp Act. (mμCi/μmol) ^{3}H	^{14}C	^{3}H/^{14}C
DL-[1-^{14}C;2-^{3}H]Glutamate	1.83	0.440	4.16	0.000	0.088	0
DL-[5-^{14}C;2-^{3}H]Glutamate	1.92	0.435	4.42	0.000	0.123	0

Table III: Incorporation and Distribution of DL-[2-^{3}H;5-^{14}C]Glutamate into Cell Protein Glutamate.

Glutamate Added Sp Act. (Ci/mol) ^{3}H	^{14}C	^{3}H/^{14}C	Glutamate Isolated from Cell Protein Sp Act. (mμCi/μmol) ^{3}H	^{14}C	^{3}H/^{14}C	% Retention of ^{3}H	% Distribution of ^{14}C in C-1 [a]
1.26	0.46	2.75	0.500	0.522	0.96	35	5.9

[a] C-1 was obtained from the total disintegration per minute from the carbon dioxide evolved by ninhydrin oxidation. Oxidation procedure is given under Materials and Methods.

look into the cell protein glutamate. To get the best incorporation, radioactive glutamate was added in a logarithmic phase of growth, and cells were harvested as soon as they reached a stationary phase. As shown in Table III, ^{14}C was indeed found to be distributed again 6% in C-1 as determined by carbon dioxide evolved by ninhydrin oxidation. Authentic [5-^{14}C]glutamic acid was oxidized under the same conditions. Only 0.75% of the radioactivity was found in carbon dioxide evolved. A lower value compared to that of I (25%) may be largely attributed to the different growth phase employed in each experiment. It is worth noting that ^{3}H on C-2 was retained 35% in this case, which shows 65% of ^{3}H was lost by an equilibrium between α-ketoglutarate.

Discussion

The biosynthesis of the maleimide ring of one of the C-nucleoside antibiotics, showdomycin, was studied with *Streptomyces showdoensis* (Elstner and Suhadolnik, 1971, 1972; Elstner et al., 1973). This study shows that C-2 to C-5 of α-ketoglutarate was incorporated asymmetrically into the maleimide ring. An antibiotic, acetylene dicarboxamide (cellocidin), was also shown to be biosynthesized by a *Streptomyces* from α-ketoglutarate in a similar way (Jones et al., 1973). In these papers, the incorporation and the distribution of radioactive mono- and dicarboxylic acids into these two antibiotics were reported. These distributions are

very similar to that described in this paper, although information about the metabolism of C-1 of glutamate was not reported in the above papers, since both antibiotics were formed only from C-2 to C-5 of α-ketoglutarate. The 2-pyrrolidone ring of the antibiotic, variotin, was also reported to come from C-2 to C-5 of glutamate, although the distribution data were not given in the study (Tanaka et al., 1962). The biosynthesis of bacterial meanaquinones (vitamin K_2) has been extensively studied and the carbon skeleton of naphthoquinone was shown to be provided with the seven carbons from shikimate and C-2 to C-4 of glutamate or α-ketoglutarate (Campbell et al., 1971). A primary addition product of shikimate and α-ketoglutarate is believed to be *o*-succinylbenzoic acid, which is a precursor of a number of plant naphthoquinones and anthraquinones (Dansette and Azerad, 1970).

To accomplish the biosynthesis of I from glutamate, the γ-carboxyl group must be reduced to the hydroxymethyl group. Also there is a complete loss of ^3H at C-2. There are two likely pathways to convert glutamate to I, and both may be able to explain this total loss of ^3H. The first pathway (A) is to always have α-ketoglutarate as an intermediate; the other pathway (B) is not to have α-ketoglutarate as an intermediate. Finding the enzyme ω-hydroxy-α-amino acid dehydrogenase from *Neurospora crassa* (Yura and Vogel, 1957, 1959) lends support to the fact that pathway B actually exists. The loss of ^3H in pathway A is obvious, but its loss in pathway B needs explanation. Tritium loss from C-2 in pathway B might be related to oxidation of C-3 and/or C-4. This hypothesis is now being currently investigated in this laboratory by the synthesis and incorporation of ^{14}C-, ^3H-, and ^{15}N-labeled α-amino-δ-hydroxyvaleric acid.

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References

Ashwell, G. Kanfer, J., and Burns, J. J. (1959), *J. Biol. Chem. 234,* 472.

Bray, G. A. (1960), *Anal. Biochem. 1,* 279.

Campbell, I. M., Robins, D. J., Kelsey, M., and Bentley, R. (1971), *Biochemistry 10,* 3069.

Dansette, P., and Azerad, R. (1970), *Biochem. Biophys. Res. Commun. 40,* 1090.

Elstner, E. F., and Suhadolnik, R. J. (1971), *Biochemistry 10,* 3608.

Elstner, E. F., and Suhadolnik, R. J. (1972), *Biochemistry 11,* 2578.

Elstner, E. F., Suhadolnik, R. J., and Allerhand, A. (1973), *J. Biol. Chem. 248,* 5385.

Isono, K., Asahi, K., and Suzuki, S. (1969), *J. Am. Chem. Soc. 91,* 7490.

Isono, K., Funayama, S., and Suhadolnik, R. J. (1975), *Biochemistry 14,* 2992.

Isono, K., Nagatsu, J., Kawashima, Y., and Suzuki, S. (1965), *Agric. Biol. Chem. 29,* 848.

Isono, K., Nagatsu, J., Kobinata, K., Sasaki, K., and Suzuki, S. (1967), *Agric. Biol. Chem. 31,* 190.

Isono, K., Suzuki, S., and Azuma, T. (1971), *Agric. Biol. Chem. 35,* 1986.

Jones, E. R. H., Keeping, J. W., Pellatt, M. G., and Thaller, V. (1973), *J. Chem. Soc., Perkin Trans. 1,* 148.

Kanfer, J., Ashwell, G., and Burns, J. J. (1960), *J. Biol. Chem. 235,* 2518.

Tanaka, N., Sashikata, K., and Umezawa, H. (1962), *J. Gen. Appl. Microbiol. 8,* 192.

Yura, T., and Vogel, H. J. (1957), *Biochim. Biophys. Acta 24,* 648.

Yura, T., and Vogel, H. J. (1959), *J. Biol. Chem. 234,* 339.