A 500-mg. sample of this material was subjected to a fifty-transfer countercurrent distribution using ethyl acetate and 2 *M* phosphate buffer (K_2HPO_4–NaH_2PO_4).[4] Analysis of the curve obtained by following the ultraviolet absorption at 265 mμ indicated a homogeneity of approximately 90%. The material was recovered from the peak (K = 0.39), and this melted after recrystallization from 5% ethanol at 155–156°. The melting point depended on the rate of heating.

For analysis, a sample of A was recrystallized repeatedly from 5% ethanol as lustrous, long prisms, m.p. 155–156°, $[\alpha]^{20}D$ −13.6° (*c* 15 mg. in 1 ml. of dimethylformamide).

Anal. Calcd. for $C_{11}H_{14}N_2O_5S$: C, 46.1; H, 4.93; N, 9.79. Found: C, 46.2; H, 4.99; N, 9.50.

The material that had remained insoluble when the aqueous suspension was extracted with ethyl acetate was also collected by filtration; wt. 1.06 g. (10%), m.p. 181–184°. There was no depression in melting point upon admixture with a reference sample of *p*-tosyl-L-asparagine, m.p. 186.5–187°, prepared through direct tosylation of L-asparagine.

The *p*-tosyl-L-isoasparagine obtained as A after extraction of the crude product with ethyl acetate was of a degree of purity suitable for the following conversion to L-isoasparagine, as well as probably for other synthetic reactions. For more complete separation from the small amount of *p*-tosylasparagine remaining in A, a second extraction, using an aqueous suspension of A, appeared sufficient. Material which melted at 155° was recovered after this procedure in 81% yield.

L-Isoasparagine (VI).—A solution of 3.0 g. of V, (A), m.p. 154–155°, in 400 ml. of liquid NH_3 was held at its boiling point, and 1.1 g. of sodium was added in portions until a lasting blue color resulted. Acetic acid (2.2 ml.) was then cautiously added, and the NH_3 was allowed to evaporate. The white residue was dissolved in a small volume of warm water, the solution was adjusted to approximately *p*H 5, and boiling ethanol was added to incipient cloudiness. While still hot, the solution was rapidly centrifuged, removing the amorphous, ninhydrin-negative precipitate which had separated. On cooling, the centrifugate deposited 0.97 g. (65%) of soft needles, $[\alpha]^{20}D$ +14.7° (*c* 15 mg. in 1 ml. of 0.1 *N* HCl). The product was examined by electrophoresis on paper (Whatman No. 1), using barbital buffer, μ 0.05, *p*H 8.5 and 21 volts/cm. In this system a mixture of L-asparagine, L-isoasparagine and L-aspartic acid is well separated in 2.25 hr. After treatment of the dried paper with ninhydrin in acidified butanol, the L-asparagine is identified near the origin as a green spot, and the L-isoasparagine and L-aspartic acid are located approximately 3.5 and 5.5 cm. from the origin toward the anode as a pale reddish-blue spot and as a purple spot, respectively. A single spot characteristic of isoasparagine was obtained for the product under these conditions.

For analysis, the material was recrystallized twice as long needles from water–ethanol and was air-dried; $[\alpha]^{20}D$ +14.9°, reported[3] $[\alpha]^{18}D$ +15.5° (*c* 1.55, 0.1 *N* HCl).

Anal. Calcd. for $C_4H_8N_2O_3 \cdot H_2O$: C, 32.0; H, 6.72; N, 18.7. Found: C, 32.2; H, 6.85; N, 18.6.

A 200-mg. sample of the L-isoasparagine was reconverted to the *p*-tosyl derivative by treatment with 400 mg. of *p*-tosyl chloride in 0.8 ml. of acetone and 3 ml. of 1 *N* sodium hydroxide for 1.5 hr. On acidification of the solution 260 mg. melting at 153–154.5° was obtained. Recrystallization yielded long prisms melting at 156–156.5°.

***p*-Toluenesulfonyl-L-asparagine.**—A solution of 12.8 g. of *p*-tosyl chloride in acetone was added with stirring to a solution of 7.2 g. of L-asparagine hydrate in 55 ml. of 1 *N* sodium hydroxide. An equal quantity of 1 *N* sodium hydroxide was added in portions over the next 40 min. When after the last addition the *p*H had fallen to 7, excess *p*-tosyl chloride was removed by filtration. The clear solution was then concentrated under reduced pressure to remove acetone.

The solution was acidified to *p*H 3 and cooled. The white crystalline material which separated was collected by filtration and dried; wt. 12 g., m.p. 183–184°. The product was recrystallized once from 30% ethanol as elongated plates. Some of these were 4.5 cm. in length, wt. 10.7 g. (78%), m.p. 186.5–187°, $[\alpha]^{20}D$ −10.3° (*c* 15 mg. in 1 ml. of dimethylformamide); reported[22,23] m.p. 175°. The melting point depended on the rate of heating.

Anal. Calcd. for $C_{11}H_{14}N_2O_5S$: C, 46.1; H, 4.93; N, 9.79. Found: C, 46.4; H, 5.07; N, 9.75.

The author wishes to thank Mr. Joseph Albert for the microanalyses reported herein.

(22) S. Berlingozzi, *Gazz. chim. ital.*, **57**, 814 (1927).

(23) Very recently, a melting point of 191° (rate 4° per minute) has been reported (M. Zaoral and J. Rudinger, *Collection Czechoslov. Chem. Commun.*, **6**, 1993 (1959)).

NEW YORK, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, OKLAHOMA AGRICULTURAL EXPERIMENT STATION, OKLAHOMA STATE UNIVERSITY]

Biosynthesis of Gliotoxin. II.[1,2] Further Studies on the Incorporation of Carbon-14 and Tritium-labeled Precursors

BY J. A. WINSTEAD[3] AND R. J. SUHADOLNIK

RECEIVED AUGUST 3, 1959

Trichoderma viride incorporated DL-phenylalanine-H^3, DL-*m*-tyrosine-H^3, DL-methionine-CH_3-C^{14}, DL-serine-3-C^{14}, DL-serine-1-C^{14} and glycine-2-C^{14} into the gliotoxin, which is secreted into the medium. These results furnish additional evidence that phenylalanine is a precursor for the indole moiety. The incorporation of *m*-tyrosine indicates that hydroxylation can occur before cyclization of the aliphatic side chain of phenylalanine. The N-methyl group of gliotoxin produced from methionine-CH_3-C^{14}, serine-3-C^{14}, glycine-2-C^{14} and serine-1-C^{14} was found to contain 72, 25, 19 and 0% of the radioactivity, respectively. Radioactive gliotoxin produced from the serine-3-C^{14} was degraded to indole-2-carboxylic acid. This degradation product contained 19% of the radioactivity. All of the radioactivity incorporated into the gliotoxin from serine-1-C^{14} and 56% from serine-3-C^{14} was in carbon atoms 3, 3a and 4. A biosynthetic pathway which accounts for all 13 carbon atoms of gliotoxin is proposed.

The incorporation of phenylalanine-1-C^{14} and phenylalanine-2-C^{14} into gliotoxin by *Trichoderma viride* has been reported.[4] Alkaline degradation of gliotoxin from phenylalanine-1-C^{14} resulted in indole-2-carboxylic acid which retained 82% of the radioactivity with essentially all of the radioactivity in the carboxyl carbon. Thus, phenylalanine appeared to be a direct precursor of the indole moiety of gliotoxin. It was considered of interest to conduct further experiments to determine if the aromatic ring of phenylalanine were incorporated into

(1) This investigation was supported in part by a research grant from the United States Atomic Energy Commission, Contract No. AT(11-1)-71, Project No. 7.

(2) Presented in part at the 135th meeting of the American Chemical Society, Boston, Mass., March 1959.

(3) From the thesis submitted by Jack A. Winstead in partial fulfillment of the requirements for the Master of Science Degree at the Oklahoma State University.

(4) R. J. Suhadolnik and R. G. Chenoweth, THIS JOURNAL, **80**, 4391 (1958).

gliotoxin and if *m*-tyrosine were a subsequent intermediate. The precursors of the remaining carbon atoms of gliotoxin were also investigated. This communication presents evidence that phenylalanine is incorporated into gliotoxin as a nine-carbon unit and that *m*-tyrosine can serve as an intermediate between phenylalanine and gliotoxin. Methionine-CH_3-C^{14}, serine-3-C^{14} and glycine-2-C^{14} served as methyl donors and were incorporated into the N-methyl group of gliotoxin. Serine-3-C^{14} and serine-1-C^{14} were shown to be incorporated into carbon atoms 3, 3a and 4.

Experimental[5]

Isotopes.—The DL-serine-3-C^{14}, DL-serine-1-C^{14}, glycine-2-C^{14} and DL-methionine-CH_3-C^{14} were obtained from the Volk Radiochemical Company. The DL-phenylalanine and DL-*m*-tyrosine were obtained from the California Foundation for Biochemical Research and were labeled with tritium by the tritium exchange method reported by Wilzbach.[6] The labile tritium in the *m*-tyrosine-H^3 was exchanged with water to a constant specific activity. A paper chromatogram[7] of the crystalline *m*-tyrosine-H^3 using Whatman No. 1 filter paper and a pyridine–butanol–water mixture (6:4:3) gave four radioactive peaks as determined by a windowless chromatogram scanner. One peak remained at the origin, and only one peak was ninhydrin positive. To purify further the tyrosine-H^3, 20 mg. of the crystalline *m*-tyrosine-H^3 was dissolved in 2 ml. of water and placed on an ion-exchange column of Dowex 50-H. The column was developed with 100 ml. of each of the following: water, 0.15 *N* HCl, 0.3 *N* HCl, 0.6 *N* HCl, 1.5 *N* HCl and 4 *N* HCl. The rate of flow was 2 ml./min., and 4-ml. fractions were collected.

The ninhydrin-positive material from the ion-exchange column, when chromatographed on paper, had one radioactive peak and was identified by comparison of the R_f with that of authentic *m*-tyrosine. The radioactivity of the purified *m*-tyrosine-H^3 solution from one fraction was determined and used in subsequent experiments.

The phenylalanine-H^3 was purified by the same procedure as the *m*-tyrosine. A paper chromatogram of the crystalline phenylalanine-H^3 indicated four radioactive components. Chromatography of the impure phenylalanine-H^3 on Dowex 50-H resulted in incomplete purification. Repetition of this procedure gave pure phenylalanine-H^3 which was eluted with 0.6–1.5 *N* HCl. It was identified by the ninhydrin test and by comparison of its R_f with the R_f of authentic phenylalanine.

Fermentation Procedure.—The fermentation procedure used was the same as reported by Suhadolnik and Chenoweth.[4] The organism used in these experiments was *T. viride*, No. 1828 NRRl which was kindly supplied by Dr. C. W. Hesseltine of the Northern Utilization Research Branch, Peoria, Illinois. The yield of isolated gliotoxin was 25 to 90 mg./l.

Administration of Isotopes.—The carbon-14 and tritium-labeled compounds used in this study were added to the medium gradually during the growing period. The compounds studied and the times of addition are shown in Table I.

Isolation of Gliotoxin.—The procedure reported[4] was used with the exception that some of the isolations were crystallized from ethanol instead of methanol and all isolations were crystallized at −10° rather than 2°. An infrared spectrum[8] of gliotoxin prepared as a potassium bromide pellet was the same as the infrared spectrum reported by Johnson.[9] The ultraviolet absorption spectrum showed an absorption maximum in agreement with that reported by Johnson.[9]

Alkaline Degradation of Gliotoxin.—A method similar to that of Bruce, *et al.*,[10] was used to isolate the N-methyl group of gliotoxin as methylamine and was applied to the gliotoxin isolated from flasks 7, 16, 17 and 26. Fifty mg. of carrier gliotoxin was added to 50 mg. of the radioactive gliotoxin isolated from these experiments. Ten ml. of 10% NaOH was added to the gliotoxin and the solution was refluxed for 1 hr. The volatile base was collected in 0.1 *N* HCl. The acidic solution of methylamine hydrochloride was taken to dryness in a rotatory evaporator and then dried *in vacuo* over phosphorus pentoxide. A white deliquescent solid melting at 220–222° was obtained. Authentic methylamine hydrochloride melted at 222–224°. Addition of pure methylamine hydrochloride caused no depression of mixed melting point. The yields were approximately 75% that of theoretical.

TABLE I

LABELED COMPOUNDS ADDED TO THE FERMENTATION MEDIUM

Flask no.	Substrate	Specific activity, mc./mmole	Amt. of compd. added Mg.	Mμc.	Time of addition, hr.
7	DL-Serine-3-C^{14}	2.02	0.26	5,000	44–53
8	DL-Serine-3-C^{14}	2.02	.26	5,000	44–53
9	DL-Serine-3-C^{14}	2.02	.26	5,000	0–49
15	Glycine-2-C^{14}	1.68	.49	10,980	23–50
16	Glycine-2-C^{14}	1.68	.49	10,980	23–50
17	DL-Methionine-CH_3-C^{14}	1.96	.39	5,170	42–65
18	DL-Methionine-CH_3-C^{14}	1.96	.39	5,170	42–65
20	DL-*m*-Tyrosine-H^3	9.60[a]	.25[b]	2,400	27–65
21	DL-*m*-Tyrosine-H^3	9.60[a]	.25[b]	2,400	27–65
24	DL-Phenylalanine-H^3	5.50[a]	2.50[b]	13,700	27–47
25	DL-Phenylalanine-H^3	5.50[a]	2.50[b]	13,700	27–47
26	DL-Serine-1-C^{14}	1.77	1.10	18,500	31–52

[a] μc./ml. [b] Ml.

The radioactive gliotoxin from flasks 9 and 26 was degraded by the method of Suhadolnik and Chenoweth[4] to yield indole-2-carboxylic acid. The product melted at 200–204° and the yields were about 10% that of theoretical.

Kuhn–Roth Degradation of Dethiogliotoxin.—Dethiogliotoxin was prepared from the gliotoxin isolated from flasks 7, 8, 15–16 and 17–18 by the method of Bell.[11] Two hundred mg. of labeled gliotoxin with 200 mg. of carrier gliotoxin was added to 100 ml. of 95% ethanol and dissolved over a steam cone. The solution was cooled to room temperature and treated under nitrogen with 0.8 g. of aluminum foil, which had been cut into 1 cm. squares, amalgamated with 5% aqueous mercuric chloride for 30–45 sec. (with constant stirring) and washed several times with water. The amalgamated aluminum was used immediately. During the initial stage of the reaction the temperature increased from 25° to about 40°. The amalgamation was almost complete after 3 hr. At this time an additional 0.4 g. of amalgamated aluminum was added, and the reaction was continued for another 4 hr. The mixture was then heated to boiling and filtered hot. The residue was treated with 50 ml. of 95% ethanol, boiled for several minutes and filtered. The combined filtrates were evaporated to a volume of 7 ml. After standing at −10° for 24 hr. the dethiogliotoxin was filtered and dried. In each of the above degradations of gliotoxin, approximately 80 mg. of dethiogliotoxin was obtained which melted between 246–250°. A second crop of approximately 40 mg. was obtained by evaporating the mother liquor to 1 ml. The combined yields from all degradations were 30–40%. Johnson and Buchanan[12] and Bell[11] reported a melting point range of 246–252°. The infrared[8] and ultraviolet absorption spectra of the dethiogliotoxin were the same as that reported by Johnson.[9]

The dethiogliotoxin samples were degraded by the Kuhn–Roth procedure,[13] a micromethod for detecting the presence

(5) All melting points are uncorrected.

(6) K. E. Wilzbach, THIS JOURNAL, **79**, 1013 (1957).

(7) Presented at the 134th meeting of the American Chemical Society, Chicago, Ill., September 1958.

(8) Courtesy of Dr. N. Deno, Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania.

(9) J. R. Johnson, "Roger Adams Symposium," John Wiley and Sons, Inc., New York, N. Y., 1954, pp. 60–90.

(10) W. F. Bruce, J. D. Dutcher, J. R. Johnson and L. L. Miller, THIS JOURNAL, **66**, 614 (1944).

(11) M. R. Bell, private communication.

(12) J. R. Johnson and J. B. Buchanan, THIS JOURNAL, **75**, 2103 (1953).

(13) R. Kuhn and H. Roth, *Ber.*, **66**, 1274 (1933).

TABLE II

INCORPORATION OF ISOTOPES INTO GLIOTOXIN[a]

Flask no.	Substrate	Gliotoxin yield, mg.	Isotope, mμc.	Incorporated, %
7	DL-Serine-3-C^{14}	350	137	2.74
8	DL-Serine-3-C^{14}	429	121	2.42
9	DL-Serine-3-C^{14}	217	36	0.72
15	Glycine-2-C^{14}	160	97	0.88
16	Glycine-2-C^{14}	159	128	1.17
17	DL-Methionine-CH_3-C^{14}	262	175	3.39
18	DL-Methionine-CH_3-C^{14}	165	90	1.74
20	DL-*m*-Tyrosine-H^3	524	739	30.8
21	DL-*m*-Tyrosine-H^3	418	1062	44.3
24	DL-Phenylalanine-H^3	310	2405	17.6
25	DL-Phenylalanine-H^3	320	1950	14.2
26	DL-Serine-1-C^{14}	485	355	1.92

[a] See Table I for amount of substrate added.

TABLE III

CARBON-14 FOUND IN THE METHYLAMINE HYDROCHLORIDE

Flask no.	Substrate added	Sample Mg.	Sample Mμc.	Specific activity of $CH_3NH_2 \cdot HCl$ av.,[a] mμc./mmole	Specific activity of gliotoxin,[b] mμc./mmole	Carbon-14 in methylamine, %
7	DL-Serine-3-C^{14}	5.12	1.21	32.0	128	25.0
		5.12	1.23			
16	Glycine-2-C^{14}	3.52	1.20	50.4	262	19.3
		3.52	1.43			
17	DL-Methionine-CH_3-C^{14}	6.20	6.90	158.0	218	72.4
		6.20	7.60			
26	DL-Serine-1-C^{14}	2.48	0.00	0.0	239	0.0
		2.48	0.00			

[a] Corrected for dilution (2-fold). [b] Based on mg. of gliotoxin and mμc. incorporated (see Table II).

TABLE IV

KUHN-ROTH DEGRADATION OF DETHIOGLIOTOXIN

Flask no.	Substrate added	Dethiogliotoxin degraded Mμc./mmole	Dethiogliotoxin degraded Mg.	Dethiogliotoxin degraded Mμc.	Distillate dethiogliotoxin, meq./mmole	Radioactivity Distillate, mμc.	Radioactivity CO_2, mμc.
7	DL-Serine-3-C^{14}	67	12.8	3.25	0.68	0.19	0.78
7	DL-Serine-3-C^{14}	67	16.8	4.26	..	..	1.08
8	DL-Serine-3-C^{14}	49	20.0	3.7	.76	.14	..
15, 16	Glycine-2-C^{14}	110	20.0	8.3	.32	.22	3.00
17, 18	DL-Methionine-CH_3-C^{14}	103	16.4	6.40	.35	.70	0.29

of C–CH_3 groups by oxidation to acetic acid. Two ml. of concentrated H_2SO_4 was added to 10–20 mg. of dethiogliotoxin. Eight ml. of 5 *N* chromic acid was then added. This mixture was refluxed for 1.5 hr. and the CO_2 evolved during this time was collected in excess NaOH. After 1.5 hr. the sample was distilled into a known volume of *N*/100 NaOH and then back titrated with *N*/100 acetic acid to a phenolphthalein end-point. The distillate was not identified. The distillate was taken to dryness, combusted and counted for radioactivity. The alkaline solution containing the CO_2 collected during reflux was evaporated to a small volume and transferred to a combustion tube. Perchloric acid (35%) was then added to the alkaline samples and the CO_2 evolved was passed through a Dry Ice–acetone trap, collected in an ionization chamber and counted. In control experiments DL-serine-3-C^{14} and DL-serine-1-C^{14} were degraded by the above procedure.

Counting Procedures.—All of the carbon-14 samples from the isolations described were combusted by the method of Van Slyke, *et al.*[14] The CO_2 was collected in an ionization chamber and counted with a vibrating reed electrometer. The tritium-containing samples were prepared for counting by the procedure of Wilzbach, *et al.*[15]

(14) D. D. Van Slyke, R. Steele and J. Plazin, *J. Biol. Chem.*, **192**, 769 (1951).

(15) K. E. Wilzbach, L. Kaplan and W. G. Brown, *Science*, **118**, 522 (1953).

Results and Discussion

By using a chemically defined fermentation medium, it has been possible to study the incorporation of tritium and carbon-14 labeled suspected precursors into gliotoxin. The yields and the incorporation of the radioisotopes into the isolated gliotoxin from the substrates studied are shown in Table II.

The incorporation of the tritium-labeled phenylalanine into gliotoxin provides additional evidence that all nine carbon atoms of phenylalanine are incorporated into gliotoxin. The presence of the hydroxyl group in the *meta*-position of phenylalanine suggests *m*-tyrosine as a possible precursor of the nine carbon fragment of gliotoxin. The data reported in Table II indicate that DL-*m*-tyrosine-H^3 is incorporated into gliotoxin and may serve as a nine-carbon intermediate between phenylalanine and gliotoxin. The data also suggest that hydroxylation can occur before cyclization of the aliphatic side chain of phenylalanine.

The results of the studies concerning the precursor for the N-methyl carbon of gliotoxin are given in Table III. The alkaline degradation of gliotoxin from DL-methionine-CH_3-C^{14} showed that 72.4% of the radioactivity was in the N-methyl group. Since serine-3-C^{14} and glycine-2-C^{14} were shown to be less efficient as methyl donors, these results indicate that the methyl group of methionine is a more direct methyl donor in gliotoxin biosynthesis.

Since the remaining three carbon atoms of gliotoxin (carbon atoms 3, 3a and 4) are structurally related to serine, it seemed reasonable that this amino acid might serve as a precursor. The studies conducted showed an incorporation of carbon-14 labeled serine into gliotoxin to the extent of 0.72–2.74%. Dutcher, Johnson and Bruce[16] reported

(16) J. D. Dutcher, J. R. Johnson and W. F. Bruce, THIS JOURNAL, **67**, 1736 (1945).

the formation of acetic acid from dethiogliotoxin by the Kuhn–Roth procedure. It was suggested that the acetic acid formed by this degradation arose from carbon atoms 3 and 3a of dethiogliotoxin. The dethiogliotoxin obtained from gliotoxin into which serine-3-C^{14} was incorporated, was degraded by the Kuhn–Roth procedure. The results are shown in Table IV. The distillate which should have contained carbon atoms 3 and 3a (as acetic acid) had comparatively little radioactivity, whereas the CO_2 collected during the period of reflux was more radioactive. Using serine as a model for carbon atoms 3, 3a and 4 of dethiogliotoxin, Johnson and Buchanan[12] reported the formation of acetic acid by the Barthel–LaForge procedure. To determine if serine gave rise to acetic acid, both serine-1-C^{14} and serine-3-C^{14} were degraded. Essentially all of the radioactivity was evolved as CO_2 during reflux.[17] On the basis of these data, it appears that the Kuhn–Roth degradation of dethiogliotoxin does not specifically yield acetic acid from carbon atoms 3 and 3a.

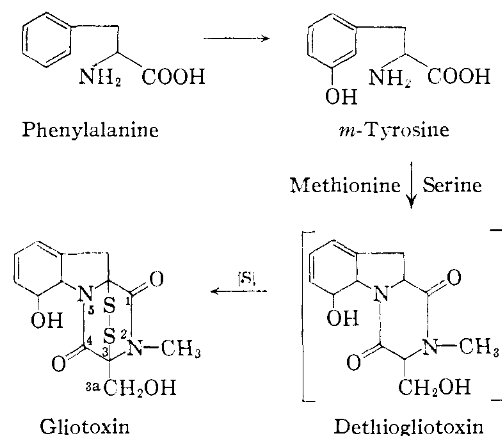
Alkaline degradation of the gliotoxin from the serine-3-C^{14} showed that 19% of the radioactivity was in the indole-2-carboxylic acid. Since 25% was found in the N-methyl group, 56% of the radioactivity is, therefore, located in carbon atoms 3, 3a and 4. The specific location of the radioactivity is being investigated further and will be reported later.

Results of experiments using serine-1-C^{14} showed that no radioactivity was present in the indole-2-carboxylic acid or the N-methyl group of gliotoxin. Thus, all of the radioactivity was in carbon atoms 3, 3a or 4. The fact that all of the radioactivity from serine-1-C^{14} is located in carbon atoms 3, 3a or 4 strongly suggests that this amino acid is a direct precursor for this portion of gliotoxin.

(17) To be published.

Since conversion of glycine to serine is known,[18] glycine-2-C^{14} was studied as the precursor for carbon atoms 3 and 3a of gliotoxin. Glycine was incorporated into gliotoxin to a smaller extent than serine. On the basis of the data obtained from the serine and glycine studies, it is postulate that glycine is converted to serine which, in turn, serves as the precursor for carbon atoms 3, 3a and 4 of gliotoxin.

Since the hydroxylation of phenylalanine can occur before cyclization of the side chain of phenylalanine, reduction of the aromatic ring probably occurs in a subsequent step. On the basis of this assumption and the data presented in this study, the following pathway for the biosynthesis of gliotoxin which accounts for all of the carbon atoms is proposed.



(18) J. S. Fruton and S. Simmonds, "General Biochemistry," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 771–778.

STILLWATER, OKLAHOMA

[CONTRIBUTION FROM THE MERCK SHARP AND DOHME RESEARCH LABORATORIES, DIVISION OF MERCK AND CO., INC.]

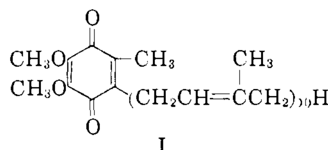
Coenzyme Q. XII. Ethoxy Homologs of Coenzyme Q_{10}. Artifact of Isolation

BY BRUCE O. LINN, NELSON R. TRENNER, BYRON H. ARISON, ROY G. WESTON, CLIFFORD H. SHUNK AND KARL FOLKERS

RECEIVED JULY 31, 1959

An artifact of coenzyme Q_{10} produced during the isolation from beef myocardial tissue has been discovered. Ethoxy and the diethoxy homologs of coenzyme Q_{10} have been characterized. Evidence for the probable mechanism of formation of these homologs indicates that Q_{10} had undergone alkali-catalyzed ethanolysis, a unique reaction of methoxy-1,4-benzoquinones. A method of isolation which avoids ethanolysis of Q_{10} is given. Comparison of our data on ethoxy homologs with those of early ubiquinone preparations shows close similarities and differentiates these compounds from coenzyme Q_{10}.

We have described[1] our initial experience with the isolation of coenzyme Q_{10} (I) from beef myocardial tissue. The product melted at 49.5–50.5°, and there was no evidence for the presence of other quinones of the same class. However, further processing did yield crystalline materials, m.p. *ca.* 45–48°, which were revealed to contain two quinones by paper chromatography. A critical examination of these lower-melting mixtures has revealed the presence of a homolog, which has been structurally elucidated. The mechanism of its origin has been determined; substantiating data were communicated.[2]



(1) B. O. Linn, A. C. Page, Jr., E. L. Wong, P. H. Gale, C. H. Shunk and K. Folkers, THIS JOURNAL, **81**, 4007 (1959).

(2) B. O. Linn, N. R. Trenner, C. H. Shunk and K. Folkers, *ibid.*, **81**, 1263 (1959).