Communications to the Editor

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Metabolism of Lipoamide and Lipoic Acid in Rabbits

Gal, *et al.*[1] reported the presence of an unidentified lipoic acid conjugate, which migrated to the front of radioautogram with the butanol system (see below), in the urine of rats following intraperitoneal injection of DL-lipoic[^{35}S] acid. A similar compound was also found by Grisebach, *et al.*[2] in the *Scenedesmus* cells after incubation with DL-lipoic-[^{35}S] acid. It was found that the conjugate had activity as a pyruvate oxidation factor[1] and upon treatment with acid or lipase, yielded lipoic acid or its sulfoxide.[2] However, nature of the labeled metabolic product, which is a relatively minor component in the urine of rats and which remains near the origin of radioautogram remained unsolved. The report of Patterson, *et al.*[3] stated that an unidentified form of lipoic acid found in the urine of rats supports the growth of *Corynebacterium* but not that of *St. faecalis*.

In the course of investigation on the metabolism of lipoamide, some unknown metabolic products which seemed to be different from hitherto described conjugate forms of lipoic acid,[1~4] were found as the main component, besides the known forms of lipoic acid, in the urine of rabbits after oral administration of lipoamide.

Male rabbits (3 kg. average weight) were orally administered with 100 mg./kg. of DL-lipoic acid or DL-lipoamide. The urine was collected from each rabbit by catheterization during six hours following the administration and submitted to chromatography with butanol saturated with 0.5*N* ammonia (butanol system). The content of unknown metabolites, lipoic acid and its analogs, on the paper was determined microbiologically[5] by a slight modification of Stokstad's method,[6] using *St. faecalis* R as the test organism and DL-lipoic acid as a standard. As shown in Table I, about 25~35% of the lipoic acid activity*[1] in the urine was due to this unknown metabolites. Fear of the artifact of lipoamide was excluded by the fact that such materials were not produced when a solution of authentic lipoic acid or lipoamide was left standing with control rabbit urine even for 20 hours at room temperature.

The unknown metabolite in the urine of rabbit administered with lipoamide was further purified in the following way. The urine was concentrated in vacuum and lipoamide, lipoic acid, etc., in the residue were eliminated by pyridine extraction. The remaining metabolite was extracted with 80% phenol. After the phenol solution was washed with water, phenol was removed by ether. The residual aqueous solution (phenol fraction) was chromatographed with isopropanol-0.5*N* ammonia (80:20) (propanol system) and used for polarographic analysis. According to the data in Fig. 1, most of the activity

*[1] Calculated from the lipoic acid activity in the urine (Table I), only 0.8~2.0% of the administered lipoamide was excreted in the urine during six hours. It has been suggested that the lipoamide administered is converted to lipoic acid in the body.[7] This conversion in the liver and blood was already described by Mori[8] and by Saito.[9]

1) E. M. Gal, D. E. Razenska : Arch. Biochem. Biophys., **89**, 253 (1960).
2) H. Grisebach, R. C. Fuller, M. Calvin : Biochem. et Biophys. Acta, **23**, 34 (1957).
3) A. Patterson, H. P. Broquist, M. H. von Saltza, A. Albrecht, E. L. R. Stokstad, T. H. Jukes : Am. J. Clin. Nutrition, **4**, 269 (1956).
4) H. Nawa, W. P. Brady, M. Koike, L. J. Reed : J. Am. Chem. Soc., **82**, 896 (1960).
5) E. Yamazaki, M. Nakamura, F. Tanaka : Vitamins (Kyoto), **20**, 540 (1960).
6) E. L. R. Stokstad : "Method of Biochemical Analysis," **3**, 23. (1956).
7) F. Tanaka : Unpublished data.
8) K. Mori : Seikagaku, **32**, 514 (1960).
9) J. Saito : Vitamins (Kyoto), **21**, 359 (1960).

Table I. Urinary Metabolites[a] after Oral Administration of Lipoamide or Lipoic Acid to Rabbits

Subst. administered	Exptl. No.	Unknown metabolites[b] Rf 0.02 (γ/cc.)	β-Lipoic acid Rf 0.25 (γ/cc.)	α-Lipoic acid Rf 0.57 (γ/cc.)	[c] Rf 0.66 (γ/cc.)	α-Lipoamide Rf 0.86 (γ/cc.)	Total (γ/cc.)	Urine volume (cc.)
Lipoamide	I	59.0	43.5		59.3	5.4	167.2	25
Lipoamide	II	15.8	14.9		34.9	0.6	66.2	36
Lipoamide	III	183.7	57.2		294.9	99.0	634.8	10
Lipoic acid	IV	376.3	113.0	133.3			622.6	22

a) Calculated as lipoic acid. The paper strips were developed by the descending technique with butanol system. Dried and cut-out papers were used for microbiological (tube method, 4-cc. scale/tube) assay.

b) Although three different metabolites (metabolite-I, II, and III) (see below) were present in this fraction, none of these metabolites were separated well by paper chromatography with the butanol system and the content represented is that of a mixture.

c) The Rf values of authentic α-lipoic acid and β-lipoamide are so similar that it is difficult to distinguish them when the sample contained either or both compounds. It is assumed that there are α-lipoic acid and a little β-lipoamide in the Rf 0.66 material.

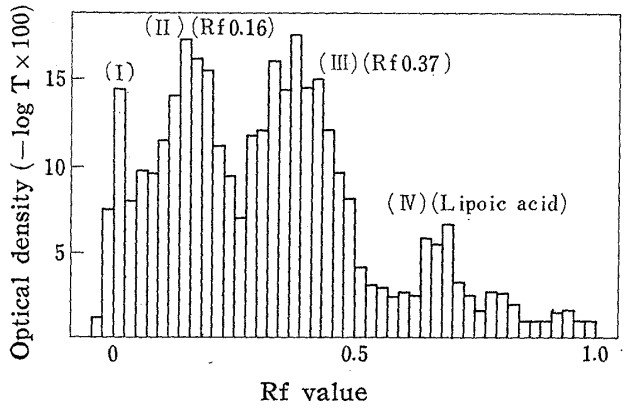

Fig. 1. Bioautography of Phenol Fraction

The paper strip was developed by descending technique with the propanol system. The lipoic acid activity on the strip was determined microbiologically as in Table I and expressed as the optical density.

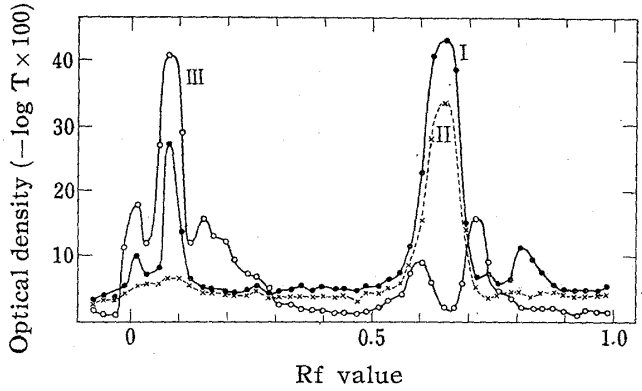

Fig. 2. Bioautography of Phenol Fraction treated with Various Reagents

The conversion of metabolites to other compound which has different Rf value

(The method of bioautography was the same as in Fig. 1)

0.2 cc. of phenol fraction containing about 30 γ/cc. of metabolite as lipoic acid was treated as follows :

I : Autoclaving with 2*N* H_2SO_4 (15 lb., 60 min.)

II : Autoclaving with 2*N* NaOH (15 lb., 60 min.)

III : Treatment with 2*N* HCl (room temperature, 30 min.)

in the phenol fraction was found on the paper corresponding to Rf 0.16 (II) and Rf 0.37 (III). Two other minor constituents (I and IV) were also observed in the phenol fraction, one of which agreed with the authentic lipoic acid (IV). The two major components changed to lipoic acid upon treatment with alkali even at room temperature. Upon treatment with acid at room temperature, they changed to a component with Rf 0.08 and also to lipoic acid by more drastic autoclaving in these acids. This conversion of the two major components to lipoic acid or some other unidentified components was effected not only with acid or alkali but also with sodium borohydride, sodium thioglycolate, iodine, sodium

thiosulfate, or potassium permanganate at room temperature, but not with hydrogen peroxide. It was also found that the phenol fraction shows two polarographic reduction waves in phosphate buffer of pH 6.8 at $E_{1/2}=-0.41$ and $E_{1/2}=-0.61$.

The two active components in the the phenol fraction were separated from each other by paper chromatography, using several different solvent systems successively. Both components were positive to potassium cyanide-nitroprusside. Details of these experiments will be published elsewhere.

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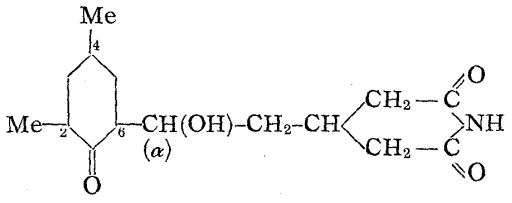
Absolute Configuration of Cycloheximide*1

Previous studies on the stereochemistry of cycloheximides made it possible to assume most of the configurations and conformations of the four isomers as illustrated in Table I and depicted in Chart 1.[1] However, it still remained to determine which of the configurations (I) or (II) is to be assigned to cycloheximide (Naramycin-A).

Table I. Configuration and Conformation of Cycloheximides

Isomers	Octant projection	Configuration	Conformation	[φ] Value at extrema of acetates
Cycloheximide (Naramycin-A)	(I) or (II)	4S:6S:αS	4-ax:6-eq	− 449° (at 312.5 mμ)
Naramycin-B	(VI)	2S:4S:6R:αS	2-ax:4-eq:6-eq	+2,923° (at 312.5 mμ)
Isocycloheximide	(V)	2R:4S:6R:αS	2-eq:4-eq:6-eq	+ 575°[b] (at 307.5 mμ)
α-Epiisocycloheximide[a]	(III)	2R:4S:6R:αR	2-eq:4-eq:6-eq	+ 449° (at 315 mμ)

a) A compound referred to as A_{II} in J. Antibiotics, **14A**, 158 (1961).
b) [φ] Value of synthesized acetyl-isocycloheximide (m.p. 166~167°).



Plane Structure of Cycloheximides

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*1 "Studies on Streptomyces Antibiotic, Cycloheximide. XVII.[2]"

1) Part IV. T. Okuda: This Bulletin, **7**, 659 (1959); Part VI. *Ibid.*, **7**, 671 (1959); Part VII. *Ibid.*, **8**, 335 (1960); Part XIV. Yakugaku Kenkyu, **33**, 532 (1961).

2) Parts XV and XVI. Presented before the 81st Annual Meeting of the Pharmaceutical Society of Japan (July, 1961). Preliminary Note. T. Okuda, M. Suzuki, Y. Egawa: J. Antibiotics, **14A**, 158 (1961).