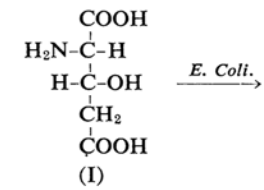
On the Absolute Configuration of l-Carnitine (Vitamin B_T)[*1]

By Takeo KANEKO and Ryonosuke YOSHIDA[*2]

(Received January 9, 1962)

The chemical structure of (−)-carnitine[1] or vitamin B_T[2,3] has been conclusively established by Tomita et al.[4] as (−)-γ-trimethyl-β-hydroxybutyrobetaine by synthesizing natural (−)-carnitine through the methylation of (−)-β-hydroxy-γ-aminobutyric acid, which was obtained by resolution of its racemic modification. The configuration of the β-asymmetric carbon atoms of (−)-β-hydroxy-γ-aminobutyric acid and (−)-carnitine, however, has not yet been established. In this paper the determination of these configurations by a chemical method is reported.

It has been known that the racemic modification of β-hydroxyglutamic acid could be decarboxylated by the action of decarboxylase and that the rate of the decarboxylation of the threo isomer is higher than that of the erythro isomer[5-7]. Recently, we resolved two racemic modifications of β-hydroxyglutamic acid to four optical isomers and determined their configurations[7]. One of the optical isomers, *threo*-β-hydroxy-L(+)-glutamic acid hydrochloride ($[\alpha]_D$ +9.5° in water), which was represented by the configurational formula I, was treated with glutamic acid decarboxylase of *E. Coli.*, and (−)-γ-amino-β-hydroxybutyric acid was isolated as the decarboxylated product of I by means of ion exchange chromatography, using Dowex 50. The melting points, specific rotations, R_f values and infrared spectra of the isolated γ-amino-β-hydroxybutyric acid and its benzoyl derivative were identical with those of the authentic specimens[*3], as is shown in Table I, and no depressions of melting point were observed.



(I)

threo-β-Hydroxy-L(+)-glutamic acid
or R(+)-Hydroxy-S-glutamic acid

[*1] Presented at the 14th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1961.

[*2] Present address: Central Institute of Ajinomoto Co., Inc., Kawasaki-shi.

1) W. L. Gulewitsch and R. Krimberg, *Z. physiol. Chem.*, **45**, 326 (1905).

2) G. Fraenkel, M. Blewet and M. Coles, *Nature*, **161**, 981 (1948).

3) E. Carter, P. K. Bhattacharyya, R. Weidmen and G. Fraenkel, *Arch. Biochem. Biophys.*, **38**, 405 (1952).

4) M. Tomita and Y. Sendju, *Z. physiol. Chem.*, **169**, 263 (1927).

5) S. Akabori, T. Kaneko, S. Sakurai and Y. Izumi, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **75**, 942 (1954).

6) W. W. Umbreit and P. Heneage, *J. Biol. Chem.*, **201**, 15 (1953).

7) T. Kaneko, R. Yoshida and H. Katsura, *J. Chem. Soc. Japan. Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **80**, 316 (1957).

[*3] The standard samples of (−)-γ-amino-β-hydroxybutyric acid and its benzoyl derivative were supplied by Dr. Y. Hirose and Mr. K. Nishimura.

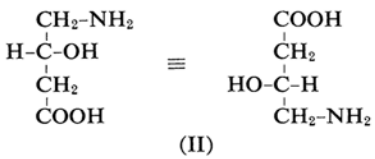
TABLE I. PHYSICAL PROPERTIES OF (−)-γ-AMINO-β-HYDROXYBUTYRIC ACID AND ITS BENZOYL DERIVATIVE

(−)-γ-Amino-β-hydroxybutyric acid	Decarboxylated product	Synthetic*3 sample	Cited in the literature[4]
M. p., °C (decomp.)	216～217	216～217	212
$[\alpha]_D$ (water)	−20.7°	−20.8°	−21.06°
R_f value			
80% phenol	0.43	0.43	
Butanol : acetic acid : water (4 : 1 : 1)	0.21	0.21	
N-Benzoyl derivative			
M. p., °C	112～113	111～112	114
$[\alpha]_D$ (water)	−11.2°	−11.9°	−11.84°

TABLE II. PHYSICAL PROPERTIES OF CARNITINE

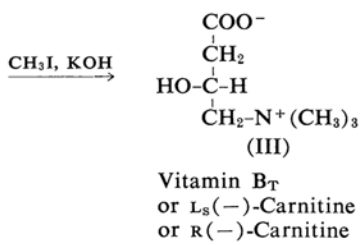
	Carnitine	a)	b)	c)
M. p., °C (decomp.)	197～198		196～198	210～212
$[\alpha]_D$ (water)	−23.9°	−20.98°	−21.5°	−30.9°
M. p., °C (decomp.)				
Chloroaurate	153～155	155	153～155	157
Chloroplatinate	228	220	216～219	

a) M. Tomita et al., *Z. physiol. Chem.*, **169**, 263 (1927).
b) H. E. Carter et al., *Arch. Biochem.*, **38**, 405 (1952).
c) E. Strack et al. *Z., physiol. Chem.*, **318**, 129 (1960).



(II)

Ls(−)-γ-Amino-β-hydroxybutyric acid
or 2,4-Didesoxy-4-amino-Lg-threonic acid
or R(−)-γ-amino-β-hydroxybutyric acid



Vitamin B_T
or Ls(−)-Carnitine
or R(−)-Carnitine

Scheme 1

Consequently, the configuration of the asymmetric carbon atom of (−)-γ-amino-β-hydroxybutyric acid must be the same as that of the β-asymmetric carbon atom of *threo*-β-hydroxy-L(+)-glutamic acid. Although the β-asymmetric carbon atom of the latter has a Ds configuration[7], a Ls or Lg configuration II to be assigned to that of (−)-γ-amino-β-hydroxybutyric acid, since the carboxyl group of (−)-γ-amino-β-hydroxybutyric acid corresponded to the γ-carboxyl group of *threo*-β-hydroxy-L(+)-glutamic acid. Strictly speaking, it should be designated as the R configuration according to the nomenclature of absolute configuration presented by Cahn, Ingold and Prelog[8].

(−)-γ-Amino-β-hydroxybutyric acid was methylated to its betaine with some modifications by Carter's method[9], which was applied to racemic amino acid. The properties of the betaine and its obtained salts were consistent

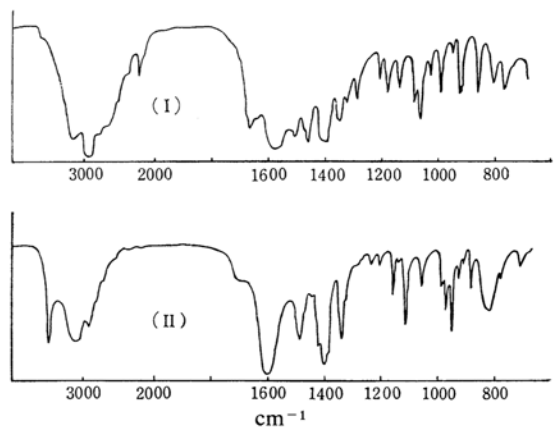


Fig. 1. Infrared spectrum of (I) R(−)-γ-amino-β-hydroxybutyric acid (Nujol), (II) R(−)-carnitine (KBr disk).

8) R. S. Cahn and C. K. Ingold, *J. Chem. Soc.*, **1951**, 612; R. S. Cahn, C. K. Ingold and V. Prelog, *Experientia*, **12**, 81 (1956).
9) H. E. Carter and P. K. Bhattacharyya, *J. Am. Chem. Soc.*, **75**, 2503 (1953).

with that of natural (−)-carnitine, as is shown in Table II.

As it is clear from these results that (−)-carnitine and (−)-γ-amino-β-hydroxybutyric acid have the same configuration, the natural (−)-carnitine may be designated as R(−)-carnitine.

Experimental*4

Decarboxylation of *threo*-β-Hydroxy-L(+)-glutamic Acid.—To 630 mg. of *threo*-β-hydroxy-L(+)-glutamic acid hydrochloride ($[\alpha]_D$ +9.5° (water)), 0.1 N sodium hydroxide was added until a pH of 5.0 was reached. To this solution, 4.5 g. of dry, powdered E. Coli. and 50 ml. of phosphate buffer (pH 5) were added, and the suspension was shaken mechanically at 37°C for 3 hr. until the theoretical amount of carbon dioxide was evolved. The reaction mixture was centrifuged, and the sediment was mixed with water and centrifuged again. The combined supernatant liquid was treated with charcoal under warming and filtered.

In another experiment, 731 mg. of *threo*-β-hydroxy-L(+)-glutamic acid hydrochloride was treated as mentioned above. The combined filtrate was passed through a column (4×50 cm.) of Dowex 2-X8 (50~100 mesh, H form), which was then washed thoroughly with water. The elution of the amino acid from the column was carried out first with 1800 ml. of 0.05 N acetic acid and then with 2000 ml. of 0.1 N acetic acid, using a 15 ml. fraction collector. An aliquot of each fraction was subjected to paper chromatography to test it for the presence of the amino acid. The combined solution of tubes number 237 to 258, which contained γ-amino-β-hydroxybutyric acid, was concentrated in vacuo, and the residue was crystallized by adding ethyl alcohol; m. p., 208~209°C (decomp.); yield, 600 mg. The product was recrystallized from water and ethyl alcohol repeatedly to give 236 mg. of pure crystals; m. p. 216~217°C (decomp.), $[\alpha]_D^{20}$ −20.7° (*c* 1.83, water). The melting point was not depressed on admixture with the authentic specimen. The R_f values of the sample were 0.43 and 0.21, using 80% phenol and *n*-butanol: acetic acid: water (4:1:1) as the solvents. Its infrared spectrum and other properties were identical with those of the authentic specimen.

Found: C, 40.17; H, 7.66; N, 11.67. Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76%.

***N*-Benzoyl-(−)-γ-amino-β-hydroxybutyric Acid.** Two hundred and eighty eight milligrams of (−)-γ-amino-β-hydroxybutyric acid and 0.5 g. of sodium carbonate were dissolved in 8 ml. of water, and the solution was cooled in an ice bath. To this solution 0.4 g. of benzoyl chloride was added, and the mixture was vigorously stirred for 2 hr. The solution was extracted with ether to remove the benzoic acid; then the aqueous layer was concentrated. (−)-*N*-Benzoyl-γ-amino-β-hydroxybutyric acid separated out as a solid which was removed by filtration; after recrystallization from water, 0.2 g. of monohydrate was obtained; m. p., 70~85°C. The anhydrous crystals were obtained by recrystallization from ethyl acetate; m. p. 112~113°C, $[\alpha]_D^{23}$ −11.2° (*c* 1.43, water), p*K*a' 4.45. The melting point was not depressed on admixture with the authentic specimen. The infrared spectrum was also identical with that of the authentic specimen.

Found: C, 59.17; H, 5.96; N, 6.39. Calcd. for $C_{11}H_{13}O_4N$: C, 59.18; H, 5.87; N, 6.28%.

The optically pure (−)-γ-amino-β-hydroxybutyric acid could be recovered by refluxing the *N*-benzoyl-derivative with 48% hydrogen bromide for 4 hr.

(−)-Carnitine.—A solution of 5.8 g. of methyl iodide in 60 ml. of methanol was added to a solution of 1.2 g. of (−)-γ-amino-β-hydroxybutyric acid ($[\alpha]_D$ −20.3° (water)) and 2.2 g. of potassium hydroxide in 10 ml. of water. After standing overnight at room temperature, the mixture was concentrated in vacuo and the residue was dissolved in 50 ml. of water. The solution was extracted four times with 50 ml. of 80% phenol[9]. After the combined phenol extract had been washed with 20 ml. of water, 600 ml. of ether was added. The aqueous layer was separated, and the ether-phenol layer was extracted four times with 50 ml. of water. The combined aqueous layer was washed with 150 ml. of ether and passed through an anion exchanger column (IR4B, 3×25 cm.), which was then washed with 500 ml. of distilled water. The alkaline fraction of the effluent was concentrated in vacuo to a syrupy residue, which crystallized on standing in a desiccator. It was filtered using ethyl alcohol-acetone (2:3); yield, 1.0 g. After recrystallization two times from the same mixed solvent, 0.5 g. of hygroscopic crystals were obtained. M. p., 197~198°C (decomp.) $[\alpha]_D^{30}$ −23.9° (*c* 0.86, water).

Found: C, 52.17; H, 9.59; N, 9.08. Calcd. for $C_7H_{15}O_3N$: C, 52.15; H, 9.38; N, 8.69%.

(−)-Carnitine chloroaurate was obtained by the usual method[4]. M. p. 153~155°C (decomp.)

Found: C. 17.02; H, 3.28; N, 2.62. Calcd. for $C_7H_{16}O_3N \cdot AuCl_4$: C, 16.77; H, 3.22; N, 2.79%.

(−)-Carnitine chloroplatinate was also obtained[4]. M. p., 228°C (decomp.)

All properties of the synthetic product were practically identical with those of natural (−)-carnitine as cited in the literature.

The authors wish to express their deep thanks to Dr. Yoshio Hirose and Mr. Kiichi Nishimura of the Research Institute for Food Chemistry, Foundational Juridical Persons, for their kind gift of authentic samples of (−)-γ-amino-β-hydroxybutyric acid and its benzoyl derivative; Dr. Setsuji Sakurai, Central Institute of Ajinomoto Co., Inc., for his kind gift of the enzyme, and Dr. Kazuo Okunuki for his advice and for affording facilities for carrying out the decarboxylation experiments. Thanks are also due to Messrs. Masakazu Okumiya and Kenzi Yoshimura for their microanalysis.

Department of Chemistry
Faculty of Science
Osaka University
Nakanoshima, Osaka

*4 All melting points are uncorrected.