In connection with synthesis of extremely unstable S-alkylthiabenzenes,[5] further studies on the electronic structure and stability of thiabenzenes are now in progress.

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5) A.G. Hortmann and R.L. Harris have recognized the generation of an unstable 1-methyl-3,5-diphenyl thiabenzene by the treatment of 1-methyl-3,5-diphenyl-2*H*-thiinium tetrafluoroborate with *t*-butyllithium in DMSO-d_6 in a standard NMR tube under nitrogen stream but were not able to isolate it (*J. Am. Chem. Soc.*, **92**, 1803 (1970)).

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Ultra-microdetermination of Amino Acids by Microbioassay, Applying Lactic Acid Assay with Lactate Dehydrogenase[1]

A new ultra-micro microbioassay method proposed in this paper was proved to be approximately 300 times as sensitive as the conventional method for glycine. The principle of Hohorst's analytical method[2] was applied to this method, which assays the lactic acid produced during the growth of lactic acid bacteria with the aid of lactate dehydrogenase (LDH) [L-lactate: NAD oxidoreductase, EC 1.1.1.27] from rabbit muscle.

In order to compare the assayable range of the conventional titrimetry,[3] which assays the lactic acid resulted from the growth of the bacteria, with that of our method (hereinafter referred to as the LDH method), the growth response of *Leuconostoc mesenteroides* p-60 to various concentration of glycine were determined as shown in Fig. 1.

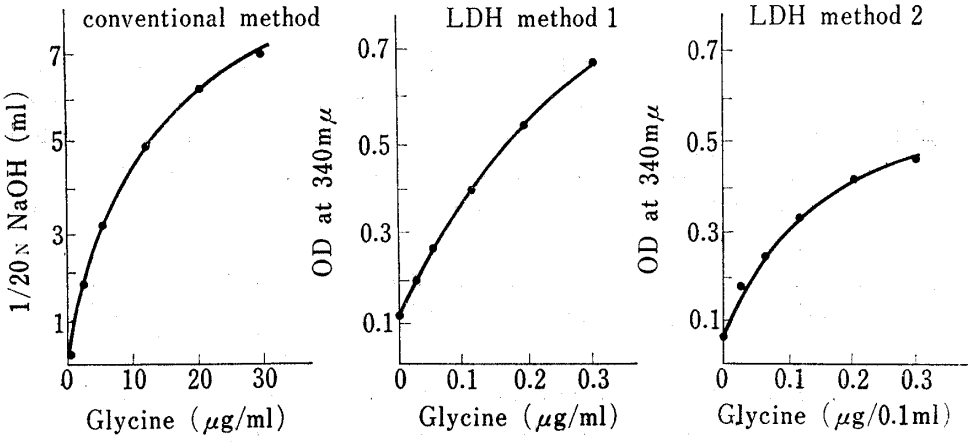

Fig. 1. Growth Responses of *Leuconostoc mesenteroides* p-60 to Various Concentrations of Glycine as Determined by the Conventional Method, LDH Method 1 and LDH Method 2

1) This work was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April 1974.
2) H.-J. Hohorst, "Method of Enzymatic Analysis," ed. by H-U. Bergmeyer, Academic Press, London/New York, 1963, p. 378.
3) G. Tamura, T. Tsunoda, J. Kirimura, and S. Miyazawa, *Nippon Nogeikagaku Kaishi*, **26**, 464 (1952).

The LDH method was performed by the following two ways: A mixture of the standard solution and the double strength basal medium[3] was inoculated with the test bacteria, and after the incubation at 33° for 48 hr the resulted lactic acid was assayed by the LDH method. In one case (LDH method 1), 1 ml of the standard solution and 1 ml of the double strength basal medium were 100 times, and 10 times as dilute as those used in the conventional method, respectively. In another case (LDH method 2), 0.1 ml of those were 10 times as dilute as used in the conventional method, respectively, and the incubation solution was diluted ten times, and assayed for lactic acid by the LDH method.

The results of the experiments shown in Fig. 1 indicate that a good growth response was achieved in the range of 0 to 0.3 μg/ml and 0 to 0.3 μg/0.1 ml respectively.

In order to compare the assays by the LDH method with those by the conventional method, 4 to 6 steps dilution of casamino acids were assayed for glycine (Table I).

Table I. Assays of Glycine in the Casamino Acid Solution by LDH Method 1 and LDH Method 2

Concentration of casamino acids	Assays of glycine			
	LDH method 1		LDH method 2	
	ng	%	ng	%
20 μg	224	1.12	198	0.98
10	115	1.15	105	1.05
5	59	1.18	53	1.06
2.5	29	1.15	29	1.16
1.25	16	1.28	14	1.12
0.625	6.5	1.04	7	1.12
	mean	1.15	mean	1.08
	S.E.[a]	±0.03	S.E.[a]	±0.02
	tit.dev.[b]	+0.07	tit.dev.[b]	±0

a) standard error b) deviation from titrimetry (conventional method)

The mean assays of glycine by the conventional method, LDH method 1 and 2 were 1.08%, 1.15% and 1.08%, with the standard errors of ±0.01%, ±0.03% and ±0.02%, and the deviation of assays by the LDH methods from the assay by the conventional method were very small, namely, +0.07% by the LDH method 1 and ±0 by the LDH method 2. It was further revealed that such ultra-minute amounts of glycine which were in the range of 6.5 to 7 ng were assayable by the LDH methods.

Further, in order to compare the recoveries by the respective assay methods with one another, the recovered glycine from casamino acid solutions were assayed (Table II).

Table II. Recovery of Glycine from Casamino Acid Solution by Conventional Method, LDH Method 1 and LDH Method 2

Method	Glycine in test solution	Glycine added	Found	Calcd.	Recovery (%)
Conventional method	0.63 μg/ml	3 μg/ml	3.7 μg/ml	3.63 μg/ml	101.9
LDH method 1	6.3 ng/ml	30 ng/ml	38 ng/ml	36.3 ng/ml	104.7
LDH method 2	6.3 ng/0.1ml	30 ng/0.1ml	38 ng/0.1ml	36.3 ng/0.1ml	104.7

This method was successfully applied to the assay of glycine in uric acid hydrolyzates[1] and in plasma samples,[4] threonine in casamino acid[1] and pantothenic acid in yeast extract,[1] respectively.

Analysis of other amino acids and vitamins by this method is now in progress and the detail paper will be presented in the near future.

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4) T. Aikawa, H. Matsutaka, H. Yamamoto, T. Okuda, E. Ishikawa, T. Kawano, and E. Matsumura, *J. Biochem.*, **74**, 1003 (1973).

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Synthesis of 5-endo-Benzoyloxy-N-[amino(lower)alkyl]bicyclo[2.2.1]heptane-2,3-di-endo-carboxylic Acid Imides as Potential Antiarrhythmia Agents

A series of 5-endo-benzoyloxy-N-[amino(lower)alkyl]bicyclo[2.2.1]heptane-2,3-di-endo-carboxylic acid imides (**1**) have been synthesized and found to possess unique pharmacological activity as antiarrhythmia agents. An example of such a compound possessing excellent activity is 5-endo-benzoyloxy-N-(3-dimethylaminopropyl)bicyclo[2.2.1]heptane-2,3-di-endo-carboxylic acid imide (**1**: Ar=C_6H_5, R=CH_3, n=3) hydrochloride.

5-endo-Hydroxybicyclo[2.2.1]heptane-2,3-di-endo-carboxylic acid γ-lactone[1] (**2**) was obtained by the acid-catalyzed lactonization of endo- or exo-*cis*-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride, but preferably the endo-*cis* isomer.

2: mp 200° (lit.[1] 203°). IR ν_{max}^{KBr} cm^{-1}: 1770 (γ-lactone), 1690 (COOH). NMR ($CDCl_3$) δ: 1.33 (d,d C^3H, $J_{2,3}$=5.0 Hz, $J_{3,4}$=2.0 Hz); 1.49 (d,t C^2H, $J_{2,3}$=5.0 Hz, $J_{1,2}$=1.5 Hz, $J_{2,6exo}$=2.0 Hz). The coupling constants for C^2H and C^3H indicated that they are exo.

2 was treated with acetyl chloride or phosphorous trichloride and then heated with alkylenediamine [$NH_2(CH_2)_nNR_2$] to give 5-endo-hydroxy-N-[amino(lower)alkyl]bicyclo[2.2.1]-heptane-2,3-di-endo-carboxylic acid imides (**3**). Acylation of **3** with benzoyl halide gave **1**.

(**3**: R=CH_3, n=3): Colorless plates, mp 154°. *Anal.* Calcd. for $C_{14}H_{22}O_3N_2 \cdot 1/3H_2O$: C, 61.76; H, 8.45; N, 10.29. Found: C, 61.93; H, 8.26; N, 10.40.

(**1**: Ar=C_6H_5, R=CH_3, n=3)·HCl: Colorless plates (hygroscopic), mp 239°. *Anal.* Calcd. for $C_{21}H_{26}O_4N_2 \cdot HCl \cdot 1/3H_2O$: C, 61.07; H, 6.83; N, 6.95. Found: C, 60.63; H, 6.88; N, 7.33. Nuclear magnetic resonance (free base in $CDCl_3$) δ: 8.0—7.3 (5H, C_6H_5), 5.17 (1H, C^5H, broad d,t $J_{5,6endo}=J_{4,5}$=4.5 Hz, $J_{5,6exo}$=10.5 Hz), 3.6—3.1 (5H, includes C^2H, C^3H, C^4H, and $CO)_2NCH_2$), 2.6—1.9 (9H, includes C^7H_2, C^6H_{endo}, and the remaining side chain CH_2). The coupling constants for C^5H indicated, from Jackman and Sternhell,[2] that C^5H is exo.

1) K. Alder, G. Stein, F.V. Buddenbrock, W. Eckardt, W. Frercks, and S. Schneider, *Ann.*, **514**, 1 (1934).
2) "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, 1969, p. 289.