[Chem. Pharm. Bull. 16(6) 984—987 (1968)] UDC 577.16 : 612.392.01

Investigations on Pantothenic Acid and Its Related Compounds. XIV.[1] Biochemical Studies. (8).[2] Effect of Pantethine Analogs on *Lactobacillus bulgaricus* B1[3]

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(Received July 6, 1967)

α-Methylpantethine, β-methylpantethine, homopantethine and oxypantetheine did not support the growth of *L. bulgaricus* B1 which required pantethine for its growth.

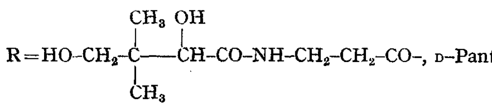
These pantethine analogs were competitive inhibitors of pantethine in this microorganism, but they stimulated the bacterial growth at their subinhibitory concentrations in the presence of a small amount of pantethine.

Magnitudes of their inhibitory effects were in the order of oxypantetheine$\geqslant$homopantethine$>$β-methylpantethine$>$α-methylpantethine on the molar basis of the reduced form.

In the preceding paper[1], the synthesis of some analogs of pantethine or pantetheine was reported. They were α- and β-methylpantethines, homopantethine and oxypantetheine, the structures of which are shown in Table I. Of these analogs microbiological activities of oxypantetheine and homopantethine were already reported by Stewart, *et al.*[5] and Felder, *et al.*[6], respectively, using *Lactobacillus helveticus* 80 which required pantethine for its growth. Stewart, *et al.*[5] found that oxypantetheine was an active, competitive inhibitor of pantetheine in this microorganism with an inhibition index of 100 to 200 at 50% inhibition, and that oxypantetheine stimulated the growth of *L. helveticus* at subinhibitory concentrations in the presence of a small amount of pantetheine. Felder, *et al.*[6] reported that homopantethine was a more potent inhibitor with a 50%-inhibition index of 60. On the other hand, Mautner and Günther[7] reported that seleno-pantethine was equally active to pantethine, on molar basis, in supporting the growth of *L. helveticus* 80, suggesting a functional similarity of sulfur and selenium.

Table I. Structures of Pantetheine or Pantethine Analogs

Pantetheine	$R\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}SH$	β-Methylpantethine	$R\text{-}NH\text{-}CH_2\text{-}CH(CH_3)\text{-}S\text{-})_2$
Pantethine	$R\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}S\text{-})_2$		
α-Methylpantethine	$R\text{-}NH\text{-}CH(CH_3)\text{-}CH_2\text{-}S\text{-})_2$	Homopantethine	$R\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}S\text{-})_2$
		Oxypantetheine	$R\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}OH$


D-Pantothenoyl

1) XIII: O. Nagase, H. Tagawa, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **16**, 977 (1968).
2) Part (7): T. Suzuki, Y. Abiko, and M. Shimizu, *J. Biochem.* (Tokyo), **62**, 642 (1967).
3) A part of this work was presented at the General Meeting of Pharmaceutical Society of Japan, April, 1967, Kyoto.
4) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*
5) C.J. Stewart, W.H. Cheldelin, and T.E. King, *J. Biol. Chem.*, **215**, 319 (1955).
6) E. Felder, L. Fumagalli, and D. Pitré, *Helv. Chim. Acta*, **46**, 752 (1963).
7) H.G. Mautner and W.H.H. Günther, *Biochim. Biophys. Acta*, **36**, 561 (1959).

Cysteamine moiety of pantetheine is important as a functional group of coenzyme A, and it seems interesting to investigate the effects of chemical modifications of this moiety on biological activities of pantetheine. The present report deals with the effects of α– and β–methylpantethines, homopantethine and oxypantetheine on a pantethine-requiring bacterium, *Lactobacillus bulgaricus* B1.

Results and Discussion

Microbiological activity of α– and β–methylpantethines, homopantethine and oxypantetheine was assayed using *L. bulgaricus* B1 as a test organism. *L. bulgaricus* B1 inocula were grown at 37° for 40 hours on the basal medium[8] supplemented with each of these pantethine analogs. The results indicated inability of these analogs in supporting the growth of *L. bulgaricus* B1. All of these analogs were found to inhibit the bacterial growth competitively with pantethine. Table II shows inhibition indexes of these compounds at 50% and 100% inhibitions, indicating that magnitudes of inhibitory action of the analogs are in the order of oxypantetheine ⩾ homopantethine > β–methylpantethine > α–methylpantethine on the molar basis of the reduced form.

Table II. Inhibitory Effects of Pantethine Analogs

Pantethine present, mμmole/tube	50%–Inhibition index				100%–Inhibition index			
	0.04	0.06	0.08	Average	0.04	0.06	0.08	Average
Oxypantetheine	50	50	45	50	180	120	130	140
Homopantethine	50	70	55	60	250	100	330	230
β–Methylpantethine	100	140	60	100	250	330	300	300
α–Methylpantethine	1000	530	600	700				>1000

Inhibition index was represented by mole of analog/mole of pantethine, in the reduced forms.

Recently, Stewart, *et al.*[9–12] demonstrated that oxy–coenzyme A, a coenzyme A analog corresponding to oxypantetheine, was a very potent, competitive inhibitor of coenzyme A in the phosphotransacetylase system[13] and that oxypantetheine–4′–phosphate was converted to oxy–coenzyme A by the actions of bovine coenzyme A–synthesizing enzymes.[14] On the basis of these findings, they[11] postulated that the inhibitory action of oxypantetheine in *L. helveticus* might be caused by an *in vivo* synthesis of oxy–coenzyme A. Although their proposition seems to be very likely, it should await evidence for the metabolic conversion of oxypantetheine to oxy–coenzyme A in this lactic acid bacterium.

It was very interesting to have observed that in the presence of a small amount (less than 1.6×10^{-8} M) of pantethine these analogs stimulated the growth of *L. bulgaricus* B1 at their subinhibitory concentrations. Fig. 1 shows the dual effect of these analogs on the bacterial growth. Such stimulation could not be observed at the full growth of *L. bulgaricus* B1 in the presence of large amounts of pantethine and inhibition was only noted, as shown in Fig. 2.

In the stimulated cultures, morphologically somewhat abnormal cells were often observed. Viable cell counting was performed with the stimulated cultures and the normal one by the

8) Y. Abiko, *J. Biochem.*, **61**, 300 (1967).
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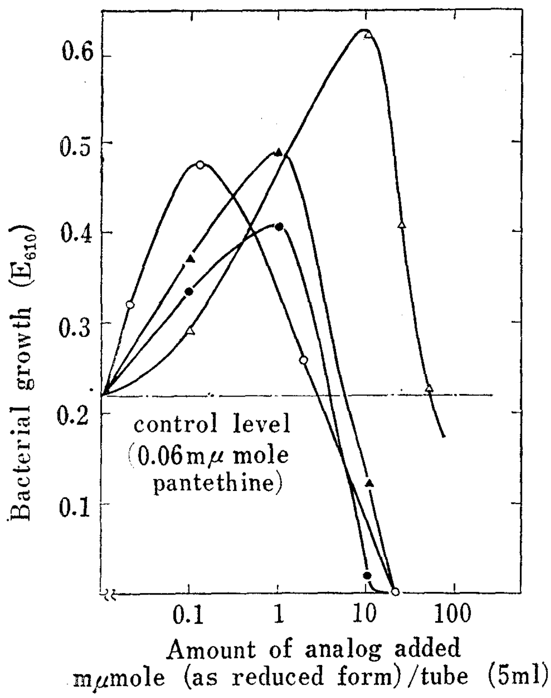

Fig. 1. Dual Effect of Pantethine Analogs on the Growth of *L. bulgaricus* Bl

L. bulgaricus B1 was grown on the basal medium supplemented with a constant, small amount (0.06 mμmole/tube) of pantethine and various amounts of each of pantethine analogs at 37° for 40 hours. ● Oxypantetheine, ○ homopantethine, ▲ β-methylpantethine, △ α-methylpantethine

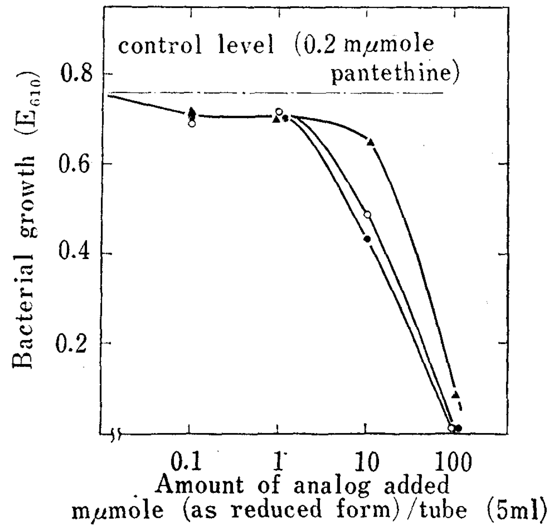

Fig. 2. Effect of Pantethine Analogs on the Growth of *L. bulgaricus* Bl in the Presence of a Large Amount of Pantethine

L. bulgaricus B1 was grown on the basal medium supplemented with 0.2 mμmole of pantethine and various amounts of each of pantethine analogs at 37° for 40 hours. ● Oxypantetheine, ○ homopantethine, ▲ β-methylpantethine

method of Irie, *et al.*[15] The stimulated cultures were found to increase in actual cell number roughly in proportion to rise in turbidity at 610 mμ, as shown in Table III. The mechanism of this stimulation remains unknown. Coenzyme A levels of the cells of the stimulated cultures were found to have an inclination to decrease (Table III), indicating that these analogs inhibited apparently the biosynthesis of coenzyme A in the cells. However, it must be noted that in the present study the intracellular coenzyme A was assayed using the phosphotransacetylase reaction,[16] which was reported to be inhibited by oxy-coenzyme A[12] and which was

Table III. Stimulatory Effects of Pantethine Analogs at Subinhibitory Concentrations

	Growth (E_{610})	Dry cell weight[a] (mg)	Viable cell count[b]	Coenzyme A level[c]
Pantethine	0.112	8.3	2.8×10^{6}	14.47
plus Oxypantetheine 2×10^{-8}M	0.250	14.0	4.9×16^{6}	8.57
Pantethine	0.475	16.4	4.2×10^{7}	11.52
plus Homopantethine 1×10^{-8}M	0.570	21.5	5.2×10^{7}	10.29
plus β-Methylpantethine 8×10^{-8}M	0.555	20.9	5.1×10^{7}	9.04

a) mg/100 ml culture b) cell number/ml culture c) coenzyme A unit/g dry cell

L. bulgaricus B1 was grown on the basal medium containing 8×10^{-9}M of pantethine with or without each of pantethine analogs at the concentrations indicated. After incubation at 37° for 23 hr, turbidity of each culture was read at 610 mμ and actual cell number was measured by the colony counting technique.[15] Bacterial cells from a 100 ml-culture were collected by centrifugation, washed twice with water, lyophilyzed and weighed. Lyophilyzed cells were assayed for coenzyme A by the phosphotransacetylase method.[16]

15) R. Irie, N. Yano, T. Morichi, and H. Kembo, *Nihon Saikingaku Zasshi* (in Japanese), **17**, 779 (1962).
16) Y. Abiko, T. Suzuki, and M. Shimizu, *J. Biochem.*, **61**, 10 (1967).

found to be inhibited also by α–methyl–coenzyme A,[17] β–methyl–coenzyme A[17] and homo–coenzyme A[18] in the preliminary experiments in our laboratory. Thus, if the coenzyme A analogs are formed from these pantethine analogs in the bacterial cells, as suggested by Stewart, *et al.*[11] and Mautner and Günther,[7,19] coenzyme A assay might be affected by them.

Stewart, *et al.*[5] reported a similar phenomenon with oxypantetheine in *L. helveticus* 80 and they speculated that this stimulation of the bacterial growth might be caused by synergistic effect of pantothenic acid which was liberated from oxypantetheine by metabolic hydrolysis. However, it was not the case with *L. bulgaricus* B1. As shown in Fig. 3, synergistic effect of pantothenic acid on the growth of *L. bulgaricus* B1 was too small to explain the stimulation of the bacterial growth by pantethine analogs, except α–methylpantethine. Although the possibility as speculated by Stewart, *et al.*[5] can not be excluded in the case of α–methylpantethine, it may be reasonable to think that all these pantethine analogs including α–methylpantethine stimulate the bacterial growth by a common mechanism different from the synergism of pantothenic acid liberated.

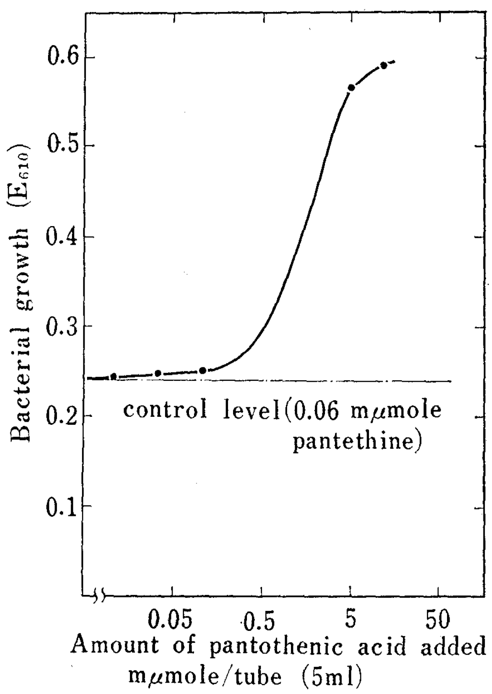

Fig. 3. Synergistic Effect of Pantothenic Acid on the Growth of *L. bulgaricus* B1

L. bulgaricus B1 was grown on the basal medium supplemented with 0.06 mμmole of pantethine and various amounts of pantothenic acid at 37° for 40 hours.

Acknowledgement The authors wish to express their gratitude to Dr. T. Ishiguro, President of this Company, for his encouragement.

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18) to be published.
19) W.H.H. Günther and H.G. Mautner, *J. Am. Chem. Soc.*, **87**, 2708 (1965).