the Bohlmann absorption band[3] was absent in its infrared (IR) spectrum also supported it. Under expectation that the *cis*-quinolizidine moiety exists in a chair-chair conformation in the molecule, the splitting with 11 and 4 H_Z of the H_C signal represents that it has an axial orientation. Moreover, an octet (J 3, 6, and 11.5 H_Z) at δ 5.15 (H_B) and a narrow triplet (J 3 H_Z) at δ 4.91 (H_X) show that these two hydrogens should have an axial and an equatorial orientation, respectively. This indicates a *cis*-relationship of two acetoxy groups on C-3 and C-4, and is consistent with the chemical evidence.[1]

Subsequently, an optical rotatory dispersion (ORD) spectrum of dehydrolythrancine-III (VIII)[1] was taken. It showed a negative Cotton effect at 312 mμ, which indicated the S configuration of C-5 *i.e.* α-H_D (equatorial C-H_D to the ring A) in the formula shown.

A *trans*-relationship between H_D and H_G is clear, since *trans*-2-carboxy-6-carboxymethyl hexahydropyridine[1] has been afforded on oxidation of lythrancine-II.

Consequently, the absolute configurations of five asymmetric centers were clarified; C-1: *R*, C-3: *R*, C-4: *S*, C-5: *S*, and C-9: *S* in lythrancine-I—-IV, and all the same except C-4 in lythrancepine-I—-III. The determination of the absolute configuration of the remaining asymmetric center C-11 and the final confirmation of the foregoing assignment was achieved by the following chemical conversions.

Jones' oxidation of lythrancepine-II (VI) gave an oxoproduct IX, mp 219—220°, $C_{29}H_{35}O_5N$, which was treated with silica gel, then was subjected to catalytic hydrogenation to give a mixture of products, from which the ketone X, mp 81—82°, $C_{29}H_{37}O_5N$ (NMR δ 3.80, dd, J 8 and 4 H_Z, C-1-H) and the desired ketone XI, mp 89—90°, $C_{29}H_{37}O_5N$, were isolated. The $LiAlH_4$ reduction of XI and subsequent formylation with acetic anhydride and formic acid afforded an O,O,N-triformate XII, mp 211—212°, $C_{30}H_{37}O_7N$, $[\alpha]_D$ +82°. This proved to be the antipode of compound XIV, mp 214—216°, $C_{30}H_{37}O_7N$, $[\alpha]_D$ −80°, derived from lythranidine (XIII), whose absolute configuration had been clarified.[4] Thus, the absolute stereochemistry of the seven bases was established as shown.

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3) F. Bohlmann, *Chem. Ber.*, **91**, 2157 (1958).
4) E. Fujita and K. Fuji, *J. Chem. Soc.* (*C*), **1971**, 1651.

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Mechanism of Utilization of Pantetheine-S-Sulfonic Acid by *Bifidobacterium bifidum*

4′-Phosphopantetheine-S-sulfonic acid (P-PaSSO_3H) and 3′-dephospho-coenzyme A-S-sulfonic acid (DP-Co$ASSO_3H$) were isolated from carrot root as growth factors for *Bifidobacterium bifidum* N4 by Yoshioka, *et al.*[1,2] They described that these new type of compounds

1) M. Yoshioka and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 178 (1971).
2) M. Yoshioka and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 186 (1971).

showed almost the same specific activity to the strain as their desulfated compounds (-SH form). We investigated the utilization of pantetheine-S-sulfonic acid ($PaSSO_3H$), P-Pa-SSO_3H, DP-$CoASSO_3H$ and coenzyme A-S-sulfonic acid ($CoASSO_3H$) by twenty strains of *B. bifidum* and *Lactobacillus plantarum* ATCC 8014, obtaining the similar results (unpublished data). Thus we were interested in the mechanism of utilization of these vitamins containing both pantothenoyl moiety and -S-SO_3H group in the molecule. The uptake and the intracellular metabolism of $PaSSO_3H$ by *B. bifidum* N4 were investigated in detail by the aid of radioactive $PaS^{35}SO_3H$ synthesized.

Time course of $PaSSO_3H$ and pantethine (PaSS) uptake by cells of *B. bifidum* N4 is shown in Fig. 1. The figure also illustrates temperature dependence of PaS-SO_3H and PaSS[3] uptake. The relative initial velocity of $PaSSO_3H$ uptake to PaSS uptake was about 0.83.

The rate of $PaS^{35}SO_3H$ uptake was maximum at pH 5.5. The rate of $PaS^{35}SO_3H$ uptake in the presence of 3% lactose was reduced to 66% of that in the absence of lactose. Ouabain, phlorizin, sodium azide, sodium fluoride, potassium cyanide, malonic acid and 2,4-dinitrophenol did not inhibited the transport. However, semicarbazide, iodoacetic acid, iodoacetamide and N-ethylmaleimide inhibited the uptake of $PaS^{35}SO_3H$.

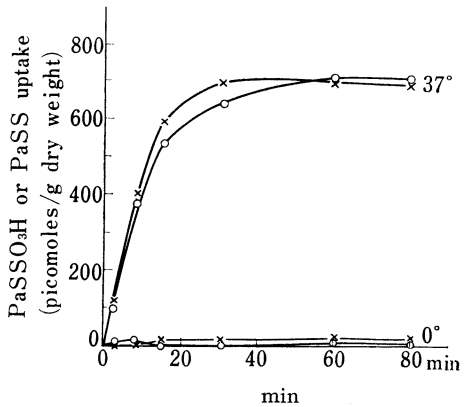

Fig. 1. Uptake of $PaSSO_3H$ and PaSS and Its Temperature Dependence

B. bifidum N4 (26 mg dry weight) was incubated in 6 ml of the basal medium[4] containing 3 nM $PaS^{35}SO_3H$ or 3 nM PaSS. The uptake was calculated from the residual amount in the medium by microbioassay[6] using *B. bifidum* N4.
—○—: $PaSSO_3H$ —×—: PaSS

The K_m for $PaS^{35}SO_3H$ uptake was 3.6×10^{-8} M and the V_{max} was 4.8×10^{-11} moles per min per g dry weight of cells. The uptake of $PaS^{35}SO_3H$ was inhibited by CoA and all of its precursors[7] including their -S-SO_3H form, and competitive inhibitionof the uptake was confirmed with PaSS (Fig. 2).

After one minute exposure of high concentration of cells to $PaS^{35}SO_3H$, $PaS^{35}SO_3H$ and $^{35}SO_4^{2-}$ were detected in the cells (Fig. 3) and in the medium. After 8 hr of cultivation, no radioactivity was remained in the cells and large amount of $^{35}SO_4^{2-}$ was accumulated in the medium, and at that time increased amount of 4′-phosphopantetheine was found in the cells.

From the results described above, it is clear that the uptake of $PaS^{35}SO_3H$ by cells of *B. bifidum* N4 proceeds by a mechanism involving active transport. Since free $PaS^{35}SO_3H$ was found in the cells of the strain after uptake of it, it is certain that $PaS^{35}SO_3H$ passes through the cell membrane as free form by a specific carrier and is then probably attacked by sulfatase to give pantetheine, which is incorporated into CoA and acyl carrier protein.

The work is in progress, and the details will be presented in the near future.

3) Recently Nishikawa and Tamura obtained the similar result in their experiment concerning the uptake of PaSS by *B. bifidum* N4.
4) M. Yoshioka, S. Yoshioka, Z. Tamura, and K. Ohta, *Japan. J. Microbiol.*, **12**, 395 (1968).
5) G.A. Bray, *Anal. Biochem.*, **1**, 279 (1960).
6) K. Samejima, M. Yoshioka, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 166 (1971).
7) Some of them were kindly provided by Central Research Laboratory, Daiichi Seiyaku Co., Ltd., Tokyo.
8) H. Nakamura, K. Samejima, and Z. Tamura, *Japan. J. Microbiol.*, **14**, 9 (1970).

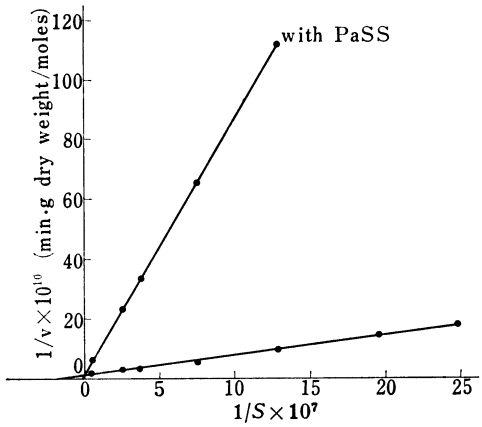

Fig. 2. Competitive Inhibition of $Pas^{35}SO_3H$ uptake by PaSS

B. bifidum N4 (36 mg dry weight/ml) was incubated in the basal medium[4] containing various concentration of $PaS^{35}SO_3H$, with or without 90 nM Pass at pH 6.8 and 37° for 5 min.

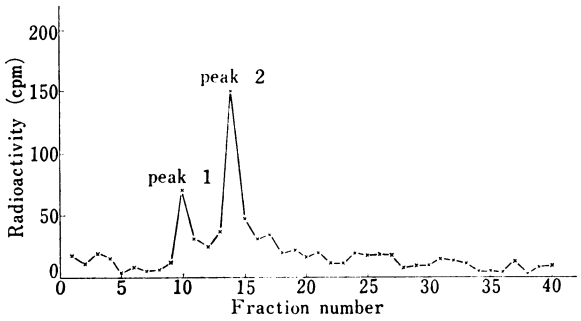

Fig. 3. Intracellular Radioactivity after One Minute Exposure to $PaS^{35}SO_3H$

The cells were filtered on a Millipore (type HA, 0.45 μ), washed three times with each 7 ml of physiological saline and destroyed by sonication. The disrupted material obtained was fractionated on QAE-Sephadex column (OH^- form, 0.9 × 11 cm) with a linear gradient of final concentration of 0.5M NH_4HCO_3. The radioactive peaks were examined by paper electrophoresis (0.1M NH_4HCO_3, pH 8) and paper chromatography (*n*-BuOH:AcOH:water, 5:2:3, v/v/v) followed by bioautography[8] and were proved to be $PaS^{35}SO_3H$ (peak 1) and $^{35}SO_4^{2-}$ (peak 2).

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