heated under reflux for 4 hr. After the completion of the reaction, 60 ml of water was added dropwise to decompose excess $LiAlH_4$. The ether layer was decanted, dried over anhydrous sodium sulfate and concentrated to dryness under reduced pressure. The oily residue was taken up in ethanolic HCl and the solvent evaporated to a residue, which was recrystallized from 30 ml of ethanol to give 4.7 g of colorless crystals of compound V hydrochloride, mp 224—225°. Recrystallization from EtOH gave colorless crystals, mp 229.5—230°. UV λ_{max}^{MeOH} mμ (ε): 232 (36100), 238 (38100), 267 (9900). IR ν_{max}^{KBr} cm^{-1}: 2617, 1603. *Anal.* Calcd. for $C_{19}H_{19}N_2Cl \cdot HCl$: C, 65.70; H, 5.82; N, 8.06; Cl, 20.41. Found: C, 66.25; H, 5.83; N, 7.93; Cl, 20.57.

An additional 2.49 g of compound V hydrochloride was obtained from the mother liquor; the total yield was 7.19 g (75.0%).

7-Chloro-1-cyclopropylmethyl-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (VI)——A solution of 1.75 g of chromic anhydride in 1.75 ml of water was added dropwise to 2.0 g of compound V in 20 ml of glacial acetic acid on cooling with stirring. The stirring was continued at room temperature for additional 12 hr. The temperature was maintained at 0—10°, while the reaction mixture was added dropwise to 90 ml of 12.4% aqueous ammonia. The mixture was extracted with 20 ml of carbon tetrachloride three times. The carbon tetrachloride layer was washed with 60 ml of water and dried over anhydrous sodium sulfate. The solvent was evaporated to a residue, which was crystallized on the treatment of 2.0 ml of isopropyl alcohol to give 1.27 g (69.9%) of the compound VI, mp 139.5—141.5°. IR ν_{max}^{KBr} cm^{-1}: 3080, 1670. UV λ_{max}^{MeOH} mμ (ε): 229 (30100), 314 (1900). *Anal.* Calcd. for $C_{19}H_{17}ON_2Cl$: C, 70.32; H, 5.28; N, 8.63; Cl, 10.92. Found: C, 70.63; H, 5.13; N, 8.28; Cl, 10.78.

From the mother liquor was given 0.11 g of the 2nd crop, mp 138—140°. The total yield was 1.38 g (76%).

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A Simpler Synthesis of D-Pantothenic Acid 4'-Phosphate[1]

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In the series of studies[3] to propagate Bifidobacterium bifidum (*Lactobacillus bifidus*) in intestines, D-pantothenic acid 4'-phosphate (III) was necessary to examine the nutritional requirement of this microbe of coenzyme A and its precursors. Although several synthetic routes were reported,[4] they were long, and if short, involved production of by-products as indicated in the direct phosphorylation of D-pantothenic acid (I) with diphenyl phosphorochloridate followed by hydrolysis with sodium hydroxide.[4e] A simpler synthesis was attempted as shown in Chart 1 on the basis of the fact that the aliphatic primary hydroxyl group was phosphorylated with dibenzyl phosphorochloridate more readily than the secondary.[5]

1) A part of this work was presented at the 87th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April 1967.
2) Location: *Hongo, Bunkyo-ku, Tokyo.*
3) M. Yoshioka, S. Yoshioka, Z. Tamura, and K. Ohta, *Japan. J. Microbiol.*, 12, 395 (1968).
4) *a*) T.E. King and F.M. Strong, *Science*, 112, 562 (1960); *b*) J. Baddiley and E.M. Thain, *J. Chem. Soc.*, 1951, 246; *c*) G.D. Novelli, "Methods in Enzymology," vol. 3, Academic Press Inc., New York, N. Y., 1957, pp. 926—928; *d*) F.R. Atherton, U.S. Patent 2870188 (1959); *e*) S. Okada, O. Nagase, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), 15, 713 (1967).
5) H.G. Khorana, "Some recent developments in the chemistry of phosphate esters of biological interest," John Wiley & Sons, Inc., New York, N. Y., 1961, p. 19.

As expected, III was easily obtained by this method using dibenzyl phosphorochloridate. In contrast to diphenyl phosphorylated pantothenic acid, the dibenzyl phosphorylated one was considered as pantothenic acid 4′-dibenzyl phosphate (II) and not its lactone, for infrared absorption at 1745 cm^{-1} assigned to lactone of pantothenic acid 4′-diphenyl phosphate (V)[4e] or carboxylic acid ester of methyl pantothenate[6] was not observed but absorption at 1735 cm^{-1} assigned to carboxylic acid of I was observed in crude II.

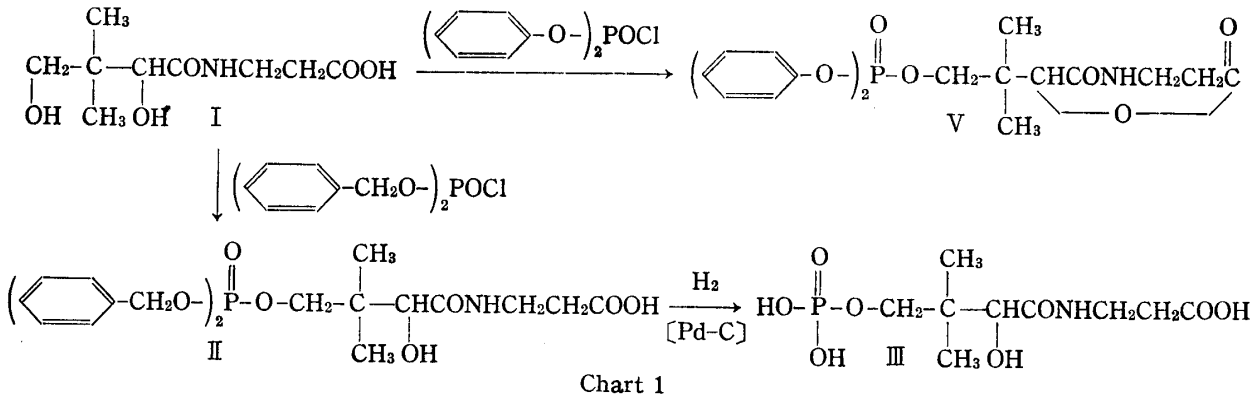

Chart 1

The biological activity of I was determined for *L. arabinosus* 17–5[7] after the intestinal phosphatase treatment of the synthesized III.[8] The result showed that no racemization of the secondary hydroxyl group occurred in the present route. By chromatographic comparsion of III (Table I) with that derived from barium D-pantetheine 4′-phosphate (IV)

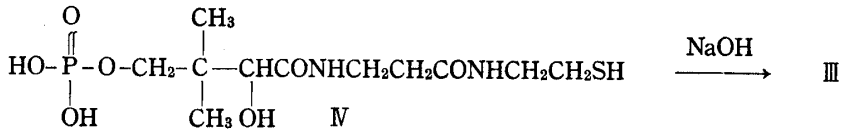

Chart 2

Table I. Chromatographic and Electrophoretic Behaviors of Synthesized III

	III[a]	III[4c),a] from IV	IV[a]	PAT[b]
Rf of PPC[c]				
in solvent 1	0.48	0.48	0.38	0.86
2	0.16	0.16	0.14	0.94
3	0.50	—	0.65	0.90
Rf of TLC[d] in solvent 1	0.46	0.46	0.43	—
Migrated distance (cm) of PEP[e] against anode				
at pH 3.5	4.2	4.2	3.7	0
at pH 7.4	8.3	8.3	5.6	0

a) Phosphate ester was detected with the Hanes and Isherwood spray,[11] followed by ultraviolet irradiation.[12]
b) D-pantethine (PAT) was used as nonmigrating marker for PEP. Disulfide was detected by the reduction with sodium cyanide, followed by spraying a solultion of sodium nitroprusside.[10]
c) Paper electrophoresis was done horizontally on Toyo Roshi No. 51A with buffers(ionic strength, 0.05) of pH 3.5 acetate-H_2SO_4 and pH 7.4 Veronal-H_2SO_4 at 12 V per cm for 2 hours.
d) Thin-layer chromatography was carried out on Abicel SF (Funakoshi Pharmaceutical Co., Ltd., Tokyo) layer by the ascending method.
e) Paper chromatography was carried out on Toyo Roshi No. 51A by the ascending method with solvent systems of (1) 1-BuOH–AcOH–H_2O (5:2:3, v/v); (2) 1-PrOH–NH_4OH (28%)–H_2O (6:3:1, v/v); (3) isobutyric acid–0.5N NH_4OH (5:3, v/v).

6) Synthesized according to King & Strong.[4a] IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 2960, 2890, 1745, 1667, 1530, 1445, 1370, 1290, 1195, 1175, 1080, 1045.
7) Kindly provided by Central Research Laboratory, Daiichi Seiyaku Co., Ltd.
8) Y. Abiko and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **15**, 884 (1967); H.R. Skeggs and L.D. Wright, *J. Biol. Chem.*, **156**, 21 (1944).

according to Novelli as shown in Chart 2,[4c] it was proved that the phosphorylated pantothenic acid of only primary hydroxyl group was obtained without contamination of pantothenic acid 2′-phosphate, for *Rf* values of both isomers were different in these solvent systems of PPC.[9]

Experimental

Synthesis of III——Calcium D-pantothenate was converted into I by ion exchange. IR ν_{max}^{Cap} cm^{-1}: 3390, 2960, 2890, 1735, 1650, 1535, 1440, 1410, 1370, 1270, 1190, 1080, 1040. I (1.76 g) was dried by evaporation of its solution in anhydrous pyridine three times, dissolved in 15 ml of pyridine at −40°, and added with dibenzyl phosphorochloridate (3.2 g). The mixture was allowed to stand for 16 hours at −20°, added with water (5 ml) and ethanol (10 ml), kept at room temperature for 10 minutes, and concentrated to a small volume under reduced pressure. Petroleum ether was added to the residual solution with shaking. The precipitate formed was separated, dissolved in methylene choride, and the solution was washed successively with 1M acetic acid, 1M potassium hydrogen carbonate, and water. The organic phase was dried over sodium sulfate and evaporated under reduced pressure. II (2.08 g) remained syrupy. IR ν_{max}^{Cap} cm^{-1}: 3387, 3045, 3020, 2960, 2890, 1735, 1670, 1525, 1495, 1456, 1380, 1265, 1212, 1183, 1080, 1013, 970, 885, 745, 700.

The syrup was dissolved in 50 ml of ethanol and reduced with hydrogen in the presence of palladium–carbon (1.0 g). After 1 hour, the catalyst was filtered off and the solvent was evaporated. The residue was dissolved in water and neutralized to pH 7.5 with 0.1 M barium hydroxide. The slight excess of alkali was removed by addition of carbon dioxide and centrifugation. The solution was concentrated to a small volume and added with acetone. The precipitate was reprecipitated with acetone in the same manner twice and washed with ether (Yield, 0.60 g, 15%).

Purification of III——The crude (100 mg) product was dissolved in 0.1 M acetic acid and applied to a column (1.2 × 20 cm) of DEAE–Sephadex (CH_3COO^- form). The column was washed with water and eluted with an increasing gradient (mixing volume, 200 ml) of ammonium carbonate (0—1.0 M). Fractions of 10 ml each were collected at a flow rate of 0.13 ml/min. Phosphorus-containing fractions were detected by the Hanes and Isherwood reagents.[11,12] The contents of tubes 4—8 were pooled and evaporated to dryness under reduced pressure. The residue was dissolved in water and passed through a column of Amberlite IR-120 resin (H^+ form). The eluate was neutralized to pH 7.5 with 0.1 M barium hydroxide and the product was precipitated by the addition of acetone. The precipitate was reprecipitated twice with acetone in the same manner and washed with ether (Yield, 50 mg, 50%). *Anal.* Calcd. for $C_9H_{15}O_8NBa_{1.5}$ P: C, 2.1.52; H, 3.01; N, 2.79; P, 6.16. Found: C, 21.31; H, 3.52; N, 2.92; P, 6.02. $[\alpha]_D^{16.5}$ +13.7 (c=1.62, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3420, 2970, 2895, 1650, 1565, 1415, 1110—1070, 977, 810.

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11) C.S. Hanes and F.A. Isherwood, *Nature*, **164**, 1107 (1949).
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