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Investigations on Pantothenic Acid and Its Related Compounds. XVIII.[1] Chemical Studies. (8).[2] Synthesis of Pantothenyl Alcohol 4'-Phosphate[3]

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In the preceding paper of this series,[1] it was reported that phosphorylation of D-pantothenyl alcohol (III) (PaOH) (D-2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutyramide)[5] is catalyzed by pantothenic acid kinase (PaA-kinase) (ATP: pantothenic acid 4'-phosphotrans-

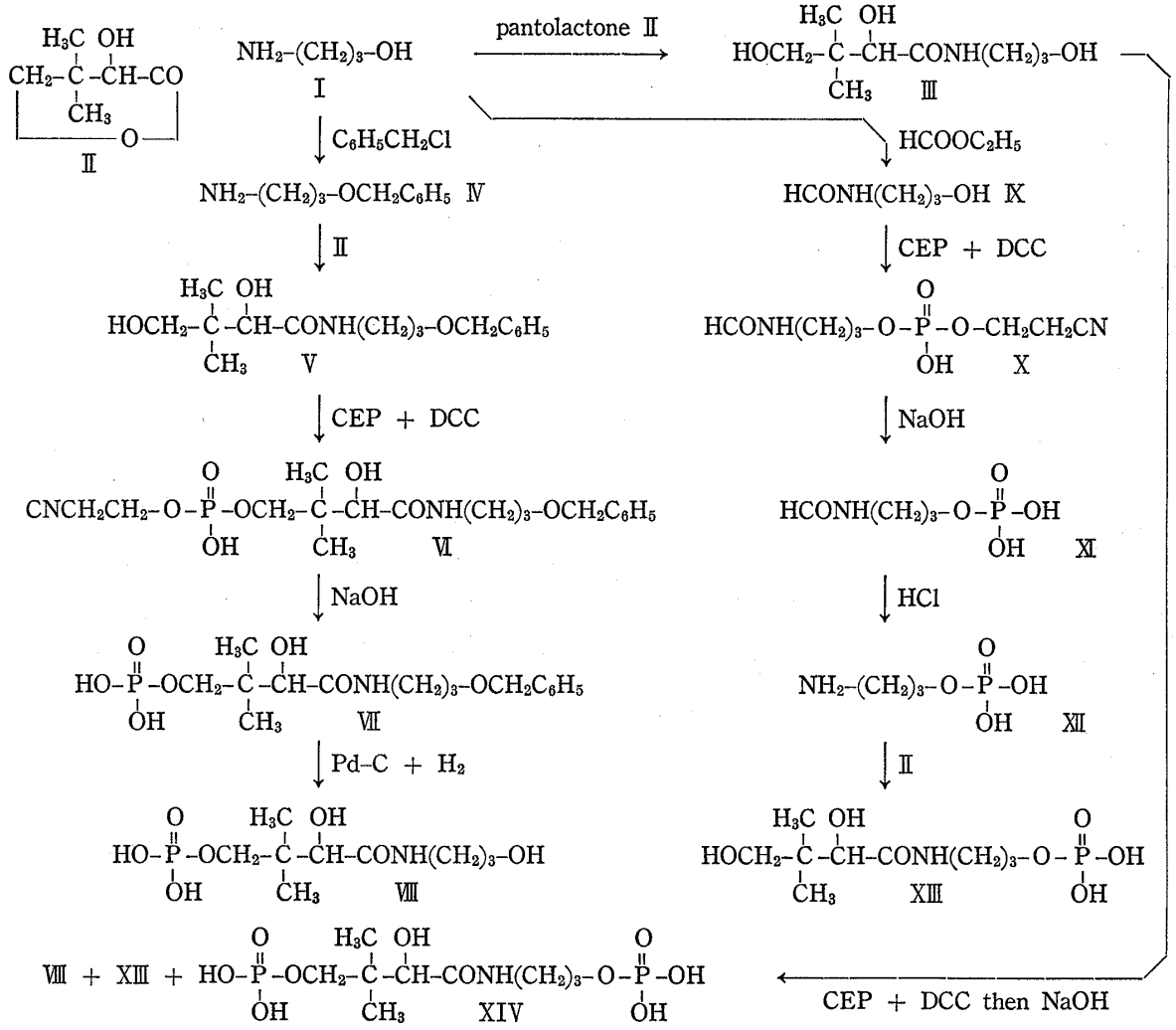

Chart 1

1) Part XVII: Y. Abiko, M. Tomikawa, Y. Hosokawa, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **17**, 200 (1969).
2) Part (7): M. Shimizu, O. Nagase, Y. Hosokawa, and H. Tagawa, *Tetrahedron*, **24**, 5241 (1968).
3) This work was delivered at the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968.
4) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*
5) In the present paper, we prescribe conventionally the primary hydroxyl group of pantoic acid moiety as 4'-OH and conveniently that of 3-aminopropanol moiety in PaOH as 3''-OH group.

ferase, EC 2.7.1.33) from rat liver. It has been known that this enzyme catalyzes specifically phosphorylation of the primary 4′-OH group in pantothenic acid,[6–8] pantetheine,[7,9] or pantothenoylcysteine[7] but not of the secondary 2′-OH group in these compounds. Because of the presence of another primary hydroxyl group at 3″-position in PaOH (III) besides the 4′-OH group, it was necessary to establish chemically the structure of the PaOH phosphate obtained enzymatically. This paper deals with the chemical synthesis of two phosphates of PaOH for comparison.

Synthesis of the 4′-phosphate was performed as shown in Chart 1. Condensation of 3-benzyloxypropylamine (IV) with D-pantolactone (II) afforded PaOH 3″-benzyl ether (V), which was phosphorylated with cyanoethyl phosphate (CEP) and dicyclohexylcarbodiimide (DCC)[10] to give VI. Mild alkaline hydrolysis of VI gave PaOH 3″-benzyl ether monophosphate (VII), debenzylation of which was effected by catalytic hydrogenation over palladium charcoal to yield the desired phosphate (VIII). In the previous report,[2] It was shown that phosphorylation of pantethine with CEP and DCC gives 4′,4″-diphosphate. Therefore, it seems reasonable to assume that the phosphate obtained in the present case would be also the 4′-phosphate but not the 2′-phosphate. As described later, this point was confirmed by comparison of the nuclear magnetic resonance (NMR) spectrum with those of PaOH and other phosphates.

3″-Phosphate (XIII) of PaOH was obtained by condensation of 3-aminopropanol-O-phosphate (XII) with pantolactone (II) as shown in Chart 1. The compound (XII) was prepared from N-(3-hydroxypropyl)formamide (IX) through initial phosphorylation with CEP and DCC, mild alkaline hydrolysis of the cyanoethyl group, and final acidic deformylation. The position of phosphate in XIII is unambiguous from the above synthetic steps. Direct phosphorylation of PaOH (III) with CEP and DCC gave the mixture of various phosphates, from which one diphosphate could be isolated besides the mixture of two monophosphates by purification with (XIV)column chromatography. The structure of the diphosphate was determined to be 4′,3″-diphosphate from analysis of its NMR spectrum as described below.

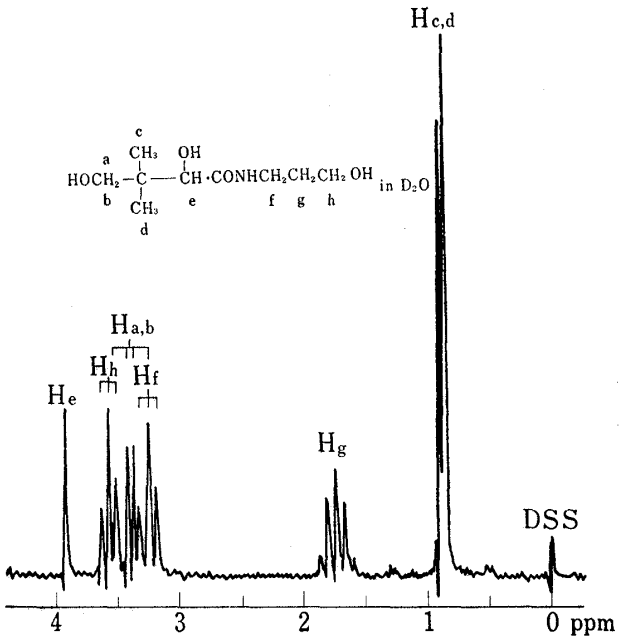

Fig. 1. NMR Spectrum of Pantothenyl Alcohol (III)

In the NMR spectrum of PaOH as shown in Fig. 1, the proton signals of pantoyl moiety are quite similar to those of pantothenic acid reported by Fritz, *et al.*[11] In the case of 3″-phosphate (XIII) (Fig. 2), the signal due to H_h– appears at lower field than that of PaOH by 0.43 ppm and consists of four peaks attributed to the spin couplings with H_g and phosphorous.[12] The signals for other protons are in good accordance with those of PaOH. The splitting pattern of 4′-phosphate (VIII) is also consistent with that of PaOH except that the

6) G.M. Brown, *J. Biol. Chem.*, **234**, 370 (1959).
7) Y. Abiko, *J. Biochem.* (Tokyo), **61**, 290 (1967).
8) Y. Abiko and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **15**, 884 (1967).
9) G.M. Brown and J.J. Reynold, *Ann. Rev. Biochem.*, **32**, 419 (1963).
10) G.M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).
11) H. Fritz and W. Löwe, *Angew. Chem.*, **74**, 751 (1962).
12) M. Tsuboi, F. Kuriyagawa, K. Matsuo, and Y. Kyogoku, *Bull. Chem. Soc. Japan*, **40**, 1813 (1967).

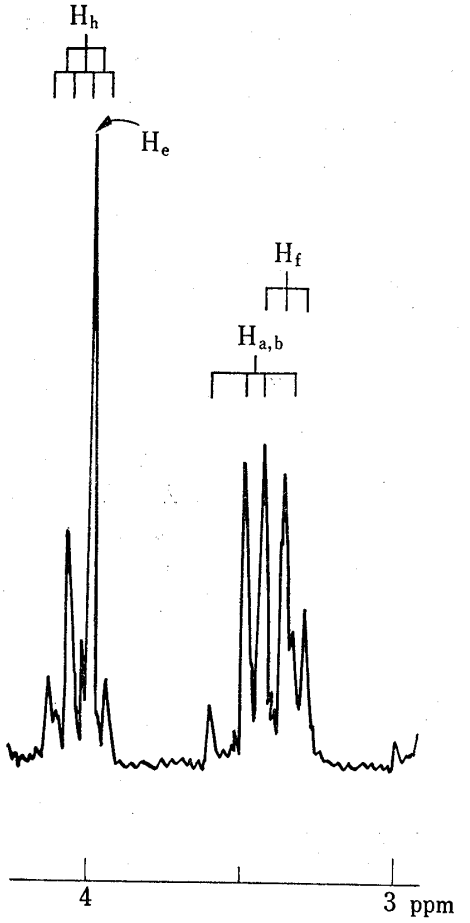

Fig. 2. NMR Spectrum of Pantothenyl Alcohol 3″-Phosphate (XIII)

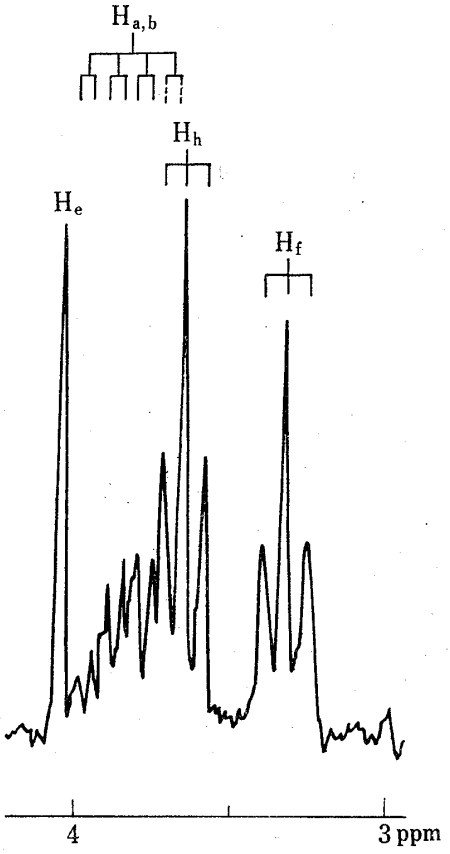

Fig. 3. NMR Spectrum of Pantothenyl Alcohol 4′-Phosphate (VIII)

signals of H_a and H_b are shifted to lower field than those of PaOH by 0.38 ppm and resplit by coupling with phosphorous (Fig. 3). Hence, it is concluded that the phosphate group of VIII is attached to 4′-position. In regard to the NMR spectrum of the diphosphate (XIV) it is found that the signals of H_a, H_b and H_h are quite similar to those of VIII and XIII, respectively. These data can give the evidence for the 4′,3″-diphosphate structure of XIV. Finally, H_e signals of VIII and XIV are not different from that of PaOH and no proton signal is observed in the lower field than that of H_e. This fact indicates that 2′-phosphate isomer is not contained in these compounds.

Rf values of 4′- and 3″-phosphate in paper and thin-layer chromatography are so close that separation between these compounds is generally difficult. But we could succeed in differentiation of both esters by descending paper chromatography using the following solvent system: pyridine–butanol–H_2O–NH_4OH. Based on the above fact, the enzymatic phosphorylation of PaOH was confirmed to be brought about exclusively at 4′-OH but not at 3″-OH as described in the preceding paper.[1]

Experimental[13]

N-(3-Benzyloxypropyl)-2,4-dihydroxy-3,3-dimethylbutyramide (V)——A reaction of D-pantolactone (II, 13 g) and 3-benzyloxypropylamine (IV, 16.5 g) was carried out at 55° for 1 hr and at room temperature over-

13) Ascending paper chromatography (PPC) on Toyo Roshi No. 51A was carried out with solvent system A, BuOH–AcOH–H_2O (5:2:3), B, PrOH–NH_4OH–H_2O (6:3:1) and descending chromatography with solvent system C, pyridine–BuOH–H_2O–NH_4OH (70:10:20:1). NMR spectra were measured on a JNM 4H-100 spectrometer at 100 Mc in D_2O with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. All evaporations were performed *in vacuo*.

night. The reaction product was purified by treatment with Amberlite IR 120 (H^+) and IRA 410 (OH^-) to give V as a viscous oil (27.4 g, 92.8%). $[\alpha]_D^{20}$ +30.9° (c=1.1, MeOH). *Anal.* Calcd. for $C_{16}H_{25}O_4N$: C, 65.06; H, 8.53; N, 4.74. Found: C, 65.20; H, 8.37; N, 4.73.

V-4-(2-cyanoethyl)phosphate (VI)——A solution of V (4.43 g, 15 mmoles), CEP (15 mmoles) and DCC (3.09 g, 15 mmoles) in anhydrous pyridine (30 ml) was left at room temperature for 24 hr and then H_2O (30 ml) was added. After 30 min dicyclohexylurea was filtered off and the filtrate was evaporated to dryness. An aqueous solution of the residue was neutralized with $Ba(OH)_2$ and concentrated to about 20 ml, and EtOH (90 ml) was added. The precipitated powder was filtered off and the filtrate was evaporated. The residue was dissolved in AcOEt (10 ml), and ether (150 ml) was added to precipitate the barium salt of VI (3.7 g, 50%). IR ν_{max}^{KBr} cm^{-1}: 1220, 1090, 1040 (PO_2^-, C–O, P–O–C). PPC: *Rf* 0.72 (solvent A), 0.85 (solvent B). *Anal.* Calcd. for $C_{19}H_{28}O_7N_2PBa_{1/2}$: C, 46.00; H, 5.69; N, 5.65. Found: C, 45.83; H, 5.81; N, 5.45.

In the etheral mother liquor, a presence of the 2′,4′-cyclic phosphate compound was suggested by PPC (*Rf* 0.97 in solvent B) and IR spectrum (1295 cm^{-1} (P=O)).

V-4-phosphate (VII)——To a solution of VI (3.7 g) in H_2O (10 ml), 4N NaOH (10 ml) was added and the mixture was stirred at 0° for 20 min. It was acidified by addition of IR 120 (H^+) and applied to a column of IR 120 (H^+) (25 ml). The eluate was neutralized with $Ba(OH)_2$ and evaporated. Addition of ether to a MeOH solution of the residue gave a precipitate of the barium salt of VII (2.5 g, 66%). IR ν_{max}^{KBr} cm^{-1}: 1095, 985 (C–O, P–O–C, PO_3^{2-}). PPC: *Rf* 0.64 (solvent B). *Anal.* Calcd. for $C_{16}H_{24}O_7NPBa$: C, 37.63; H, 4.74; N, 2.74. Found: C, 38.10; H, 4.97; N, 2.85.

Pantothenyl Alcohol 4′-Phosphate (2-Hydroxy-N-(3-hydroxypropyl)-3,3-dimethyl-4-phosphonoxybutyramide) (VIII)——A mixture of free acid of VII (from 2.5 g of the barium salt) and 10% Pd–C (5 g) in MeOH (100 ml) was shaken in H_2 stream until H_2 uptake ceased (40 min). The catalyst was filtered off and the filtrate was evaporated. After neutralization with $Ba(OH)_2$ and evaporation of the solvent, the residue was dissolved in MeOH and ether was added to give the barium salt of VIII (1.7 g, 83%). $[\alpha]_D^{23}$ +13.5° (c=1.0, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3380, 1643, 1540, 1080, 978. PPC: *Rf* 0.40 (solvent A), 0.45 (solvent B), and distance travelled for 48 hr 17 cm (solvent C). NMR δ: 0.95, 1.01 (each 3H, singlet, CH_3), 1.76 (2H, pentaplet, J=6.9 cps, H_g), 3.30 (2H, triplet, J=6.9 cps, H_f), 3.63 (2H, triplet, J=6.9 cps, H_h), 3.80 (2H, multiplet, $J_{a,b}$=10.2 cps, J_{HP}=5.1 cps), 4.01 (1H, singlet, H_e). *Anal.* Calcd. for $C_9H_{18}O_7NPBa\cdot H_2O$: C, 24.65; H, 4.60; N, 3.19. Found: C, 24.67; H, 4.69; N, 3.22.

2-Cyanoethyl 3-Formamidopropyl Phosphate (X)——A reaction of IX (3.1 g, 30 mmoles) with CEP (60 mmoles) and DCC (24.8 g, 120 mmoles) was performed in anhydrous pyridine at room temperature overnight. Working-up as described for VI gave the barium salt of X (5.42 g, 59.3%). IR ν_{max}^{KBr} cm^{-1}: 1230, 1085, 1060 (PO_2^-, P–O–C). PPC: *Rf* 0.40 (solvent A), 0.60 (solvent B). *Anal.* Calcd. for $C_7H_{12}O_5N_2PBa_{1/2}$: C, 27.94; H, 4.31. Found: C, 27.67; H, 3.98.

3-Aminopropanol-O-phosphate (XII)——X (5.42 g) was hydrolyzed in 2N NaOH (54 ml) at 0° for 20 min. Treatment of the product with IR 120 (H^+) and evaporation of the solvent gave XI as an oil (3.5 g). PPC: *Rf* 0.40 (solvent A), 0.29 (solvent B). XI was dissolved in 1N HCl–MeOH (43 ml) and set aside overnight at room temperature. After removal of the solvent the residue was dissolved in H_2O and passed through a IR 120 (H^+) column (100 ml). The column was washed with H_2O and eluted with 0.03N HCl (900 ml). The eluate was concentrated to give the hydrochloride of XII as an oil (1.2 g). *Anal.* Calcd. for $C_3H_{11}O_4NPCl$: N, 7.32. Found: N, 7.04.

Pantothenyl Alcohol 3″-Phosphate (2,4-Dihydroxy-3,3-dimethyl-N-(3-phosphonoxypropyl)butyramide) (XIII)——A solution of XII (1.2 g), Et_2NH (1.65 g) and II (0.98 g) in MeOH was refluxed for 7 hr and then concentrated. Treatment with IR 120 (H^+), evaporation of the filtrate and precipitation from MeOH solution with ether gave an oil (1.5 g). Its aqueous solution was neutralized with $Ba(OH)_2$ and chromatographed on a DEAE-Sephadex A-25 (Cl^-) column (2.6×30 cm) with 2000 ml linear gradient of 0.03—0.075N LiCl in H_2O, and 20 ml fractions were collected. Fraction Nos. 35—60 were pooled and concentrated to dryness. The residue was thoroughly stirred with MeOH and acetone (1:15) to give the lithium salt of XIII (1.2 g, 62.5%). $[\alpha]_D^{22}$ +22.6° (c=1.0, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3360, 1640, 1535, 1090, 1020. PPC: *Rf* 0.40 (solvent A), 0.45 (solvent B), and distance travelled for 48 hr 15.5 cm (solvent C). NMR δ: 0.90, 0.94 (each 3H, s, CH_3), 1.89 (2H, pentaplet, J=6.9 cps, H_g), 3.34 (2H, t, H_f), 3.46 (2H, AB_q, $\Delta_{a,b}$=11.1 cps, J=11.3 cps), 3.99 (1H, s, H_e), 4.03 (2H, q, $J_{g,h}$ =6.9 cps, J_hP =6.4 cps). *Anal.* Calcd. for $C_9H_{18}O_7NPLi_2\cdot 1/2H_2O$: C, 35.31; H, 6.26; N, 4.58. Found: C, 35.68; H, 6.15; N, 4.57.

D-Pantothenyl Alcohol (III)——It was prepared by the method of Bonati, *et al.*[14] and purified by IR 120 (H^+) and IRA 410 (OH^-). $[\alpha]_{20}^{D}$ +28.8° (c=1.8, H_2O). NMR δ: 0.89, 0.92 (each 3H, s, CH_3), 1.74 (2H, pentaplet, J=6.9 cps, H_g), 3.28 (2H, t, H_f), 3.42 (2H, AB_q, $\Delta_{a,b}$=11.1 cps, J=11.3 cps), 3.60 (2H, t, H_h), 3.95 (1H, s, H_e).

Phosphorylation of III——III (1.64 g) was phosphorylated with equimolar CEP and DCC, and the product was hydrolyzed with NaOH and worked up in the same way as described above to yield the barium

14) F. Bonati and D. Pitré, *Farmaco* (Pavia) *Ed. Sci.*, **14**, 43 (1959)[*C.A.*, **53**, 17897g (1959)].

salt (1.96 g). It was chromatographed on cellulose powder (320 g) using a solvent PrOH–NH_4OH–H_2O (6:3:1). Fractions of each 20 ml were collected every 15 min. Concentration of fraction Nos. 42—55, treatment with IR 120 (H^+), neutralization with $Ba(OH)_2$, evaporation of the solvent and addition of ether to the MeOH solution of the residue gave a barium salt (60 mg). Its *Rf* values of PPC and NMR spectrum showed that it was a mixture of VIII and XIII. *Anal.* Calcd. for $C_9H_{18}O_7NPBa \cdot 2H_2O$: C, 23.67; H, 4.86; N, 3.07. Found: C, 23.51; H, 4.97; N, 3.40. Fraction Nos. 95—180 were pooled and treated similarly to give the barium salt of XIV (110 mg) which was precipitated from aqueous solution with EtOH. PPC: *Rf* 0.25 (solvent A), 0.12 (solvent B). NMR δ: 0.94, 0.99 (each 3H, s, CH_3), 1.82 (2H, t, $J=6.9$ cps, H_g), 3.33 (2H, t, H_f), 3.78 (2H, m, H_a, H_b), 3.99 (2H, q, H_h), 4.00 (1H, s, H_e). *Anal.* Calcd. for $C_9H_{17}O_{10}NP_2Ba_2 \cdot 5H_2O$: N, 1.93; P, 8.54. Found: N, 2.33; P, 8,30.

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On the Double Bond Nature (endo or exo) in the Condensation Products of Cyclic Ketones with Cyanoacetate or Cyanoacetic Acid

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In 1948 in their brilliant synthesis of morphinan, Grewe, *et al.*[2] condensed ethyl 2-oxocyclohexanecarboxylate with ethyl cyanoacetate under the Cope's conditions[3] and obtained a condensation product in good yield. However, they made no mention about the nature of the double bond and the existence of isomers in their condensation product (Chart 1).

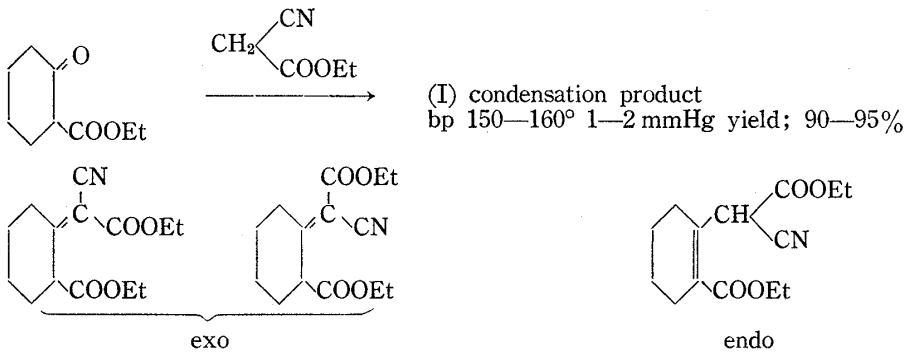

Chart 1

In simpler cases matters are rather straightforward. Thus, when cyclic ketones and cyanoacetic acid are Cope–condensed followed by decarboxylation, there are obtained in good yield unsaturated nitriles, in which the double bond is located "endo" as judged from their ultraviolet (UV) spectral data[4] (Chart 2).

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1) Location: *a) 2-2-50 Kawagishi Toda, Saitama; b) 130 shiba shirokane-Sankocho, Minato-ku, Tokyo.*
2) R. Grewe and A. Mondon, *Chem. Ber.*, **81**, 297 (1948).
3) A. C. Cope, C. A. Hofmann, C. Wyckoff and E. Hordeubergh, *J. Am. Chem. Soc.*, **63**, 3452 (1941).
4) S. Saito, *Chem. Pharm. Bull.* (Tokyo), **4**, 237 (1956).