

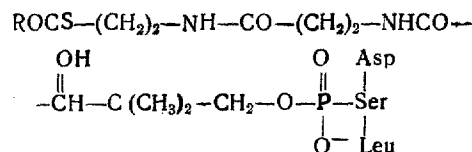
## INVESTIGATIONS IN THE FIELD OF ACYL GROUP CARRIERS

## VII. Synthesis of D-(+)-2'-O-Mesylpantothenonitrile 4'-Phosphate

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Khimiya Prirodnkh Soedinenii, Vol. 5, No. 3, pp. 170-174, 1969

In recent years it has been shown that in the biosynthesis of saturated fatty acids in various biological systems the coenzyme of acylation (CoA) is replaced by a protein-lie compound which is called an acyl carrier protein (ACP) [1]. This coenzyme contains 4'-phosphopantetheine as the prosthetic group, this being connected by a phosphodiester bond with the hydroxyl group of the serine residue of the protein [2].



4'-Phosphopantetheine is also present in coenzyme A; however, the enzymes that catalyze the synthesis of fatty acids possess a higher specificity for thioesters of ACP than for thioesters of coenzyme A. The reason for this specificity is still unclear, and additional investigations are required to establish the primary and secondary structures of ACP.

The present paper gives the results on the preparation of substances that can be used as the starting materials in the synthesis of compounds modelling ACP. To form a phosphodiester bond between the pantetheine molecule and serine it was necessary to synthesize a derivative of pantothenic acid with a blocked secondary hydroxyl group. We have previously proposed two routes for the preparation of such compounds [3,4]. Continuing investigations in this field, we have synthesized optically active 2'-O-mesylpantothenonitrile (II), 2'-O-tosylpantothenonitrile, 2'-O-mesylpantothenonitrile 4'-phosphate (III), and pantothenonitrile 2',4'-cyclophosphate (V).

Compound II was obtained by condensing 2'-O-mesylpantolactone (I) with  $\beta$ -aminopropionitrile at 50°C without a solvent. The structure of the crystalline nitrile (II) was confirmed by its IR and PMR spectra. In the IR spectrum of compound II, the absorption band at 1780  $\text{cm}^{-1}$  characteristic for a lactone has disappeared and intense bands have appeared in the 1530  $\text{cm}^{-1}$  region (amide II) and the 1665  $\text{cm}^{-1}$  region (amide I), together with a band at 2260  $\text{cm}^{-1}$  corresponding to the nitrile group. The NMR spectrum (Fig. 1) has a triplet from the protons of the methylene groups of

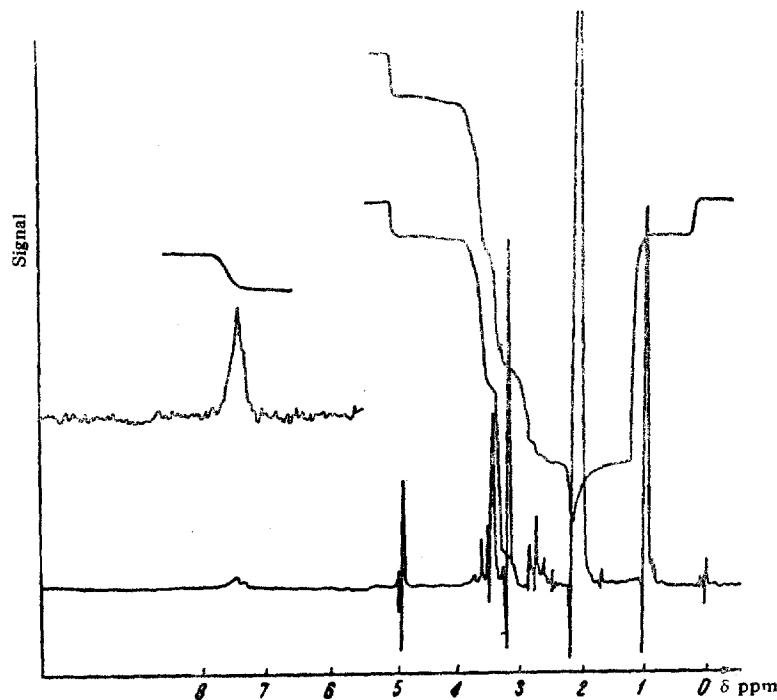


Fig. 1. PMR spectrum of D-(+)-2'-O-mesylpantothenonitrile (in acetonitrile solution).

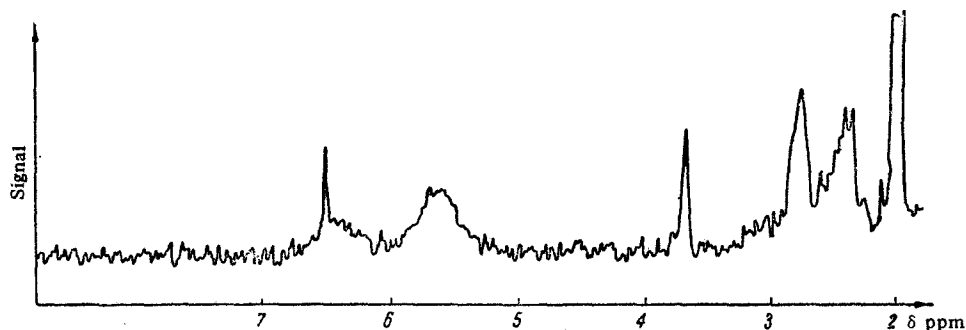
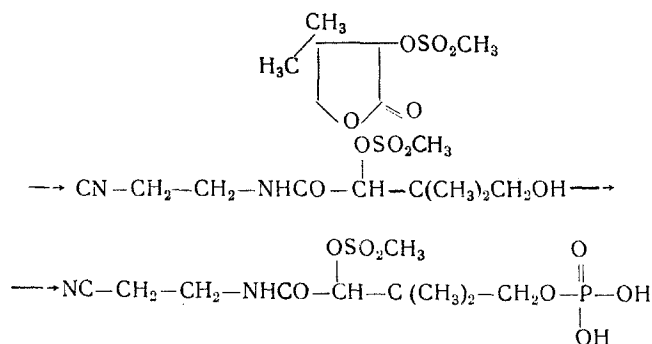


Fig. 2. PMR spectrum of D-(+)-2'-O-mesylpantothenonitrile (in acetonitrile solution).

$\text{CH}_2\text{-CN}$  with a chemical shift of 2.7 ppm and a spin-spin coupling constant  $J = 5.3$  Hz and of  $\text{CH}_2\text{-N}$  with  $\delta = 3.5$  ppm and  $J = 5.3$  Hz; a signal in the 7.6 ppm region due to the NH proton; and a signal with  $\delta = 4.9$  ppm due to the proton of the hydroxyl group; a singlet of the protons of the hydroxymethylene group with  $\delta = 3.5$  ppm is also observed.

2'-O-Mesylpantothenonitrile (II) was phosphorylated with phosphorus oxychloride in pyridine; the phosphate (III) was formed.



The addition of barium hydroxide to the reaction mixture led to the formation of the barium salt of the phosphate (III), which was purified by reprecipitation from methanol. By chromatography on ion-exchange resin, the free acid was obtained in the form of an oily substance. The individuality of this compound was confirmed by paper chromatography. The curve of the potentiometric titration of the phosphate (III) has two points of inflection, which shows the presence of two hydroxyl groups in the phosphate residue. The IR spectrum of the compound has broad bands in the  $2300\text{--}2700\text{ cm}^{-1}$  relating to  $\text{P-OH}$  stretching vibrations and also bands in the regions of  $1230\text{ cm}^{-1}$  ( $\text{P=O}$ ) and  $1050\text{ cm}^{-1}$  ( $\text{P-O-C}$ ). The PMR spectrum (Fig. 2) has a singlet (6.6 ppm) of the two hydroxyls of the phosphoryl group and signals of the methylene groups of  $\text{CH}_2\text{-CN}$  and  $\text{CH}_2\text{-N}$  (2.4 and 2.8 ppm).

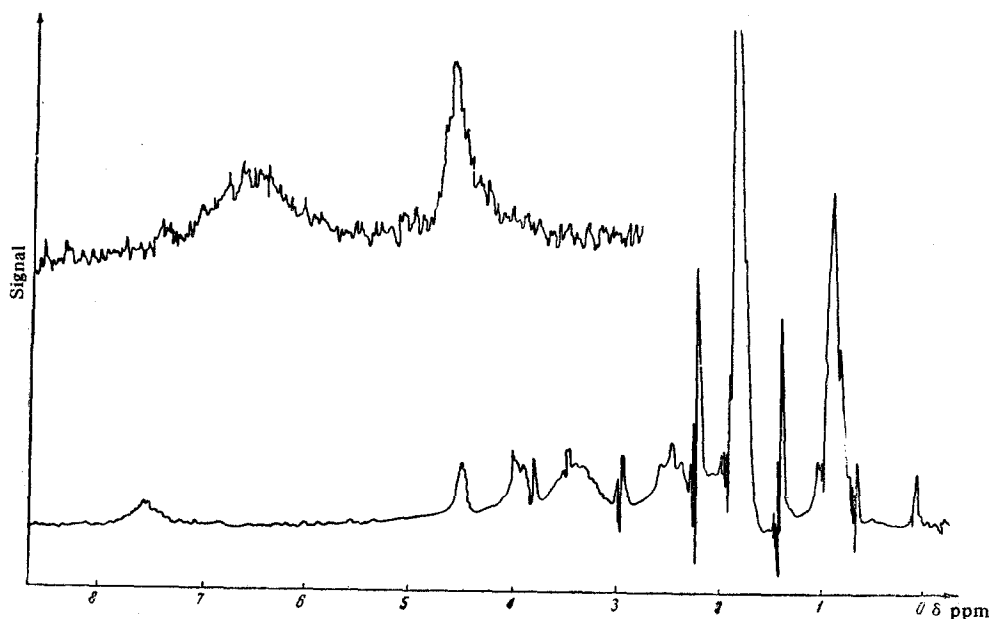
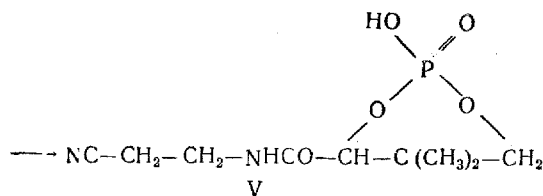


Fig. 3. PMR spectrum of D-(+)-pantothenonitrile 2',4'-cyclophosphate (in acetonitrile solution).

The phosphorylation of pantothenonitrile (IV), which contains two free hydroxyl groups, with phosphorus oxychloride led to the cyclic phosphate V, as was shown by means of potentiometric titration.



The IR spectrum of compound (V) exhibited absorption bands reflecting its structure. The features of the PMR spectra (Fig. 3) show that there is one hydroxyl group in the phosphate V (singlet with  $\delta = 4.6$  ppm).

### Experimental

D-(+)-2'-O-Mesylpantothenonitrile (II). A mixture of 4.16 g of D-(+)-O-mesylpantolactone [4] with mp 55–56° C,  $[\alpha]_D^{20} +20.2^\circ$  (c 2; chloroform) and 1.4 g of  $\beta$ -aminopropionitrile was heated at 50° C for 4 hr. On cooling, the substance crystallized. Yield 4.92 g (89.6%), mp 102–103° C (from ethyl acetate)  $[\alpha]_D^{20} +17.8^\circ$  (c 2; water).

Found, %: C 42.87, 42.94; H 6.49, 6.39; N 10.37, 10.21; S 11.42, 11.55. Calculated for  $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_5\text{S}$ , %: C 43.15; H 6.52; N 10.07; S 11.52.

D-(+)-2'-O-Tosylpantothenonitrile was obtained similarly. Yield 73%; mp 60–61° C;  $[\alpha]_D^{20} +16.4^\circ$  (c 2; water).

Found, %: C 54.28, 54.17; H 6.37, 6.45; N 7.67, 7.74; S 8.97, 8.69. Calculated for  $\text{C}_{16}\text{H}_{22}\text{N}_2\text{O}_5\text{S}$ , %: C 54.23; H 6.26; N 7.91; S 9.04.

D-(+)-2'-O-Mesylpantothenonitrile 4'-phosphate (III). In drops, 0.7 ml of phosphorus oxychloride was added at –5 to –10° C to a solution of 1.15 g of 2'-O-mesylpantothenonitrile in 3 ml of pyridine. The mixture was stirred at –5° C for 30 min and then at 20° C for 2 hr and poured onto ice. After 1 hr, the pyridine was evaporated off in vacuum, the residue was dissolved in 10 ml of water, and the solution was brought to pH 7.5 with barium hydroxide. The precipitate formed was separated off, and the filtrate was washed with ether (2  $\times$  30 ml) and evaporated. The substance was taken up in methanol, the insoluble residue was separated off, and ether was added until the solution became turbid (200 ml). After cooling to 0° C, the precipitate was collected by centrifuging and it was redissolved in 20 ml of methanol and reprecipitated with 200 ml of ether. A 10% aqueous solution of the barium salt was passed through a column of the resin Amberlite IR-120 ( $\text{H}^+$  form), which was then washed with water to neutrality. The eluate and the washing liquid were evaporated to dryness. Yield 1.12 g (78.2%);  $[\alpha]_D^{20} +17.3^\circ$  (c 2; water).

Found, %: C 30.23, 30.28; H 5.88, 6.02; P 8.21, 8.02. Calculated for  $\text{C}_{10}\text{H}_{19}\text{N}_2\text{O}_8\text{PS} \cdot 2\text{H}_2\text{O}$ , %: C 30.48; H 5.88; P 7.85.

D-(+)-Pantothenonitrile 2',4'-cyclophosphate (V). In drops, 3 ml of phosphorus oxychloride was added at –5 to –10° C to a solution of 6.0 g of D-(+)-pantothenonitrile [5] with  $[\alpha]_D^{20} +30.2^\circ$  (c 1.75; water) in 16 ml of pyridine.

The substance was isolated in a similar manner to compound III. Yield 5.44 g (76.2%);  $[\alpha]_D^{20} +19.4^\circ$  (c 2; water).

Found, %: C 36.37, 36.45; H 6.36, 6.55; P 9.83, 9.95. Calculated for  $\text{C}_9\text{H}_{15}\text{N}_2\text{O}_5\text{P} \cdot 2\text{H}_2\text{O}$ , %: C 36.25; H 6.42; P 10.38.

The PMR spectra were taken on a "Hitachi" H-60 instrument.

### Conclusions

D-(+)-2'-O-Mesyl- and D-(+)-2'-O-tosylpantothenonitriles, and also D-(+)-D'-O-mesylpantothenonitrile 4'-phosphate and D-(+)-pantothenonitrile 2',4'-cyclophosphate have been synthesized.

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29 July 1967

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