ACYL GROUP CARRIERS.

XVII. SYNTHESIS OF ANALOGUES OF 3'-DEPHOSPHOCOENZYME A WITH MODIFIED PYROPHOSPHATE GROUPS

V. M. Kopelevich, L. N. Bulanova, Yu. V. Belousova, A. V. Lysenkova, V. F. Pozdnev, and V. I. Gunar

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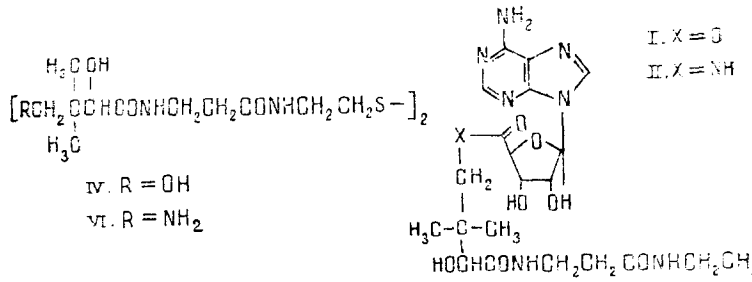
The synthesis has been performed of dephosphocoenzyme A, 4',4"-di-O-(2',3'-O-isopropylideneadenosineuronyl)pantethine, and of 4',4"-di(2',3'-isopropylideneadenosineuronylamino)-4',4"-dideoxypantethine from 2',3'-O-isopropylidene adenosineuronic acid, using as condensing agents the tert-butyl dicarbonate–pyridine and the N,N'-dicyclohexylcarbodiimide–N-hydroxysuccinimide systems, respectively.

Coenzyme A (CoA) acts as a carrier of acyl residues which are bound to its SH group [1]. To study the role of the other functional groups of CoA in enzymatic reactions the synthesis has been performed of a number of its analogues both in the nucleoside and in the pantetheine moieties of the molecule and their interaction with a number of CoA-dependent enzymes has been studied [2, 3]. Only some structural features that the CoA molecule must possess for binding with the enzyme were established, and, in particular, the role of the phosphate bridge linking the adenosine and the pantethine residues was not investigated.

In continuation of our work on the study of the properties of pantothenic acid derivatives [4, 5] we have performed the synthesis of analogues of 3'-dephospho-CoA of a new type in which, with the retention of the adenine and pantetheine parts of the molecule the pyrophosphate bond between them has been replaced by ester and carboxamide groupings. The structures of compounds (I and II) which we selected for study are given below.



As the initial compound for the synthesis of (I) and (II) we used 2',3'-O-isopropylideneadenosineuronic acid (III), a convenient method for obtaining which by the oxidation of 2',3'-O-isopropylideneadenosine with potassium permanganate in an alkaline medium has been developed by Prasad et al. [6]. We proposed to obtain the 2',3'-O-isopropylidene derivative (I) by the acid chloride method described for the synthesis of alkyl esters of (II) [6]. However, the interaction of the acid chloride of (III), obtained by the reaction of (III) with thionyl chloride, with D-pantethine (IV) did not lead to the desired product, and D-pantolactone and β-alethine were isolated from the reaction mixture, which showed the decomposition of (IV) owing, apparently, to the formation of a certain amount of hydrochloric acid from the thionyl chloride through trace amounts of water. The addition of triethylamine to the reaction mixture did not give satisfactory results.

In view of this, we studied the possibility of activating the carboxy group of (III) with the aid of the tert-butyl dicarbonate (Boc_2O)–pyridine system, which, as has been shown previously by one of us, gives good results in the acylation of primary [7] and secondary [8] alcohols. In actual fact, it was found that when the acid (III) was condensed with

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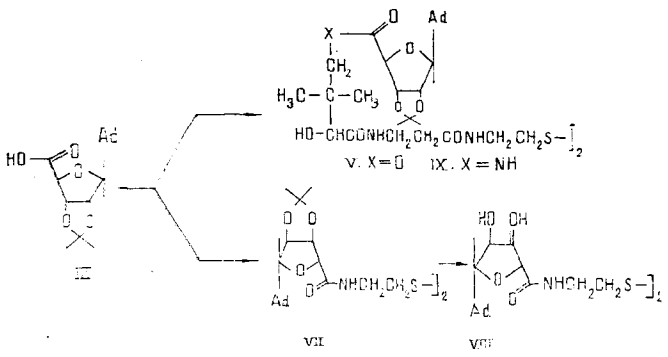
D-pantethine in dioxane–pyridine in the presence of Boc_2O and triethylamine for 96 h, 2',3'-O-isopropylidene–I (V) was obtained in satisfactory yield. Compound (V) was isolated with the aid of extraction from aqueous solutions with chloroform, which proved possible because of the fairly high lipophilicity of the pantethine residue and the presence of the lipophilic protective group in the adenosine residue. The structure of compound (V) was confirmed by its IR spectrum in which there were absorption bands in the regions of (cm^{-1}) 1745 (COOR), 1660 (amide I) and 1535 (amide II). The UV spectrum had the maximum at 260 nm that is characteristic for adenosine derivatives.



In the synthesis of the analogue (II) from (III) and 4'-amino-4'-deoxypantethine (VI), a method for obtaining which we have described previously [9], we used N,N'-dicyclohexylcarbodiimide (DCHC) as the condensing agent. And although an attempt to obtain the N-hydroxysuccinimide ester of the acid (III) with the aid of DCHC was unsuccessful, direct condensation of (III) with cystamine (used as a model compound) at 0°C in the presence of DCHC and N-hydroxysuccinimide led to the formation of the desired amide derivative, di(2',3'-O-isopropylideneadenosineuronyl)cystamine (VII) with a yield of 49%.

The IR spectrum of compound (VII) contained absorption bands of an amide carbonyl group at 1665 and 1575 cm^{-1}, confirming its structure. The elimination of the isopropylidene protecting group from the adenosine moiety of the molecule of (VII) was effected by treatment with 88% formic acid without the cleavage of the amide bond, and gave N,N'-di(adenosineuronyl)-cystamine (VIII).

2',3'-O-Isopropylidene–II (IX) was obtained by a similar route using compound (VI).

Attempts to eliminate the isopropylidene protecting group from compounds (V) and (IX) by acid hydrolysis with formic, acetic, trifluoroacetic, and hydrochloric acids of various concentrations were unsuccessful and led to the complete [in the case of (V)] or the partial [in the case of (IX)] decomposition of the substance.

EXPERIMENTAL

IR spectra were measured on a Perkin-Elmer model 180 spectrophotometer, and thin-layer chromatography was carried out on standard Silufol UV-254 plates (Czechoslovakia) in the following systems: 1) acetone–dioxane–25% ammonia (9:9:2); 2) butan-1-ol–acetic acid–water (5:2:3); and 3) isopropanol–25% ammonia-water (7:1:2).

4',4"-Di-O-(2',3'-O-isopropylideneadenosineuronyl)pantethine (V). A solution of 0.277 g of D-panthetine in 20 ml of dry pyridine was treated with 0.321 g of 2',3'-O-isopropylideneadenosineuronic acid, 0.654 g of tert-butyl dicarbonate, and 1 ml of triethylamine. The reaction mixture was stirred at 20°C for 96 h and was evaporated in vacuum, the residue was treated with 30 ml of chloroform and 20 ml of a 5% aqueous solution of sodium carbonate, the chloroform layer was separated off, the aqueous solution was extracted with chloroform (2 × 30 ml), and the chloroform solutions were combined and dried over sodium sulfate.

This gave 0.22 g (38%) of the oily ester (V), R_f 0.87 (system 1), 0.93 (system 3). IR spectrum, thin film, ν, cm^{-1}: 3320, 3190, 3090 (NH, OH), 1745 (ester C=O), 1660 (amide I), 1600, 1580 (C=N, C=C), 1535 (amide II), 1335, 1100 (C–O), 705 (C–S). Found, %: C 49.08; H 5.61; N 16.48; S 5.86. $C_{48}H_{68}N_{14}O_{16}S_2$. Calculated, %: C 49.64; H 5.90; N 16.89; S 5.52.

N,N'-Di(2',3'-O-isopropylideneadenosineuronyl)-cystamine (VII). A suspension of 0.23 g of cystamine hydrochloride in 3 ml of ethanol was treated with 2.3 ml of a 4.7% solution of sodium methanolate. The mixture was stirred for 30 min, the solid matter was separated off, and the filtrate was evaporated to dryness in a vacuum. The residue was treated with

a solution of 0.64 g of 2',3'-O-isopropylideneadenosine-5'-uronic acid in 20 ml of dimethylformamide cooled to 0°C, 0.45 g of N-hydroxysuccinimide, and 0.45 g of N,N'-dicyclohexylcarbodiimide. The mixture was stirred at 0°C for 2 h at 20°C for 10 h and, after the addition of a few drops of acetic acid, it was stirred for another 30 min, the precipitate was filtered off, and the filtrate was evaporated to dryness in vacuum. The residue was dissolved in 15 ml of chloroform and the solution was washed with water (3 × 5 ml), 5% sodium bicarbonate solution (3 × 5 ml) and with water to neutrality. The chloroform solution was dried over sodium sulfate, the solvent was driven off in vacuum, and the residue was dried in vacuum.

This gave 0.37 g (47.8%) of (VII). R_f 0.77 (system 1), 0.71 (system 2), 0.83 (system 3). IR spectrum with paraffin oil, ν, cm^{-1}: 3320 (NH), 1665 (amide I), 1575 (amide II). UV spectrum [water, λ_{max} nm (ε)]: 260 (11,000). Found, %: C 47.32; H 5.21; N 22.08; S 8.31. $C_{30}H_{38}N_{12}O_8S_2$. Calculated, %: C 47.48; H 5.05; N 22.15; S 8.45.

N,N'-Di(adenosineuronyl)cystamine (VIII). A solution of 0.2 g of compound (VI) in 2 ml of 88% formic acid was heated at 60°C for 4 h. The acid was driven off in vacuum and the residue was dried by evaporation with methanol. This gave 0.17 g (94.4%) of substance (VIII). R_f 0.37 (system 1), 0.34 (system 3). UV spectrum [water, λ_{max}, nm (ε)]: 260 (7970). Found, %: C 42.64; H 4.51; N 24.92; S 9.31. $C_{24}H_{30}N_{12}O_8S_2$. Calculated, %: C 42.47; H 4.46; N 24.77; S 9.45.

4',4''-Di(2',3'-O-isopropylideneadenosineuronylamino)-4',4''-dideoxypantethine (IX). A solution of 0.22 g of 4',4''-diamino-4',4''-dideoxypantethine trifluoroacetate [9] in 3 ml of dimethylformamide was treated with 0.08 ml of triethylamine, the mixture was stirred at 20°C for 30 min, and 0.16 g of 2',3'-O-isopropylideneadenosineuronic acid, 0.1 g of N-hydroxysuccinimide, and after cooling to 0°C, 0.13 g of N,N'-dicyclohexylcarbodiimide were added. After a working-up procedure as described for the synthesis of (VII), 0.13 g (39.4%) of (IX) was obtained. R_f 0.81 (system 1), 0.71 (system 2). IR spectrum, paraffin oil, ν, cm^{-1}: 3400 (NH, OH), 1640 (amide I), 1560 (amide II). Found, %: C 49.54; H 6.13; N 19.25; S 5.47. $C_{48}H_{70}N_{16}O_{14}S_2$. Calculated, %: C 49.73; H 6.09; N 19.33; S 5.53.

CONCLUSION

The synthesis has been effected of analogues of dephosphocoenzyme A, 4',4''-di-O-(2',3'-O-isopropylideneadenosineuronyl)pantethine and 4',4''-di(2',3'-O-isopropylideneadenosineuronylamino)-4',4''-dideoxypantethine from 2',3'-O-isopropylideneadenosineuronic acid using the Boc_2O—pyridine and the DCHC—N-hydroxysuccinimide systems as condensing agents, respectively.

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