NATURAL AND SYNTHETIC PANTOTHENIC ACID DERIVATIVES

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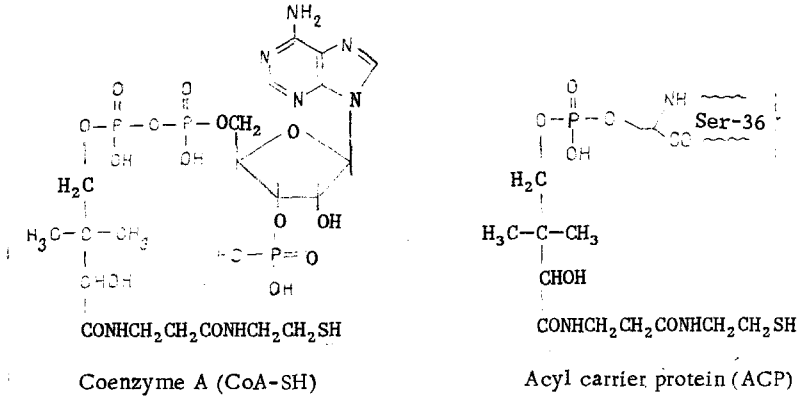
A review is given of the latest advances on the chemical synthesis and isolation of natural pantothenic acid, including bioprecursors of CoA. The value of the individual sections of the CoA molecule for the manifestation of coenzyme activity is discussed.

D-(+)-Pantothenic acid (PA), vitamin B_3, which is widespread in natural sources [1], is a component part of coenzyme A (CoA) which participates in numerous biochemical reactions of the activation and transfer of acyl groups and plays a central role in the energy metabolism [2]. The structure of CoA (Fig. 1) was determined by F. Lipmann's group in 1952 [3], and this coenzyme has long been considered as the sole coenzyme of PA. However, in the middle 60's when the acyl carrier protein (ACP) was discovered [4], and up to the present time, several new natural derivatives of PA have been characterized. Five types of proteins covalently bound with PA derivatives have been detected: 1) ACP (Fig. 1) [5] and one or two subunits of the analogous synthetase complex of eukaryotes [6]; 2) the acyl carrier subunit of the citrate and citramate lyase complex from Klebsiella [7] and Rhodopseudomonas [8]; 3) one of the subunits of the bacterial enzyme complex synthesizing various peptide antibiotics, such as gramicidin and tyrocidin [9]; 4) the patulin-synthesizing complex in fungi [6]; and 5) a subunit of the mitochondrial ATPase of fungi [10]. In cases 1, 3, and 4 the structure of the PA derivative was determined as 4'-phosphopantetheine which is bound to protein by a phosphodiester bond, while the prosthetic group of citrate and citramalate lyase was phosphoribosyldephospho-CoA. It has been reported that 4'-phosphopantetheine is a cofactor of guanosine-triphosphate-dependent acyl-CoA synthetase [11], and the presence of mitochondrial proteins covalently bound with 4'-phosphopantetheine that possibly participate in the transfer of acyl group has also been detected in Neurospora crassa [12]. Two CoA-containing nucleotides that inhibit DNA-dependent RNA polymerase have been isolated from E. coli, in which they are present in small amount, and have been characterized [13]. One of the nucleotides contains CoA and glutathione linked with one another by a disulfide bond, while the other is a dimer of CoA containing two equivalents of glutamic acid [14]. Finally, there is information on the identification in natural material of new growth factors for certain species of bacteria that contain PA [15]. The facts given permit the conclusion that in the living organism PA is subjected to various biochemical transformations with the production not only of CoA but also of other "active forms," and this also explains its vitamin activity.



Coenzyme A (CoA-SH) Acyl carrier protein (ACP)

Fig. 1

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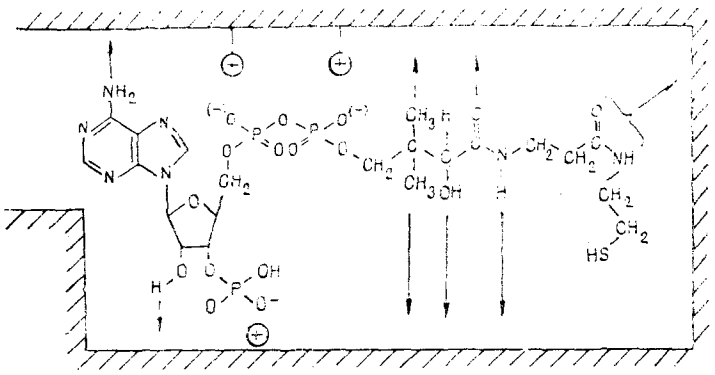


Fig. 2. Schematic model of the binding of CoA with a protein. The arrows show possible CoA-protein binding sections.

TABLE 1. Synthesis of Pantethine by Method B

Condensing agent	Yield, %	Literature
$[Me_2N^+=CHCl]\,Cl^-$	85	26
Diphenyl phosphorazidate	77	27
Benzene- or toluenesulfonyl chloride	80	28
$CH_2C_6H_4X$	—	29
$ClCH_2CN$	79.5	30
DCHC–1-hydroxybenzotriazole	95	31
DCHC–substituted phenols	85	32
DCHC–hydroxypyridines	85	32
DCHC–$(PhO)_2POH$, $(EtO)_2POH$	75	33
DCHC–$FeCl_3$ or $AlCl_3$	—	34
DCHC–aqueous pyridine	75	35
DCHC–aqueous pyridine–surface-active agent	81	36
DCHC–2-mercaptobenzimidazole	93	37
N,N'-Carbonyldiimidazole, N,N'-carbonylbis(2-phenyl- or 2-hexylimidazole)	50	38
Di-2-pyridyl disulfide and triphenylphosphine	60	39

The biochemical functions of CoA [2, 16, 17] and ACP [4, 17] have been discussed in detail in a number of reviews and monographs. Apart from this, generalizing papers on the synthesis of CoA and its bioprecursors [18, 19] and analogs [20] have been limited to the literature published up to 1970, while there are practically no reviews on the synthesis of growth factors. During the last 10-15 years, a considerable number of publications has appeared on the synthesis of pantethine and on the microbiological production of CoA, on the study of the specificity of CoA in relation to certain CoA-dependent enzymes, and also on the use of PA derivatives in medical practice. In view of this, in the present review we have analyzed certain directions and results of recent years on the chemistry and biochemistry of natural and synthetic derivatives of PA and CoA.

CHEMICAL METHODS OF SYNTHESIZING CoA AND ITS BIOPRECURSORS AND ANALOGS

D-Pantethine

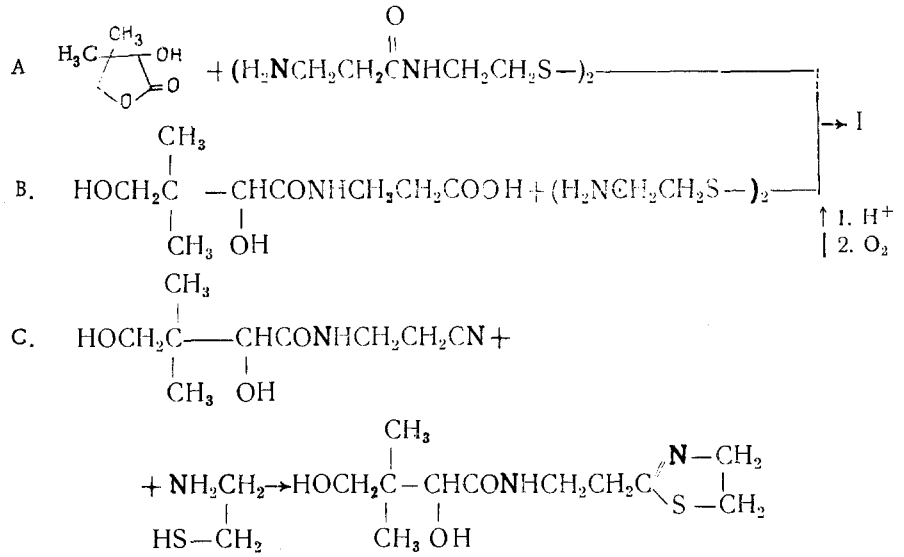
Research workers have shown considerable interest in a natural metabolite of PA, D-pantethine (I).

$$[HOCH_2\underset{I}{C}(CH_3)_2CHOHCONHCH_2CH_2CONHCH_2CH_2S-]_2$$

Investigations on pantethine can be divided into two stages. The first stage is connected with the development of the complete synthesis of CoA (1947-1965) using pantethine as an intermediate, and the second (from 1970 to the present time) relates to the period of the creation from pantethine of the new drug pantosin, which is being produced in Japan and is widely used for the normalization of the lipid metabolism in atherosclerosis, sugar diabetes, and some other diseases [17].

These synthetic methods developed for obtaining pantethine can be divided into three main groups: A) the condensation of D-pantolactone with β-alethine (II); B) the reaction of functional derivatives of PA with cystamine; and, c) the reaction of pantothenonitrile with cysteamine, followed by the opening of the thiazoline ring.

Scheme 1



A. The condensation of D-pantolactone with substance (II) and its derivatives (Scheme 1) takes place both in alcoholic solution and in the absence of a solvent at 100-150°C [19]. The key problem of this method is the synthesis of the β-alethine. Several methods have been proposed which are based on the use for protecting the amino group of β-alanine of phthaloyl and benzyloxycarbonyl groupings [18, 19], and also of formyl [21] and chloroacetyl [22] groups; the acid chloride or mixed-anhydride method is usually used for forming the amide bond between the protected β-alanine and cystamine [18, 19]. A more convenient method of obtaining β-alethine consists in the condensation of the N-hydroxysuccinimide ester of N-tert-butyloxycarbonyl-β-alanine with cystamine [23], which has also been used for the synthesis of the analogs of (II), N,N'-bis(glycyl- and γ-aminobutyryl) cystamines [24].

B. The methods of peptide chemistry, consisting in the formation and subsequent aminolysis of activated PA derivatives, are used for obtaining pantethine from PA (Scheme 1). Since the activation of the COOH group of PA by the formation of a mixed anhydride with carbonic acid that was originally used [19, 25] did not lead to satisfactory yields, in recent years more effective methods of activating the COOH group have been developed (see Table 1 and the references cited therein). Compound (I) has been synthesized in high yield by using a fairly mild reagent, $[Me_2N^+=CHCl]Cl^-$, to obtain the acid chloride of PA and condensing it with cystamine. Methods have been developed for obtaining activated derivatives of PA with phosphorus-containing acids and, in particular, with diphenyl phosphorazidate and phosphorocyanidate in the presence of a tertiary alkylamine, which have a number of advantages over carboxylic anhydrides due to the high selectivity of such anhydrides in the aminolysis reaction, which gives good results when unprotected hydroxy groups are present. Examples have been described of the use of sulfonyl chlorides for obtaining the corresponding mixed anhydrides of PA and their use in the synthesis of pantethine.

In early investigations of the synthesis of (I), the aminolysis of the methyl ester of PA was used, but this did not give satisfactory results. Later this method was improved by the use of the cyanomethyl ester of PA, which possesses a higher reactivity than the methyl ester, and also of a number of substituted phenyl and benzyl esters containing strong electron-accepting groups. The carbodiimide method using N,N'-dicyclohexylcarbodiimide (DCHC) and the carbonyldiimidazole method have also found use in the synthesis of pantethine. The possibilities of the DCHC method have been considerably expanded by the use of various additives which have effectively suppressed the formation of a by-product of the reaction, an N-acylcyclohexylurea, and have led to an increase in the yield of desired product.

C. A feature of the third method for the synthesis of pantethine is the use as the starting material of D-pantothenonitrile which, on treatment with cysteamine, is converted

into an intermediate thiazoline derivative capable of being transformed by acid hydrolysis into pantethine (Scheme 1). This method is discussed in detail in [19].

Apparently, all the methods of synthesis described are suitable for the industrial production of (I) although preference must be given to route B, where the choice of condensing agent (Table 1) is largely determined by its availability, since the yields of desired product are fairly high in practically all cases. It must be mentioned that investigations on the synthesis of pantethine are being carried out mainly by Japanese pharmaceutical firms, and a number of methods for which patents have been granted cannot be regarded as adequately effective and suitable for industrial purposes. Ion-exchange chromatography on a cation-exchanger and anion-exchanger is usually used for the isolation of pantethine, the ion-exchanger retaining traces of the initial compounds and permitting a pure preparation to be obtained in high yield [40]. The lyophilization process is used in the drying of pantethine solutions [41, 42].

S-Sulphopantetheine

At the end of the 1960's, Japanese workers [43] isolated from carrots new growth factors for bifidobacteria, which are permanently in the intestine of a healthy human being and play an important role in the functioning of the gastrointestinal tract, particularly in children during an early period of life [44]. As the result of a complex multistage process, all the stages were subjected to careful analysis, five components of the growth factor consisting of PA derivatives were isolated and identified: D-pantetheine, D-pantetheine 4'-phosphate, D-pantethine 4',4'-diphosphate, D-S-sulfopantetheine 4'-phosphate (III), and S-sulfo-3'-dephosphocoenzyme A [45]; the last two compounds were new PA derivatives. On treatment with alkaline phosphatase, the phosphate (III) was converted into S-sulfopantetheine (IV)

$$ROCH_2C(CH_3)_2CHOHCONHCH_2CH_2CONHCH_2CH_2SSO_3H$$
III. R = P(O)(OH)$_2$; IV. R = H

It was established that all five substances present in the growth factor, and also (IV), possessed practically the same growth activity for the strain Bifidobacterium bifidum No. 4 [46]. However, apparently, in vivo the main substances of carrots as a source of growth factor for bifidobacteria must be considered to be S-sulfo derivatives of pantetheine, since the other components are readily hydrolyzed in the upper sections of the small intestine to PA [47], while the S-sulfonates, which possess acidic properties, are more resistant to hydrolysis and are capable of reaching the boundary of the small and large intestines, where they exert a stimulating action on the growth of the bifidobacteria [48]. In 1974, brief information was given in Japanese patents [49, 50] of the possibility of the clinical use of the sulfonates (III) and (IV) as food additives and medicinal preparations for children aged 2-4 months, being fed artificially, for correcting the bifidus flora. In view of the fact that S-sulfopantetheine also exhibited a high vitamin activity [51], it was assumed that it can find use in therapeutic practice in those fields where PA and pantethine are used [51, 52]. The first reports of such a possibility appeared in 1983 with information on the addition of substance (IV) to pharmaceutical salves, shampoos, plasters, etc., for decreasing skin irritation [53], and also on the use of preparations of (IV) for lowering the level of cholesterol in the blood, for regulating the fatty metabolism, and for the treatment and prophylaxis of atherosclerosis [54, 55]. The use of cosmetic preparations containing the sulfonate (IV) for preventing skin pigmentation [56] and for removing pigment spots from the face [57] has been described. Substance (IV) can be used as a fodder additive in the fattening of young agricultural animals [58].

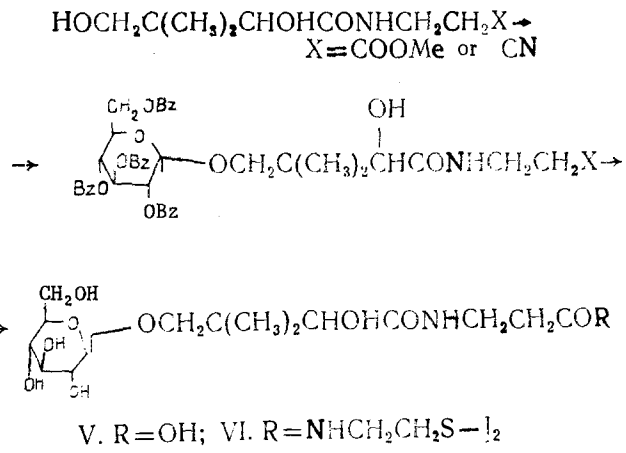
Compound (IV) was first synthesized by the sulfonation of D-pantethine with alkali-metal sulfites and bisulfites in the presence of catalytic amounts of copper, iron, or cobalt ions [50]. In recent years, a number of more rational approaches to the production of (IV) based on methods proposed previously for the synthesis of (I) have been developed. The condensation of the mixed anhydride of D-pantothenic and carbonic acids with S-sulfocysteamine in aqueous alkaline solution leads to substance (IV) with a yield of 60% [51, 59]; the L-isomer of (IV) has been synthesized by an analogous scheme [51]. Another method, based on the use of the mixed anhydride of PA and carbonic acid, consists in the production of N-D-pantothenoxylethyleneimide and the opening of the aziridine ring with sodium thiosulfate, leading to the desired product with a yield of 55% [51, 59]. On the interaction of PA or its salts with S-sulfocysteamine in the presence of DCHC with the addition of 1-hydroxybenzotriazole or of N-hydroxysuccinimide, the sulfate (IV) is obtained with a yield of 85% [60]. To obtain (IV)

from D-pantolactone, S-sulfoaletheine was synthesized by the interaction of an N-protected β-alanine (for example, N-phthaloyl-β-alanine) with S-sulfocysteamine in the presence of carbodiimides and N-hydroxy compounds [61] or by the acid chloride method [62], and also by the reaction of aziridine derivatives of N-protected β-alanine with sodium thiosulfate [61].

Glycosides of PA and of Pantetheine

At the beginning of the 1970's, the first representatives of a new group of natural PA derivatives were isolated from tomato juice [63] that possessed a 100 times greater activity for the growth of various lactic acid bacteria than the vitamin itself [64-66]. It was established [63] that the growth factor contains one residue each of glucose and PA and is, in fact, 4'-O-(β-D-glucopyranosyl)-D-PA (V). The synthesis of (V) was achieved by the glycosylation of the methyl ester of D-PA with tetra-O-benzoyl-α-D-glucopyranosyl bromide in the presence of $Hg(CN)_2$ in a mixture of nitromethane and benzene selectively at the 4'-position with the formation of tetra-O-benzoyl-(V), followed by hydrolysis with sodium methanolate [65]. When tetra-O-acetyl-α-D-glucopyranosyl bromide was used for glycosylation, a mixture of the corresponding 4'-O- and 2',4'-di-O-glucosides was formed, from which the individual substance were isolated, and these were hydrolyzed with sodium methanolate to (V) and 2',4'-O-di-(β-D-glucopyranosyl)-PA [67]. The synthesis of 4'-O-β-D-ribofuranosyl, β-D-galactopyranosyl, β-maltosyl, β-cellobiosyl, and α-D-glucopyranosyl derivatives of DL-PA and also of 4'-O-(β-D-glucopyranosyl)-DL-pantethine (VI) was effected in the same laboratory [67, 68]. For the preparation of (VI) a variant of the thiazoline method (method C) [67, 69, 70] was used (Scheme 2).

Scheme 2



V. R=OH; VI. R=$NHCH_2CH_2S-]_2$

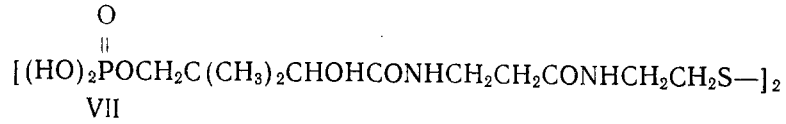
There are reports on the possibility of the enzymatic production of glucoside (V) [71] and of its α-anomer [72] with the aid of various glycosidases and by the use of certain yeast cultures with maltose as the source of carbohydrates.

The detection in natural sources of carbohydrate derivatives of PA raises the question of the physiological significance of these compounds. Since glucoside (V) and also its α-anomer and PA 4'-O-β-galactoside and 4'-O-β-D-cellobioside [67] are more effective as growth factors for a number of microorganisms than the vitamin itself, their activity is apparently not connected directly with their conversion into CoA. It is possible that the high activity of PA glycosides is due to their specific transport through biological membranes in which an important role is played by the β-glycosidic residue. In recent years, a β-glycosidic derivative of PA has been detected in canine urine, which shows the possibility of a new pathway for its metabolism in higher animals [73]. Although the significance of PA glycosides has not yet been established, their high growth activity and resistance to the action of acids, alkalis, and heating even today gives grounds for studying the possibility of using such substances as drugs and cosmetic preparations and as fodder additives [74].

D-Pantethine 4'-Phosphate and CoA

The structure of CoA proposed by Lipmann [3] was confirmed by its complete chemical synthesis achieved by various methods, a detailed review of which has been given previously [18, 19]. Only a few supplementary methods of obtaining CoA and the main intermediate in its synthesis, D-pantethine 4'-phosphate (VII), have been published. Thus, for example, the synthesis of the latter substance has been effected by the reaction of D-pantethine with 2-dimethyl-

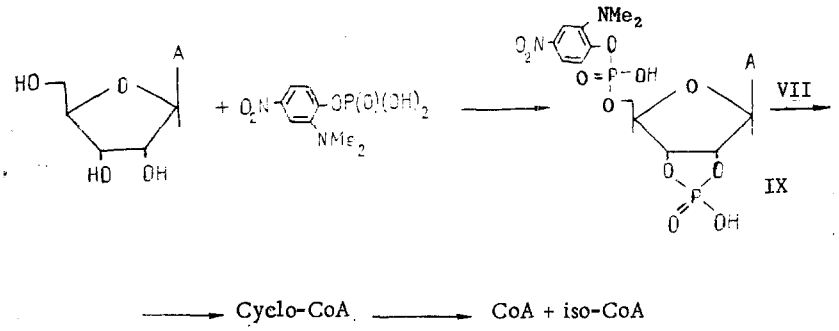
amino-4-nitrophenyl phosphate (VIII) [75]. The same phosphorylating agent has been used for synthesis of S-sulfopantetheine 4'-phosphate (III) [76], which was previously obtained by sulfonating (VII) [48] and by the phosphorylation of S-sulfopantetheine (IV) with 2-cyanoethyl phosphate in the presence of DCHC [77].



VII

The synthesis of 2',3'-cyclo-CoA has been carried out with the use of the reagent (VIII) by the condensation of 2',3'-cyclophosphoadenosine 5'-(2-dimethylamino-4-nitrophenyl phosphate) (IX) with the phosphate (VII) in a yield of 93%. Cyclo-CoA was converted by treatment with 0.1 M hydrochloric acid into a mixture of CoA and iso-CoA in a ratio of 1:1 [78] (Scheme 3).

Scheme 3



Redox condensation with triphenylphosphine and di-2-pyridyl disulfide led to pantethine and adenosine 2',3'-cyclophosphate 5'-phosphoromorpholidate; condensation of the latter with the phosphate (VII) by Khorana's method followed by treatment with ribonuclease led to CoA [79]. The overall yield of CoA by the above-mentioned methods did not exceed 25% (calculated on the PA). It must be mentioned that the methods for the chemical synthesis of CoA that have been developed up to the present time have not found practical application because of their relative complexity, multistage nature, and low yield. Therefore the commercial CoA preparations for biochemical investigations and medical use that are marketed by a number of foreign firms are obtained from natural sources.

While the demand for CoA and its numerous S-acyl derivatives for research purposes is rising continuously, the question of their therapeutic use has not yet been definitively answered. On the basis of information on the metabolic functions of CoA it may be expected that it will have a broad spectrum of pharmacological activity, and there is information in the literature on the experimental and clinical study of CoA [17], but the use of CoA preparations in medical practice has so far been limited, which is largely connected with the high cost of the CoA. In order that the use of preparations based on CoA should become economically acceptable great attention has been devoted in the last decade to the development of a cheaper microbiological method of synthesizing CoA. It has been established that the biosynthesis of CoA in microorganisms from PA, L-cysteine, and ATP takes place in five stages [80]. As a result of the screening of various yeasts and bacteria it has been found that the greatest capacity for accumulating CoA is possessed by Brevibacterium ammoniagenes 12071, which contains, with a high degree of activity, all five enzymes required for the synthesis [81, 82]. With the use of cells of these microorganisms as catalysts, a new preparative synthesis CoA from its precursors has been developed. However, the most promising method for the industrial production of CoA is a combination of the chemical synthesis of phosphorylated PA derivatives followed by their enzymatic conversion into the desired coenzyme.

CoA ANALOGS AND THEIR INTERACTION WITH CoA-DEPENDENT ENZYMES

Lynen et al. [2] have shown that the thiol group of CoA plays a central role in the process of transferring acyl groups to an appropriate acceptor and in the activation of a methylene group linked to a thioacyl grouping in condensation reactions. The mechanism of reactions with the participation of CoA has been explained on the basis of ideas of theoretical organic chemistry [83]. To determine the value of the other functional groups and individual sec-

TABLE 2. Simplified Chemical Structures of CoA and Its Analogs

Trivial name	Chemical structure
CoA	A—3′—P—5′—P—P—$CH_2C(CH_3)_2CHOHCONHCH_2CH_2$—$CONHCH_2CH_2SH$
1. Oxy-CoA	A———$CONHCH_2CH_2OH$
2. Seleno-CoA	A———$CONHCH_2CH_2Se$
3. Dethio-CoA	A———$CONHCH_2CH_3$
4. α-Methyl-CoA	A———$CONHCH(CH_3)CH_2SH$
5. β-Methyl-CoA	A———$CONHCH_2CH(CH_3)SH$
6. Homo-CoA	A———$CONHCH_2CH_2CH_2SH$
7. Decysteamine-CoA amide	A———$CONHCH_2CH_2CONH_2$
8. Decysteamine-CoA	A———$CONHCH_2CH_2COOH$
9. Decysteamine-CoA nitrile	A———$CONHCH_2CH_2CN$
10. De-β-alanine-CoA	A———$CONHCH_2CH_2SH$
11. Dealetheine-CoA amide	A———$CONH_2$
12. Pantoethyl-CoA	A———$C(CH_3)(C_2H_5)$———SH
13. Keto-CoA	A———C(O)———SH
14. Guanosino-CoA	G———SH
15. Inosino-CoA	I———SH
16. Cytidino-CoA	C———SH
17. Pyrophospho-CoA	A 3′—PP———SH

tions of the CoA molecule in the manifestation of coenzyme activity, a large number of CoA analogs have been synthesized and the influence of structural changes on the occurrence of the enzymatic reactions has been studied. These investigations have brought us closer to an understanding of the value of certain sections of CoA in its interaction with enzymes and the mechanism of the reaction itself.

Modification of the SH Group

The replacement of the SH group in the pantetheine molecule by an OH group gave its oxygen analog, oxypantetheine, which possessed a competitive growth-inhibiting activity in relation to pantetheine-dependent microorganisms [84]. Oxy-CoA (Table 2, No. 1) which has been obtained from D-oxypantetheine by both chemical [85] and enzymatic [86] methods, exhibited a high competitive inhibiting activity in the phosphotransacetylase reactions [87], with K_i $3.5 \cdot 10^{-7}$ M. An isosteric analog of CoA, seleno-CoA (Table 2, No. 2) in which the sulfur atom has been replaced by selenium was synthesized by Khorana's method [88]. The most complicated problem in its production was the separation of the 3'- from 2'-isomer. Because of the high sensitivity of selenols to oxidation, with the formation of diselenides, before separation on a column of ECTEOLA-cellulose the isomers were converted into their benzoyl derivatives. On desulfuration, CoA forms dethio-CoA (Table 2, No. 3), which, however, cannot be isolated in the analytically pure form [89]. Later, dethiopantetheine and its 4'-phosphate and 3'-dephosphodethio-CoA were synthesized [90], and the latter two compounds were converted enzymatically into dethio-CoA. The conversion of oxypantetheine and dethiopantetheine 4'-phosphates into the corresponding CoA analogs with the aid of enzymes biosynthesizing CoA shows that the SH group is not essential for the enzymatic reactions taking place in the last stages of the biosynthesis of CoA. Both analogs (Nos. 2 and 3) are competitive inhibitors of CoA in the phosphotransacetylase reaction.

Modification of the Cysteamine Moiety

The starting compound used for the synthesis of α- and β-methyl-CoA's (Table 2, Nos. 4 and 5) is P^1-(3'-phosphoadenosin-5'-yl)-P^2-(D-pantothenonitrile-4'-yl)pyrophosphate, which reacts with racemic α- and β-methylcysteamines to form the corresponding thiazolines, the hydrolysis of which leads to the desired substances [91]. In the preparation of homo-CoA (Table 2, No. 6), containing homocysteamine in place of cysteamine, a combination of Khorana's method for the formation of a pyrophosphate bond between homopantethine 4'-phosphate and an adenosine nucleotide and Michelson's method for opening a 2',3'-cyclophosphate was used [92]. These compounds proved to be competitive inhibitors of CoA; only α-methyl-CoA possessed a weak coenzyme activity.

TABLE 3. Interaction of Acetyl-CoA Synthetase with Biopre-cursors and Analogs of CoA

Substance	K_m, M	V_{max}, rel. units	Concentration of inhibitor, M	Inhibition, %
GA	$1{,}25 \cdot 10^{-4}$	1.0		
PA			$4{,}0 \cdot 10^{-3}$	7
4'-Phospho- PA			$4{,}0 \cdot 10^{-3}$	55
4'-Phosphopantetheine	$8{,}3 \cdot 10^{-4}$	1.25		
Dephospho-CoA	$2{,}0 \cdot 10^{-4}$	0,94		
Xa	$1{,}0 \cdot 10^{-3}$	0,7		
Xb	$2{,}5 \cdot 10^{-3}$	0,08		
Xc			$1{,}0 \cdot 10^{-3}$	5
Xd			$1{,}0 \cdot 10^{-3}$	5
Xe	$7{,}0 \cdot 10^{-4}$	0,8		
Xf			$1{,}0 \cdot 10^{-3}$	5
XI			$1{,}0 \cdot 10^{-3}$	5

Analogs with a Shortened Pantetheine Moiety

Japanese workers have synthesized a group of CoA analogs (Table 2, Nos. 7-11) lacking one of the sections of the pantetheine molecule by a combination of Khorana's and Michelson's methods [93]. These analogs proved to be powerful competitive inhibitors, more active than oxy- or dethio-CoA.

Modification of the Hydroxy Acid Moiety

Among PA derivatives with a changed pantoic moiety the greatest interest is presented by 2-keto- and β-ethylnorpantothenic acids, which possess a high growth activity for lactic acid bacteria. It has been shown [94] that labeled β-ethylnor-PA introduced into L. arabinosus 17-5 cells is converted into the corresponding homologs of 4'-phosphopantetheine and CoA, fulfilling in the cell the same biological functions as the natural coenzyme. Pantoethyl-CoA (Table 2, No. 12), obtained chemically by a combination of the morpholide and thiazoline methods, possessed practically complete CoA activity in the phosphotransacetylase reaction [95]. Keto-CoA (Table 2, No. 13), obtained from 2',2"-diketopantethine [96] exhibited both an inhibiting and a coenzyme activity (37% of the activity of CoA) [93]; the stereoisomer with respect to the 2'-hydroxy acid moiety, L-CoA, possessed a similar activity [93].

Modification of the Nucleoside Moiety

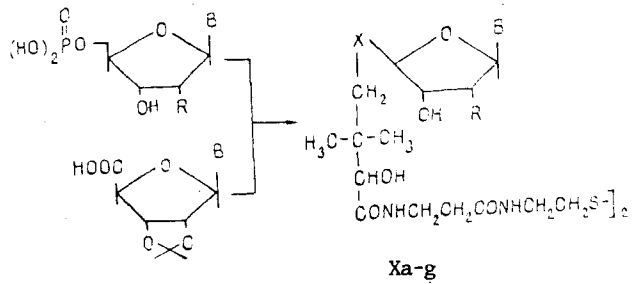
Guanosino-CoA (Table 2, No. 14), in which adenosine had been replaced by guanosine, has been synthesized by the morpholide and the thiazoline methods [97]. An attempt to obtain inosino-CoA (Table 2, No. 15) by the deamination of CoA with the aid of sodium nitrite was unsuccessful and led to the formation of an unidentified substance which was possibly formed as a result of the oxidation of the SH group. The deamination of the disulfide form of CoA followed by reduction enabled inosino-CoA to be synthesized with a yield of 64% [98]; the thiazoline method has also been used successfully for its synthesis [98]. It has been shown that neither analog replaces CoA in the phosphotransacetylase reaction [99], while cytidino-CoA (Table 2, No. 16), synthesized by Khorana's method [100], was a substrate in this reaction with a V_{max} corresponding to 1.6% of V_{max} for CoA, which shows the importance of the NH_2 group of the nucleoside moiety in binding with the apoenzyme.

Modification of the Pyrophosphate and 3'-Phosphate Groupings

To elucidate the role of the pyrophosphate group of CoA in enzymatic reactions, the analog dephospho-CoA in which the pyrophosphates section had been shortened by one phosphate unit with the retention of the adenine and pantetheine moieties of the molecule (Xa) and also its analogs modified in the ribose residue (Xb) or in the heterocyclic base (Xc-e) were synthesized. Compounds (Xa-e) were obtained by condensing the peracetylated derivatives of adenosine, 2'-deoxyadenosine, guanosine, inosine, and cytidine 5'-monophosphates with pantethine

in the presence of DCHC followed by deacetylation with a mixture of aqueous ammonia and pyridine [101, 102]. The analogs (Xf, g) in which the pyrophosphate bond had been replaced by ester and carboxamide groupings, respectively, were obtained for the same purpose [101, 103, 104] (Scheme 4).

Scheme 4



Xa-g

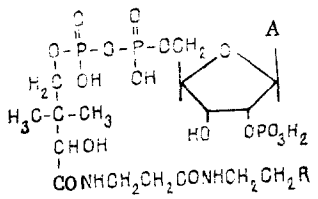
Xa–e X=$CH_2OP(OH)O$
a. B=adenine, R=OH
b. B=adenine, R=H
c. B=guanine, R=OH
d. B=xanthine, R=OH
e. B=cytosine, R=OH
f. X=COO. B=adenine, R=OH
g. X=CONH, B=adenine, R=OH

The synthesis of compound (Xf) and its 2',3'-O-isopropylidene derivative (XI) was carried out by the condensation of adenosine 5'-(ribofuranuronic acid) or its isopropylidene derivative with D-pantethine using tert-butyl pyrocarbonate as condensing agent [104]. The condensation of 2',3'-O-isopropylideneadenosine 5'-(ribofuranuronic acid) with 4'-amino-4'-deoxypantethine in the presence of DCHC-N-hydroxysuccinimide led to the formation of 2',3'-O-isopropylidene-(Xg) [104]. Compounds (Xa), (Xf), and (Xg) did not replace CoA in the phosphotransacetylase reaction [101], while in the N-acetyltransferase reaction the phosphodiester analog exhibited appreciable coenzyme activity [101]. The interaction of the analogs (X) with acetyl-CoA synthetase, which catalyzes the acetylation of the SH group of CoA, was also investigated. The choice of this enzyme was determined by the key importance of CoA in biological acetylation reactions, and also by the possibility of obtaining the enzyme in a highly purified state [105]. In the first stage of the work, the substrate and inhibitor properties of nonnucleoside precursors of CoA were studied. As can be seen from Table 3, pantothenic acid and its 4'-phosphate showed an inhibiting effect on the activity of the enzyme, while D-pantetheine 4'-phosphate, and also dephospho-CoA replaced CoA in S-acetylation reactions fairly effectively [102]. The replacement of the pyrophosphate bond by a phosphodiester bond [compound (Xa)] lowered the affinity and decreased V_{max} in comparison with dephospho-CoA, while the elimination of the hydroxyl in the 2'-position of the ribose residue [compound (Xb)] led to an appreciable fall in affinity [103]. When the adenine base in compound (Xa) was replaced by guanine or hypoxanthine [compounds (Xc) and (Xd)], but not by cytosine [compound (Xe)], complete loss of substrate properties was observed [102]; the elimination of the pyrophosphate moiety from the molecule of dephospho-CoA [compound (Xf)] also led to the loss of substrate properties [103]. Compounds (Xc, d, f) and (XI) weakly inhibited the S-acetylation reaction. On the basis of the results obtained, it may be concluded that the CoA-binding section of the active center of acetyl-CoA synthetase contains at least three main loci: a hydrophobic region of the binding of the adenine ring, and cationic regions binding the pyrophosphate moiety and the hydroxyl in position 2' of the ribose residue [103].

3-Pyrophospho-CoA (Table 2, No. 17) has been obtained enzymatically from 3'-dephospho-CoA, and in the phosphotransacetylase reaction it exhibited twice the activity of the natural coenzyme [106].

Analogs of Acetyl-CoA

Two investigations have been devoted to obtaining analogs of the most important CoA derivative, S-acetyl-CoA, the analogs concerned being acetamidodethio-CoA (XII) and acetonyldethio-CoA (XIII), in the synthesis of which the key intermediates were acetamidodethiopantetheine (XIV) and acetonyldethiopantetheine (XV).



XII. R=NHCOCH$_3$
XIII. R=CH$_2$COCH$_3$

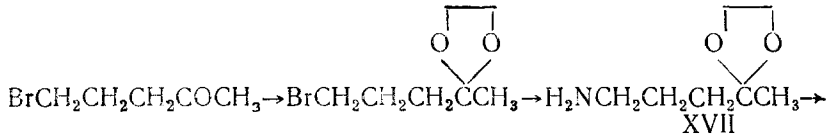
For the synthesis of the analog (XIV), ethylenediamine was acetylated with the aid of ethyl acetate, giving N-acetamidoethylamine, which was condensed with the p-nitrophenyl ester of carbobenzoxy-β-alanine with the formation of the amide (XVI). Elimination of the protective group from (XVI) by treatment with HBr/AcOH and condensation of the resulting aletheine analog with D-pantolactone led to (XIV) [107].

$$NH_2CH_2CH_2NHCOCH_3 + C_6H_5CH_2OCONHCH_2CH_2COOC_6H_4NO_2\text{-}p \rightarrow$$

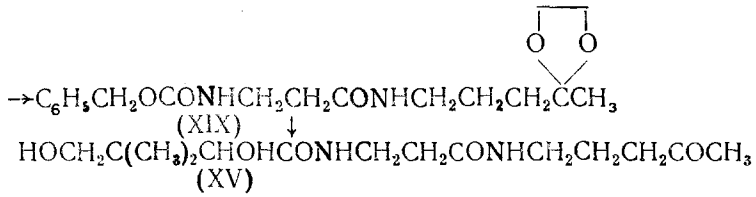
$$\rightarrow \underset{(XVI)}{C_6H_5CH_2OCONHCH_2CH_2CONHCH_2CH_2NHCOCH_3} \downarrow$$

$$\underset{(XIV)}{HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2CONHCH_2CH_2NHCOCH_3}$$

In the synthesis of compound (XV), the initial 5-bromopentan-2-one was converted into the ketal (XVII), the treatment of which with an excess of aqueous NH_3 led to 2-(3-aminopropyl)-2-methyl-1,3-dioxolane (XVIII). The reaction of the amine (XVIII) and the p-nitrophenyl ester of carboxy-β-alanine gave the protected derivative (XIX), from which, by catalytic hydrogenolysis followed by condensation of the free amine formed with pantolactone and purification on ion-exchange resins in the H^+ and OH^- forms (during which the ketal protection was eliminated), led to (XV) [108].



XVII



The phosphorylation of (XIV) and (XV) with the aid of β-cyanoethyl phosphate and DCHC led to their 4'-phosphates, from which the analogs (XII) and (XIII), respectively, were synthesized by Khorana's method. It was found [107] that (XII) is a competitive inhibitor of acetyl-CoA in the phosphotransacetylase reaction, and that the analogs (XII) [107] and (XIII) [108] inhibit citrate synthase. In contrast to this, acetyl-CoA carboxylase uses (XIII) as a substrate [108].

On the basis of information available in the literature concerning the influence of modifications to the structure of CoA on the phosphotransacetylase reaction, it is possible to draw certain preliminary conclusions concerning the specificity of the phosphotransacetylase with respect to various sections of the coenzyme molecule. Apparently, normal functioning as a cofactor requires the complete natural structure of CoA, and any changes in the molecule lead to the partial or complete loss of coenzyme activity. The only exceptions are pantoethyl-CoA and 3'-pyrophospho-CoA; the mechanism of the enhancement of catalysis with the latter analog is unclear and requires additional results, particularly in light of the fact that its activity with succinic acid thiokinase did not exceed the activity of either CoA or of 3'-dephospho-CoA [109]. A kinetic study of the analogs obtained has shown that an unchanged nucleotide moiety (3'-phosphoadenosine 5'-pyrophosphate) is necessary for the binding of CoA with phosphotransacetylase, while the pantetheine moiety mainly makes its contribution to the strength of the binding of the cofactor with the enzyme. The structure of the PA molecule is probably more specific for the enzymes involved in the biosynthesis of CoA. An important element of the structure is the adenine moiety of the coenzyme and, in particular, the presence of an amino group in position 6 of the adenine residue.

The absence of the results of x-ray structural analysis for the study of the active sites of CoA-dependent enzymes and their interaction with the substrates and the CoA (because of the difficulty of obtaining suitable crystals) makes the investigation of the nature of the bond between the apoenzyme and the coenzyme difficult. However, by using the results of a study of the chemical and coenzyme properties of CoA and its analogs and also of the conformation of CoA [110] and PA [111], it is possible to suggest the schematic working model of the binding of CoA with a protein that is shown in Fig. 2. In the CoA molecule there are hydrophobic sections that interact with hydrophobic regions of the protein, and hydrophilic sections capable of forming hydrogen bonds; the formation of ionic (or hydrogen) bonds of the phosphate groups of the CoA with the protein is also possible. To construct a stricter model of the active sites of the CoA-dependent enzymes requires additional information on the capacity of various sections of the CoA for binding with enzymes, which can be obtained with the aid of modified coenzyme analogs used as probes.

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POLYSACCHARIDES OF PLANT TISSUE CULTURES.

III. ENZYMATIC HYDROLYSIS OF INDUSTRIAL WASTES OF THE BIOMASS OF A GINSENG TISSUE CULTURE

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UDC 581.143.6:547.917

Questions of the enzymatic hydrolysis of industrial wastes of the biomass of a ginseng tissue culture by Pektofoetidin P10× (I), Tsellyulaza P10× (II), and Tsellokoningin P10× (III) are discussed. The optimum conditions permitting the hydrolysis of the wastes by the following respective percentages have been selected: (I) 62; (II) 43; (III) 54. Subsequent treatment of the wastes with mineral acids raises the total degree of hydrolysis to 84-88%. The monocarbohydrate compositions of the hydrolysates have been studied by chromatographic methods.

The development of waste-free industrial processes requires the search for methods of rationally utilizing large-tonnage secondary resources of nonfood character. These are, in the first place, cotton lint, grape vines, prunings of fruit trees, the tops and stems of vegetable crops, sawdust, straw, and wastes from the processing of aromatic and medicinal plants [1-4], and also a new type of waste – the biomass of ginseng, rose-root stonecrop, and yam, the industrial cultivation of which has been developed intensively in the last few years [5-8].

*Student I. B. Smirnova took part in the work.

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