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Abstract

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SYNTHESIS OF P-AMINO ACID DERIVATIVES OF ADENYLIC ACID

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It is known that amino acid and peptide derivatives of nucleic acids and nucleotides play an important role in a number of biochemical processes, including the directed synthesis of protein. However, the chemistry of these compounds is only just beginning to develop. Among the various types of amino acid and peptide derivatives, a special role in biochemical processes apparently belongs to compounds in which amino acids are linked with mononucleotides through a phosphoric acid residue. The synthesis and study of the properties of such compounds were the subject of the present work.

We obtained two types of amino acid derivatives of adenosine 5′-phosphate:

(I)

$$\text{adenosine acetonide residue} - CH_2 - O - P(=O)(OCH_2C_6H_5) - NH - CH(R) - COOR$$

and

(II)

$$\text{adenosine acetonide residue} - CH_2 - O - P(=O)(OCH_2C_6H_5) - O - C(=O) - CH(R) - NH - COO - Bz$$

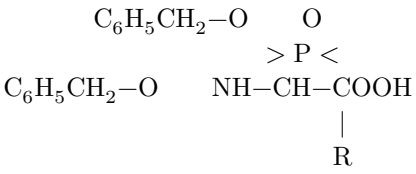
Compounds of type (I), containing a phosphoamide bond between the nucleotide and the amino acid, have not been described in the literature, although the possibility of the existence of this type of bond between nucleotides and amino acids in the organism has already been discussed ([1]).

We carried out the synthesis of these compounds from 2′ : 3′-isopropylideneadenosine-5′-benzyl phosphite (III) [(2)] by treating it with an amino acid ester in the presence of CCl_4*.

$$2' : 3'\text{-isopropylideneadenosine-}5'\text{-benzyl phosphite (III)} + NH_2{-}CH(R){-}COOR' + CCl_4 \longrightarrow$$
$$\longrightarrow (I) + CHCl_3 + HCl$$

The constants and yields of the obtained esters of *N*-adenylamino acids are given in Table 1.

As follows from the literature data [4], for the *P*—*N* bond in compounds of the type



there is a characteristic frequency in the infrared region of 11.38—11.60 μ. The esters of *N*-adenylamino acids synthesized by us also have in the infrared a characteristic frequency in the region 11.48—11.59 μ (see Table 1).

Table 1

Name of substance	Yield, %	M.p. (with decomp.), °C	Absorption in the UV, λ_{max}, mμ	Absorption in the UV, ε	Characteristic frequency in the IR region, μ
Ethyl ester of *N*-(2′:3′-isopropylideneadenosine-5′-benzylphospho)phenylalanine	62.5	88–90	260	13250	11.59
Methyl ester of *N*-(2′:3′-isopropylideneadenosine-5′-benzylphospho)glycine	52	60–63	260	11500	11.48

Name of substance	Yield, %	M.p. (with decomp.), °C	Absorption in the UV, λ_{max}, mμ	Absorption in the UV, ε	Characteristic frequency in the IR region, μ
Methyl ester of *N*-(2′:3′-isopropylideneadenosine-5′-benzylphospho)leucine	44	68–72	260	9800	11.56

The proposed method for the synthesis of esters of *N*-adenylamino acids is simple to carry out and provides sufficiently high yields; therefore it may be considered preparative and, apparently, extended to other nucleotides.

To study the stability of the $P \sim N$ bond in the compounds obtained, and also to obtain additional evidence for the structure of compounds of type (I), they were hydrolyzed with alkali and acid. It was shown that the $P \sim N$ bond in (I) is readily hydrolyzed by acid (0.1 *N*, 30 min, 100°) and more slowly by alkali (2 *N*, 30 min, 100°). In the hydrolysates, amino acids and products of nucleotide destruction were detected chromatographically.

Recently, methods for the synthesis of mixed anhydrides of adenylic acid and amino acids have been studied intensively. It has been established that mixed anhydrides are formed as a result of the interaction of adenylic acid or its salt with amino acid chloranhydrides [5], thiophenyl esters of amino acids [6], mixed anhydrides of amino acids and carbonic acid ester [7], and acid α-azides [8].

* The amine phosphorylation reaction developed by Todd and co-workers [3] was used; it consists in the action of phosphorous acid esters on primary amines in the presence of CCl_4.

We succeeded in obtaining mixed anhydrides of adenylic acid with Cbz-leucine and Cbz-glycine from 2′:3′-isopropylideneadenosine 5′-benzyl phosphate according to the scheme:

$$\text{(III)} \xrightarrow{\text{succinimidyl chloride}} \text{2':3'-isopropylideneadenosine-5'-}O\text{-}P(=O)(OCH_2C_6H_5)Cl$$

$$\xrightarrow{\text{AgOCOCH(R)NH-Cbz}} \text{(II)} \xrightarrow{\text{NH}_2\text{CH(R')COOR''}} \text{CbzNHCH(R)CONHCH(R')COOR''}$$

Since we were interested in the ability of (II) to react with amino acids with formation of peptides, we did not isolate (II), but introduced it further

into reaction with an amino acid ester. In this way the anhydrides of 2′:3′-isopropylideneadenosine 5′-benzylphosphoric acid with Cbz-glycine (IIa) and Cbz-leucine (IIb) were obtained. IIa and IIb react, respectively, with the methyl esters of phenylalanine and glycine with formation of the esters of Cbz-glycylphenylalanine and Cbz-leucylglycine. Formation of the dipeptides was established chromatographically.

Experimental Part

Ethyl ester of N-(2′:3′-isopropylideneadenosine-5′-benzylphospho)phenylalanine (Ia)

To a solution of 0.08 g of dry 2′:3′-isopropylideneadenosine 5′-benzyl phosphite (III) (?) in 5 ml of abs. CCl_4 was added 0.1 g of freshly prepared ethyl ester of phenylalanine in 2 ml of abs. CCl_4. The reaction mixture was left overnight, evaporated in vacuo, and the residual oil was dissolved in chloroform. The chloroform solution was washed with water, 0.1 N HCl, concentrated $NaHCO_3$ solution, and again with water. After drying over $MgSO_4$ the chloroform was evaporated in vacuo, and the oil was dissolved in 1 ml of benzene. The benzene solution was poured, with stirring, into dry petroleum ether (b.p. 40–60°). The precipitate was separated by decantation. The substance was purified by twofold reprecipitation and dried in vacuo (5 mm) at 37° over P_2O_5. Yield of (Ia) 0.069 g (62.5%). M.p. 88–90° (with decomp.).

$R_f = 0.91$ in the system: *n*-butanol—water—acetic acid (5:3:2) (system (I)).

$R_f = 0.93$ in the system: *n*-butanol saturated with water (system (II)).

	Found, %:	*C* 53.45;	*H* 5.96;	*N* 11.76;	*P* 4.20
$C_{31}H_{37}O_8N_6P \cdot 3H_2O$.	Calculated, %:	*C* 53.14;	*H* 6.14;	*N* 11.90;	*P* 4.30

Absorption in the UV (95% C_2H_5OH): λ_{max} 259–260 mμ (13250). Characteristic frequency in the IR: 11.59 μ.

Hydrolysis of (Ia) with acid. A solution of 5 mg of (Ia) in 2 ml of 0.1 N H_2SO_4 was boiled for several hours. At definite intervals, samples of the hydrolysate were chromatographed on an ascending chromatogram in system (I). After 30 min, the disappearance of the initial compound (Ia) and the appearance of phenylalanine ($R_f = 0.72$) and of a compound absorbing in the UV,* with $R_f = 0.47$, were detected chromatographically in the hydrolysate. The aqueous-alcoholic eluate of the spot with $R_f = 0.47$ has maximum absorption in the UV at 258–260 mμ.

* Detected by viewing the chromatogram in Brumberg' s chemoscope.

Hydrolysis of (Ia) with alkali. Boiling (Ia) with 0.1 *N* NaOH for 2 h does not lead to hydrolysis of the P—N bond. In the hydrolysate, only the starting

compound was detected chromatographically. When (Ia) was boiled with 2 N NaOH for 30 min, phenylalanine was split off and detected chromatographically ($R_f = 0.72$ in system (I)).

Methyl ester of N-(2′:3′-isopropylideneadenosine-5′-benzylphospho)glycine (Ib)

(Ib) was obtained from (III) and methyl glycinate analogously to the synthesis of (Ia). Yield of (Ib) 52%, m.p. 60-63° (with decomposition), $R_f = 0.91$ in system (I).

$C_{23}H_{29}O_8N_6P \cdot 3H_2O$.	Found %:	N 13.62; P 4.76
	Calculated %:	N 13.95; P 5.14

Absorption in the UV (96% C_2H_5OH): λ_{max}260 mμ (ε 11500). Characteristic frequency in the IR, 11.48 μ.

Hydrolysis of (Ib) with acid (0.1 N H_2SO_4) and with alkali (2 N NaOH) proceeds analogously to the hydrolysis of (Ia).

In the hydrolysates, glycocoll was detected chromatographically ($R_f = 0.37$ in system (I)).

Methyl ester of N-(2′:3′-isopropylideneadenosine-5′-benzylphospho)leucine (Iv)

Obtained analogously to (Ia) from (III) and methyl leucinate. Yield (Iv) 44%. M.p. 68-72° (with decomposition), $R_f = 0.93$ in system (I).

$C_{27}H_{37}O_8N_6P \cdot 3H_2O$.	Found %:	N 12.2; P 4.3
	Calculated %:	N 12.8; P 4.7

Hydrolysis of (Iv) with acid (0.1 N H_2SO_4) and with alkali (2 N NaOH), as in the case of (Ia) and (Ib), leads to cleavage of the amino acid. Leucine was detected chromatographically ($R_f = 0.73$ in system (I)).

Mixed anhydride of 2′:3′-isopropylideneadenosine-5′-benzylphosphoric acid and carbobenzoxyleucine (IIa)

To a solution of 0.2 g of (III) in 5 ml of abs. benzene and 3 ml of abs. CH_3CN, 0.10 g of chlorosuccinimide[9] was added. After 2 h the solution was filtered. To the filtrate, 0.07 g of the dry Ag salt of carbobenzoxyleucine[10] in 5 ml of abs. dioxane was added. The mixture was left overnight. The precipitate was filtered off and evaporated in vacuo. The oil was triturated with abs. ether. To the ethereal extract of (IIa), 0.1 g of methyl glycinate was added. The solution was left for 24 h, evaporated, and chromatographed in the system: n-butanol saturated with ammonia (system (III)). After drying, the chromatogram was developed with benzidine. A spot with $R_f = 0.93$ was detected; methyl

carbobenzoxyleucylglycinate, applied to the chromatogram as a control, also has $R_f = 0.93$.

Mixed anhydride of 2′:3′-isopropylideneadenosine-5′-benzylphosphoric acid and carbobenzoxyglycine

Obtained analogously from (III) and the Ag salt of glycine.

The anhydride was extracted with ether and introduced into reaction with methyl phenylalaninate. Chromatographically, in system (III), methyl carbobenzoxyglycylphenylalaninate was detected ($R_f = 0.67$).

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Note: Figure translations are in progress. See original paper for figures.