Communications to the Editor

Chem. Pharm. Bull.
33(1) 432—435 (1985)

PREPARATION OF CYCLIC AMP DERIVATIVES BY INTRAMOLECULAR PHOSPHODIESTER BOND FORMATION CATALYZED BY Pb^{2+} ION

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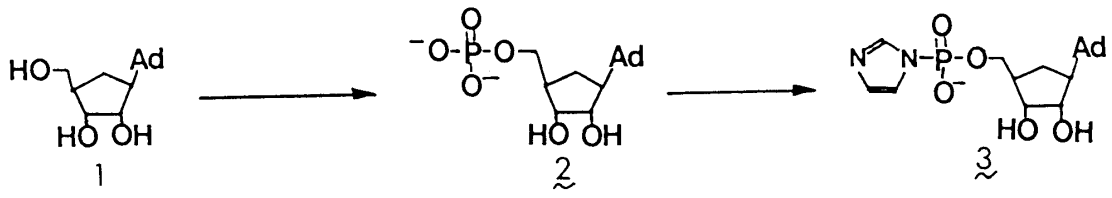
Cyclic AMP derivatives containing a carbocyclic sugar moiety were prepared by lead-ion catalyzed intramolecular phosphodiester-bond formation of carbocyclic nucleoside-5'-phosphorimidazolide in aqueous solution. The catalytic effect of other metal ions and the properties of the cyclic AMP derivatives are described.

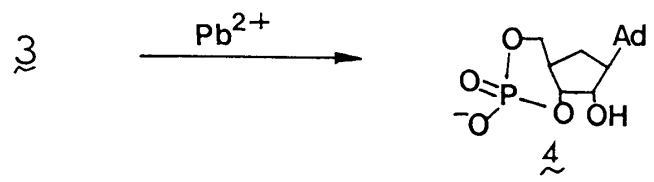
KEYWORDS - aristeromycin; neplanocin A; phosphorimidazolide; phosphodiester-bond formation; Pb^{2+} ion; cyclic AMP analog

Cyclic AMP is generally recognized as an unusual nucleotide that plays important roles in the regulation of living cells.[1] Several cyclic AMP derivatives have been prepared and used as tools for the study of functions of cyclic AMP.[2] Here we wish to report the novel synthesis and properties of cyclic AMP derivatives containing carbocyclic nucleosides.

Several years ago, we reported the lead-ion catalyzed phosphodiester-bond formation of adenosine-5'-phosphorimidazolide to give a small amount of cyclic AMP and a large amount of oligonucleotides.[3] During a study of a series of the metal-ion catalyzed phosphodiester-bond formations in aqueous solution, we found that lead-ion catalyzed the formation of cyclic AMP derivatives from carbocylic nucleoside-5'-phosphorimidazolide in high yields.

In the first, aristeromycin (1) was chosen as a starting material for the cyclic AMP derivative, as it is structurally related to adenosine and has an antagonist effect on adenosine.[4,5] Aristeromycin was phosphorylated with phosphorus oxychloride (5 eq) in triethyl phosphate at 0°C for 3 h to





give aristeromycin-5'-phosphate (2) in 45% yield.[6] Aristeromycin-5'-phosphorimidazolide (3) was prepared from 2 and imidazole (10 eq) using dipyridyl disulfide and triphenylphosphine as condensing agents.[7] The compound 3 was obtained as a sodium salt in 98% yield.

Intramolecular phosphodiester bond formation from 3 was carried out in aqueous solution using lead nitrate as a catalyst. To a solution (0.95 ml) of 3 (0.05 mmol) in 0.2 M N-ethylmorpholine buffer (pH 7.0) was added lead nitrate solution (0.25 M x 0.05 ml, 0.0125 mmol) with stirring. The reaction mixture was kept at room temperature for 5 days with stirring. Then it was treated with Diaion CR-10 (1 g) and filtered to remove the Pb^{2+} ion. The resulting solution was applied to a QAE-Sephadex A-25 (HCO_3 form) column and eluted with a linear gradient of triethyl ammonium bicarbonate buffer (H_2O - 0.5 M). The UV absorbing fractions were collected and evaporated under vacuum. Lyophilization of the residue gave aristeromycin cyclic 3',5'-phosphate (4) as a triethylammonium salt. The isolated yield of 4 was 94% from 3 based on UV absorption. The hydrolyzed product 2 and 5',5'-diaristeromycin pyrophosphate were obtained in 4.7% and 0.4%, respectively, as minor by-products.

Table I shows the effect of several metal ions on the cyclic phosphodiester bond formation, which was carried out in the same way as the lead-ion catalyzed reaction. The compound 3 was hydrolyzed to 2 in aqueous solution and a very small amount of 4 was formed when the metal ion was absent.

Table I. Reaction of Aristeromycin 5'-phosphorimidazolide (3) in the Presence of Several Metal Ions

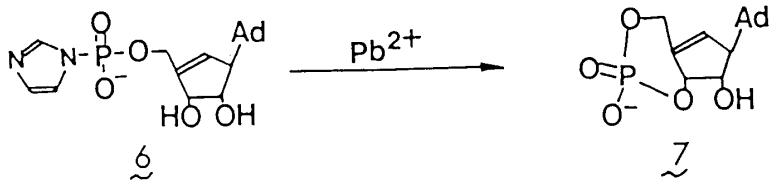
Metal ion	Yield (%)				
	P<Aris(4)	pAris(2)	ArisppAris	ImpAris(3)	Oligomers
Pb^{2+}	94.4	4.7	1.8		
Co^{2+}	9.9	72.2	3.1	10.8	4.0
Zn^{2+}	5.8	85.0	1.4	5.7	2.4
Mn^{2+}	5.7	85.6	1.3	10.7	1.9
Ni^{2+}	3.0	70.5	3.4	21.8	1.3
Cu^{2+}	3.3	85.9	6.3	0.8	3.7
Cd^{2+}	6.4	75.1	2.2	14.4	1.8
Ca^{2+}	2.3	83.2	2.6	11.6	0.3
Mg^{2+}	1.8	86.7	1.6	9.5	0.3
None	1.9	72.7	2.4	22.7	0.2

Reactions were carried out at room temperature for 5 days. The compound 3 (0.05 M) and metal salt (0.0125 M) were used in 0.2 M N-ethylmorpholine buffer (pH 7.0).

Metal ions other than the Pb^{2+} ion have little effect on the formation of cyclic phosphate. Co^{2+}, Zn^{2+}, Mn^{2+} and Cd^{2+} enhance the cyclic phosphate

formation, though the yields of 4 were less than 10%. Small amounts of oligonucleotides were formed along with 4.

In a similar way, neplanocin A cyclic 3',5'-phosphate was obtained by Pb^{2+} ion catalyzed phosphdiester bond formation. Phosphorylation of neplanocin A with phosphorus oxychloride (4 eq) in triethyl phosphate at 0°C for 2 h gave neplanocin A 5'-phosphate (5)[6] which was condensed with imidazole (10 eq) using dipyridyl disulfide and triphenylphosphine to yield neplanocin A 5'-phosphorimidazolide (6). Cyclization of 6 by lead nitrate catalysis in neutral aqueous solution gave neplanocin A cyclic 3',5'-phosphate (7) which was purified by QAE-Sephadex A-25 column chromatography. The isolated yield of 7 was 70%.



6 7

The characterization of 4 and 7 was carried out by TLC, paper chromatography and NMR (Table II.). The TLC mobilities of 4 ahd 7 were similar to that of cyclic AMP, while the Rf values of 2 and 5 were similar to that of adenylic acid in the three TLC systems. The very small H1'-H2' coupling constant of 4 and 7 indicates that the compounds possess the cyclic phosphate structure.[8]

Table II. TLC Mobilities and NMR of the Compounds

Compound	Rf value[a] I	II	III	NMR[b] (Adenine proton) H-2	H-8	Coupling constant J1'-2'(Hz)
p<Aris (4)	0.59	0.30	0.51	8.25	8.25	1[c]
p<Nep (7)	0.53	0.32	0.51	7.96	8.24	1[c]
Cyclic AMP	0.58	0.31	0.51	8.23	8.23	1[c]
pAris (2)	0.31	0.46	0.50	8.24	8.40	9.3
pNep (5)	0.32	0.48	0.49	8.26	8.60	1[c]
5'-AMP	0.31	0.49	0.49	8.24	8.57	5.8

a) I; Cellulose F: 1-propanol-conc. ammonia-water (55:10:36).
II; Cellulose F: saturated ammonium sulfate-0.1 M sodium acetate-2-propanol (79:19:2).
III; PEI-cellulose F: 0.25 M ammonium bicarbonate.

b) NMR were taken in D_2O buffered with 0.1 M phosphate (pH 6.7) at 25°C. Chemical shifts are given in δ ppm using TSP-d_4 as an internal standard.

c) Coupling constant of H1'-H2' was too small to measure.

The advantages of the present method are; (1) cyclic phosphodiester bond formation in aqueous solution, (2) using no protecting group, (3) very simple and (4) providing a sufficient amount of 4 and 7 for biological studies. Coordination of 3 or 6 to the Pb^{2+} ion so as to promote the cyclization and activation of 3'-OH group by complexation could enhance the intramolecular cyclic phosphate formation.

Antagonist activity of 4 and 7 against cyclic AMP and cyclic GMP was studied in regard to the inhibition of phosphodiesterase of cyclic AMP and cyclic GMP. The results are shown in Table III along with the activity of the typical inhibitor of cyclic AMP phosphodiesterase. The compounds 4 and 7 strongly inhibit cyclic AMP phosphodiesterase. Further studies on the biological activity of 4 and 7 are in progress.

Table III. Inhibition of Phosphodiesterase of Cyclic AMP and Cyclic GMP

Compound	Inhibition of cyclic PDE (IC_{50};μg/ml)	
	Cyclic AMP	Cyclic GMP
Isobutylmethylxanthine(IBMX)	1.3	
Theophylline	65	
p<Aris (4)	18.5	90
p<Nep (7)	7.8	13

We are very grateful to Takeda Chemical Industries, Ltd. for the supply of aristeromycin and to Toyo Jozo Co., Ltd. for a gift of neplanocin A. We thank Dr. M. Kaneko of Sankyo Co., Ltd. for the biological study of the compounds.

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(Received November 12, 1984)