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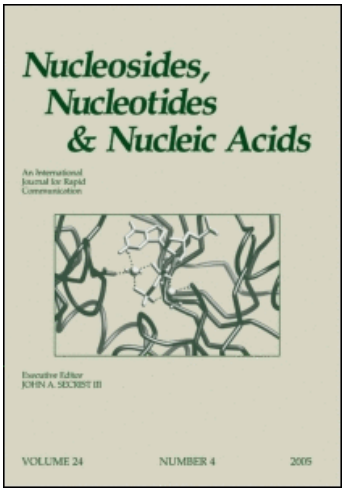

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Synthesis and Properties of Mixed Anhydrides of AMP, ADP and ATP with Mesitoic Acid

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NUCLEOSIDES & NUCLEOTIDES, 2(3), 203-219 (1983)

SYNTHESIS AND PROPERTIES OF MIXED ANHYDRIDES OF AMP, ADP AND ATP WITH MESITOIC ACID

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Abstract. New affinity reagents for ATP-dependent enzymes are described. Optimal conditions are evolved for the synthesis of mixed anhydrides of AMP, ADP, ATP with mesitoic acid ($MsCOp_nA$, n = 1-3) and for their $1,N^6$-etheno, 2',3'-dialdehyde and photoactive analogues. UV, CD and fluorescence spectra of the compounds have been analyzed. Hydrolysis of $MsCOp_nA$ (n = 1-3) and their etheno analogues over a wide pH range has been carried out.

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Mixed anhydrides of adenosine-5'-mono-, di- and triphosphates with mesitoic acid, $MsCOp_nA$ (n = 1-3), have been widely used as specific inhibitors for studying the mechanism of action and structure of mitochondrial [1] and myosin [2] ATPases, as well as of some tRNA-synthetases [3,4]. The specificity of these mixed anhydrides described by us previously [5] is that they are sufficiently stable in aqueous solutions and on interaction with nucleophiles behave, unlike other acylphosphates, as phosphorylating agents. The ability of $MsCOp_nA$ (n = 1-3) to transfer a nucleotide residue, the ease of synthesis, and the high hydrolytic stability have made them good affinity labelling agents for ATP-dependent enzymes. In order to follow the incorporation into the active site of ATPase, fluorescent analogues of $MsCOp_nA$ (n = 1-3) were needed. To this end, $1,N^6$-etheno derivatives of $MsCOp_nA$

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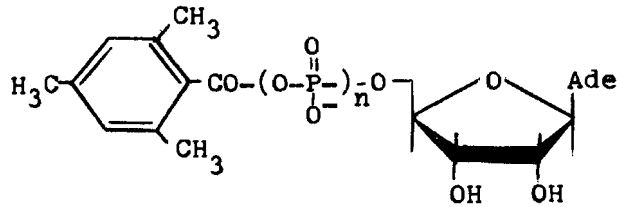
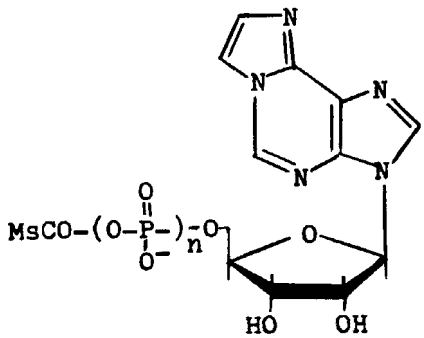
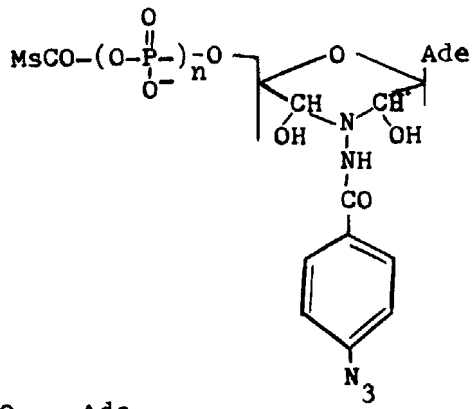


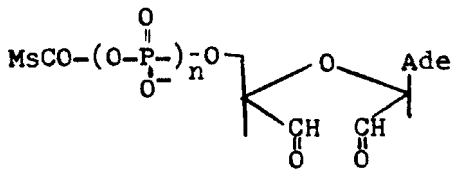
$MsCOp_nA$



Ia-c



IIIa-c



IIa-c

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(compounds I a-c) were synthesized and their absorption fluorescence properties were studied.

Then, to study the topography of the active sites and the quaternary structure of enzymes consisting of several subunits, we synthesized doubly modified structural analogues of AMP, ADP and ATP containing, besides the MsCO-group, another reaction center, i.e. 2',3'-dialdehyde groups (compounds II a-c) or an p-arylazide group (compounds III a-c).

The present work is concerned with the optimal conditions for the synthesis of $MsCOp_nA$ (n = 1-3), of doubly modi-

fied derivatives of AMP, ADP and ATP (compounds I-III), and also with a study of their hydrolytic stability.

RESULTS AND DISCUSSION

Synthesis of $MsCOp_2A$ and $MsCOp_3A$

It was found that treatment of ADP and ATP with a 5-10-fold excess of MsCOCl resulted in the formation of a number of acylated and non-acylated products of decomposition of the starting adenosine-5'-polyphosphates, with the cleavage of the pyrophosphate bonds being more pronounced if the excess of MsCOCl was greater. The major by-product was MsCOpA. ^{31}P NMR pulsed spectroscopy was used to establish that MsCOCl acylated not only the terminal, but also the internal phosphate groups of ADP and ATP. This reactions are extremely fast.

FIG. 1b shows a ^{31}P NMR spectrum of the reaction mixture obtained by treatment at 20° of ADP in pyridine solution with a 5-fold excess of MsCOCl recorded 2 min after addition of anhydride. No signal of the starting ADP is seen (FIG. 1a). In the spectrum there are signals at 8,4 ppm that correspond to MsCOpA [6] and a multiplet in the 18-24 ppm reguion. As the formation of the new anhydride bond shift signal of phosphate by $\sim$10 ppm upfield, [6] the multiplet should indicate a compound with two anhydride bonds, i.e. α,β-di-mesitoyl-ADP. With water being added to the reaction mixture, the multiplet at 18-24 ppm gradually disappears. As seen in FIG. 1c, in 19 hrs there appear doublets with the centres at 11,8 ppm and 19,7 ppm, which are assigned, when referred to the control sample, to $MsCOp_2A$. There are still signals from MsCOpA (δ=8,4 ppm), pA (δ=0,3 ppm) and inorganic phosphate (δ=0 ppm).

The data presented demonstrate that polyacyl derivatives are unstable and readily hydrolyze when the reaction mixture

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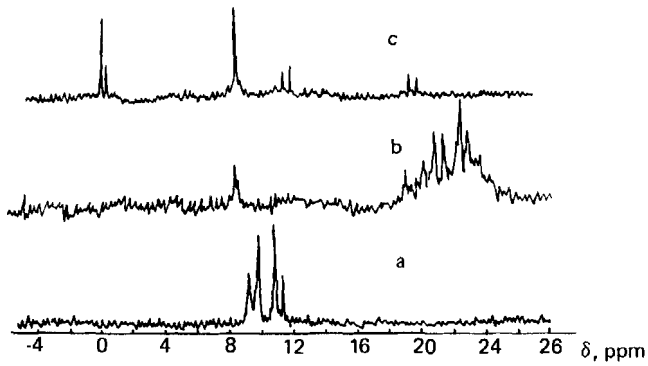

FIG. 1. A ^{31}P NMR spectrum at 20° a) 0,15 M solution ADP in pyridine; b) 0,15 M solution ADP + 0,75 M MsCOCl 2 min after the reagents were mixed; c) reaction mixture 0,15 M ADP with 0,75 M MsCOCl 19 h after addition of an excess of water.

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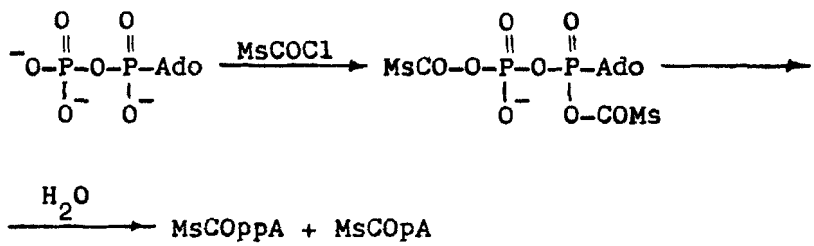
is supplemented with water. The MsCO-group remains intact only at the terminal monosubstituted phosphate groups. Therefore the scheme of the reactions of ADP with excess MsCOCl may be presented as follows:



^{31}P NMR spectroscopy was also used to demonstrate that ATP reacts with MsCOCl in the same way.

Apparently, decomposition of ADP and ATP resulting from treatment with MsCOCl cannot be avoided completely, since labile polyacyl derivatives are always formed in this case. We could establish that optimal conditions for $MsCOp_2A$ synthesis were: a two-fold excess of MsCOCl, a low temperature (+2-0°C) and a short time of reaction (2-3 min). $MsCOp_3A$

forms with a maximal yield of 50-60% under the same conditions when a three-fold excess of MsCOCl is used.

$MsCOp_nA$ (n = 1-3) was separated by preparative paper chromatography followed by electrophoresis. The solutions obtained after elution were kept frozen or lyophilized. The yields and characteristics of the compounds obtained are represented in TABLE 1.

Synthesis of $1,N^6$-Etheno Analogues of $MsCOp_nA$ (n = 1-3)

Compounds I a-c were synthesized according to the following scheme:

$$p_nA \xrightarrow{MsCOCl} MsCOp_nA \xrightarrow{ClCH_2CHO} MsCOp_n\,\varepsilon A$$

The second step of the synthesis, i.e. modification of the mixed anhydrides with chloroacetaldehyde, was carried out in 0.1 M citrate buffer (pH 4.5) for 4 hr at 37° [7]. This reaction is readily reproducible and always gives a quantitative yield of end-products. Compounds I a-c were isolated by preparative paper chromatography in system B (see TABLE 1). The homogeneity of the compounds was demonstrated by microcolumn chromatography on DEAE-cellulose in 7 M urea. When treated with snake venom phosphodiesterase compounds I a-c are hydrolyzed to εAMP.

It was reported previously [5] that upon synthesis of mixed anhydrides of mono- and oligonucleotides with mesitoic acid, heterocyclic bases remain unmodified. Hence, for the synthesis of compounds I a-c an attempt to change the sequence of reactions was made (see scheme above), i.e. to introduce first the $1,N^6$-etheno group, then to modify the terminal phosphate in $1,N^6$-ethenoadenosine-5'-mono-, di- and triphosphates with MsCOCl. This sequence of reactions would allow commercial εAMP, εADP and εATP to be used for the synthesis of compounds I a-c. However, after the treatment of

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TABLE 1. Some characteristics of mixed anhydrides of AMP, ADP and ATP with mesitoic acid and of their analogues

Compound	Yield, per cent	R_f in systems *)						UV spectra in water	
		A		B	C		D	max	min
		PC	TLC (silica-gel)	PC	PC	TLC (silica-gel)	TLC (cellulose)		
MsCOpA	96	0.74	0.71	0.69	0.81		0.74	260	234.7
$MsCOp_2A$	60	0.65	0.29	0.59	0.71		0.63	260	235
$MsCOp_3A$	52	0.53	0.10	0.52	0.62		0.55	260	234.7
I-a	93			0.74			0.64	258.3; 265; 275	
I-b	90			0.62			0.58	258.3; 265; 275	
I-c	90			0.56			0.48	258.3; 265; 275	
II-a	95				0.88	0.79		260	234.7
II-b	90				0.84	0.75		260	235
II-c	90				0.76	0.71		260	235
III-a	95	0.83	0.74					268.7	234.7
III-b	90	0.74	0.69					268.7	235
III-c	85	0.64	0.66					269.3	234.3

*) Chromatographic systems: A, ethanol–1 M ammonium acetate, pH 7.5 (7:3); B, ethanol–1 M ammonium acetate, pH 3.2 (7:3); C, ethanol–1 M lithium acetate, pH 4.4 (7:3); D, isobutyric acid–conc. NH_4OH–water (75:1:24).

ε_{AMP} and ε_{ADP} with MsCOCl under standard conditions, in addition to compounds Ia and Ib, 30-40% of nonfluorescing UV-absorbing products were detected by paper chromatography. In accordance with the literature data [8], it can be suggested that the by-products form as a result of acylation with MsCOCl of a newly formed imidazole heterocycle of 1,N^6-ethenoadenosine.

Synthesis of 2',3'-Dialdehyde and Photosensitive Analogues of $MsCOp_nA$

Compounds II a-c were obtained by oxidation of $MsCOp_nA$ [9]. However, if the 2',3'-cis-diol groups of AMP, ADP and ATP are oxidized quantitatively with $NaIO_4$ within 30 min, oxidation of their mixed anhydrides ($MsCOp_nA$) takes 2-2.5 hrs. The anhydride bonds of $MsCOp_nA$ remain stable during oxidation. The presence of dialdehyde groups is confirmed by a typical color reaction with phenylhydrazine.

Compounds III a-c were obtained by treatment of compounds II a-c in aqueous dioxane with a 3.5-fold excess of p-azidobenzoylhydrazide. On paper chromatography, compounds III a-c have close values on R_f in various systems. Using microcolumn separation of DEAE-cellulose we found out that compounds II a-c and III a-c are eluted at the same salt concentration as $MsCOp_nA$.

Physico-Chemical Properties of $MsCOp_nA$ and their Analogues

As seen in TABLE 1, UV spectra of $MsCOp_nA$ are similar to those of the corresponding adenosine-5'-mono- and polyphosphates, but their A_{max}/A_{min} ratios are somewhat different. Thus, if for AMP, ADP and ATP A_{260}/A_{235} is 3.7, for $MsCOp_nA$ this ratio is 2.0.

The UV spectra of compounds I a-c are qualitatively similar to the parent AMP, having three peaks and a broad

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shoulder in the region of 290-310 nm. [10] The ratio of absorption intensities of compounds I a-c $A_{258}/A_{265}/A_{275}$ is 1,15:1,10:1 and differs from that for ATP which is 1:1,16:1,14.

The UV spectra of 2',3'-dialdehyde derivatives of compounds II a-c are analogous with the spectra of $MsCOp_nA$, whereas for compounds III a-c λ_{max} is shifted to a long wavelength region (see TABLE 1).

Compounds I a-c displayed an intensive fluorescence in neutral and alkaline solutions. Their spectra are analogous to those of εA (λ^{max}_{fluor} = 415 nm, λ^{max}_{exc} = 300 nm) [11]. As pH decreases, the fluorescence intensity of compounds I a-c becomes lower (FIG. 2), but the fluorescence and excitation maxima do not change. It is the neutral form of $1,N^6$-etheno-adenine that is the fluorescing chromophore in these compounds [12].

Relative quantum yields of compounds I a-c measured with εATP (φ = 0.59) [11] and 9-aminoacridine (φ = 0.98) [13] as standards are 0.2. The quantum yields of compounds I a-c ob-

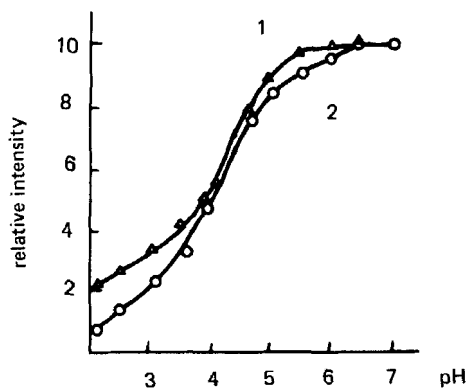

FIG. 2. pH dependence of the relative intensity of fluorescence (λ_{exc} 310 nm; λ_{em} 415 nm): 1 - MsCOp εA; 2 - $MsCOp_2\varepsilon A$.

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tained by us are lower than that of ε_{ATP}. Such quenching of emission can be ascribed to the specificity of the secondary structure of these compounds. A similar dependence between the fluorescence intensity and structure was revealed by Tolman et al. on studying dinucleoside phosphates [14]. Intensive fluorescence allows compounds I a-c (φ = 0.2) to be detected in concentrations lower than 10^{-8}M, so that they can be used for studying enzyme systems.

FIG. 3 shows CD spectra of $MsCOp_nA$ (n = 1-3) and of their 1,N^6-etheno and 2',3'-dialdehyde analogues. As follows from FIG. 3A, MsCOpA has a maximal amplitude of the positive Cotton effect, which is connected with the hydrophobic interaction between the adenine heterocycle and the mesitoic acid residue. The longer the pyrophosphate chain, the weaker the interaction, the lower is the amplitude. A similar effect was observed previously for aromatic phosphoamides of nucleotides [15]. The same regularity is observed for 1,N^6-etheno analogues of $MsCOp_nA$, compounds I a-c (FIG. 3B).

The pattern of the CD spectra of compounds II a-c (FIG. 3C) is similar to that of $MsCOp_nA$ (n = 1-3). However, there seems to be no correlation between the positive maximum of the Cotton effect and the length of the pyrophosphate chain of compounds II a-c, perhaps due to the less rigid carbohydrate structure resulting from oxidation of the 2',3'-cis-diol group.

Hydrolysis Studies

Partial decomposition of $MsCOp_nA$ is observed on evaporation under reduced pressure (bath temperature 40-50°C). The decomposition is greater in $MsCOp_3A$ compared to $MsCOp_2A$. The electron-acceptor character of the MsCO-group influences the stability of the whole pyrophosphate chain in $MsCOp_nA$. To characterize the compounds obtained, hydrolysis was stu-

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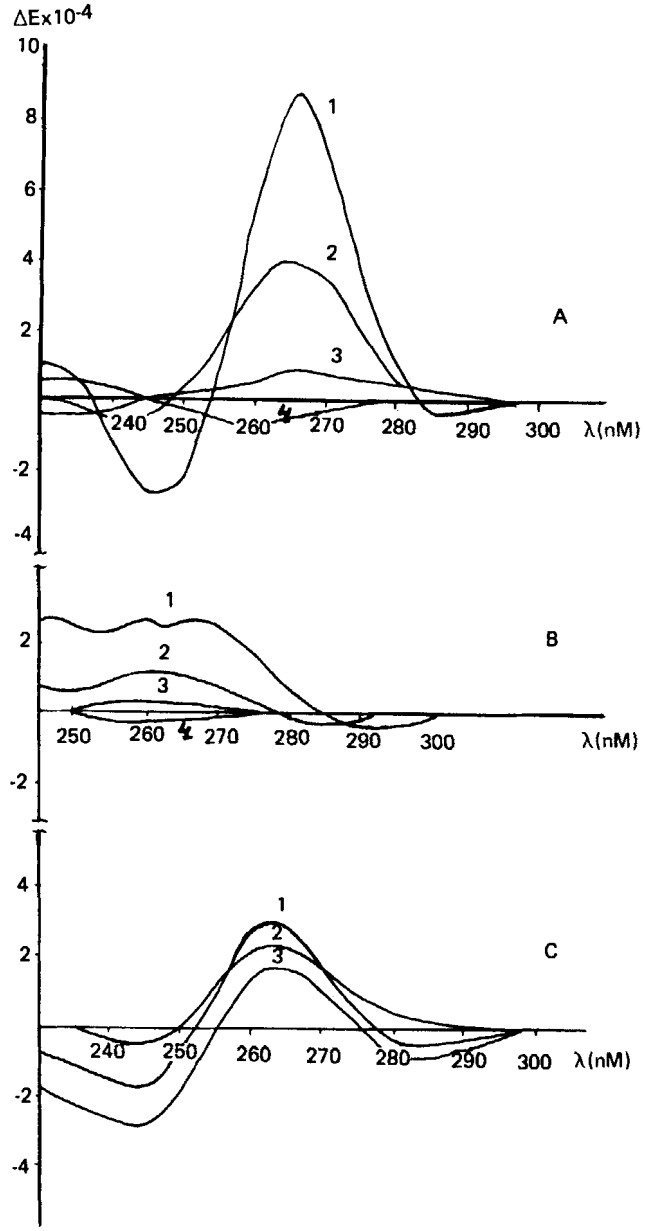

FIG. 3. CD curves recorded in 0.01 M phosphate buffer, pH 7.0 at 20°C; A: 1, MsCOpA; 2, $MsCOp_2A$; 3, $MsCOp_3A$; 4, ATP. B: 1, Ia; 2, Ib; 3, Ic; 4, ATP. C: 1, IIa; 2, IIc; 3, IIb.

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died over a wide pH range. We used 1 N HCl, 1 N NaOH, phosphate buffers (pH from 4.5 to 10.1) and buffer mixtures 1-3 that were supplemented with Mg^{++}, Ca^{++}, K^{+} etc. for ATPases [1,2]. The hydrolysis was followed by paper chromatography.

On incubation of $MsCOp_nA$ in buffer solutions (pH 4.5-10) at 37° no decomposition products were found in the first two days. After a longer incubation (4-5 days) AMP, ADP and ATP slowly accumulated in hydrolyzates. It is the phosphate directly bound to the mesitoic acid residue that is the first to react with water:

$$MsCOp_nA \xrightarrow{H_2O} MsCOOH + p_nA \qquad (n = 1\text{-}3)$$

TABLE 2 illustrates the percentage of $MsCOp_nA$ and their $1,N^6$-etheno analogues (compounds I a-c) in the hydrolyzates analyzed 15 days later. As expected, introduction of the $1,N^6$-etheno group to $MsCOp_nA$ does not affect the stability of anhydride bonds. Therefore the hydrolysis will be discussed with $MsCOp_nA$ as an example. At pH 4.5-6 it is MsCOpA that is the most stable, at pH 6.5-10 MsCOpA, $MsCOp_2A$ and $MsCOp_3A$ are rather similar in stability. At pH $\geqslant$ 7 the newly formed ADP and ATP eventually split and the main end-product of $MsCOp_2A$ and $MsCOp_3A$ after 15 day hydrolysis (37°) is AMP.

In buffer mixtures 1-3 the hydrolytic stability of $MsCOp_3A$, the most labile of $MsCOp_nA$, was studied. It was found that at 37° within two days $MsCOp_3A$ hydrolyzes by 2-3% in buffers 1 and 3, and in buffer 2, containing Mg^{++}, by 8-10%.

In 1 N HCl total hydrolysis of the mixed anhydride bonds in MsCOpA at 37°C takes 10 hr, in $MsCOp_2A$ 7 hr and in $MsCOp_3A$ 5 hr. Incubation of these compounds in 1 N HCl for 24 hr results in 16-20% cleavage of the glycosidic bond.

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TABLE 2. Percentage of $MsCOp_nA$ and their $1,N^6$-etheno analogues after 15 day hydrolysis in phosphate buffers

Compound	pH								
	4.5	5.5	6.0	6.5	7.0	7.5	8.0	9.3	10.1
MsCOpA	92	85	80	78	75	72	70	64	59
Ia	88				80				55
$MsCOp_2A$	74	81	84	87	91	92	90	76	68
Ib	70				92				65
$MsCOp_3A$	65	70	73	75	75	75	74	70	67
Ic	65				73				

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On hydrolysis of MsCOpA, $MsCOp_2A$ and $MsCOp_3A$ in 1 N NaOH, besides AMP, ADP and ATP, new compounds having (on paper chromatography in system A) R_f 0.54, 0.35 and 0.28, respectively, were found. UV spectra of these compounds are similar to those of $MsCOp_nA$. When subjected to microcolumn separation on DEAE-cellulose, these compounds are eluted at the same salt concentration as AMP, ADP and ATP. The time-course of their accumulation is shown in FIG. 4. On hydrolysis of $MsCOp_3A$, as seen in FIG. 4, the amount of the new product reaches 56% after 4 hr and then diminishes.

On hydrolysis of MsCOpA the degree of formation of the product having R_f 0.28 is the lowest. A day later these products are not to be found in alkaline hydrolyzates. On this basis we have ascribed these products the following structure - 2'(3')-O-mesitoyl-AMP, 2'(3')-O-mesitoyl-ADP and 2'(3')-O-mesitoyl-ATP. In 1 N NaOH (pH>12), when the hydroxyl groups of ribose dissociate to a great extent, the transfer of the MsCO-group from phosphate to ribose seems to be quite plau-

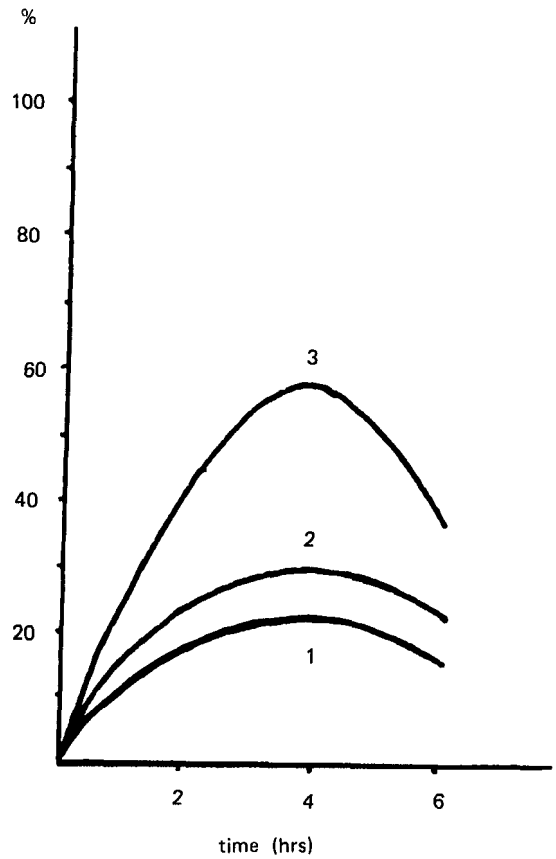

FIG. 4. Kinetic curves of the percentage of 2'(3')-O-mesitoyl-AMP (1), 2'(3')-O-mesitoyl-ADP (2) and 2'(3')-O-mesitoyl-ATP (3) on hydrolysis of $MsCOp_nA$ in 1 N NaOH at 37°C.

sible. The chromatographic patterns of the new compounds detected in the alkaline hydrolyses of MsCOpA and $MsCOp_3A$ proved to be identical with 2'(3')-O-mesitoyl-AMP and 2'(3')-O-mesitoyl-ATP were prepared by treatment of AMP and ATP, respectively, with the imidazolidate of mesitoic acid [16].

EXPERIMENTAL

General. ADP and ATP ("Serva") were purified before use by DEAE-cellulose chromatography at 4° in a triethylammonium bicarbonate concentration gradient (0.05 ⟶ 0.35 M).

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Ascending paper chromatography was performed on FN-1 (Filtrak, DDR). Paper electrophoresis was carried out in 0.05 M TEAB and phosphate buffers at pH 7.5 and 35 v/cm.

Microcolumn chromatography was performed on DEAE-cellulose in a NaCl concentration gradient (0 ——→ 0.18 M) in 7 M urea.

For optical studies 15 μmol portions of compounds I a-c were purified by paper electrophoresis and gel filtration on a Biogel P-2 column (1.3x33 cm). The elution rate was 30 mL/h.

Absorption spectra were recorded in "Cary 16" and "Specord" (DDR) spectrophotometers. For AMP ε_{260} = 13000, for MsCOOH ε_{260} = 630.

Fluorescence was measured with "Aminco-SPR-1000 CS" and "Aminco-Bowman" spectrofluorimeters at 20°C. CD spectra were recorded with a "Jouan-II" (France) dichrograph.

The ^{31}P NMR spectra were taken with a Bruker HX-90 pulse spectrometer operating at 36.43 MHz. The chemical shifts are reported in ppm related to external 85% H_3PO_4 and are judged to be $\pm$0.1 ppm accurate.

All experiments with compounds III a-c were carried out in the dark. To induce photodecomposition of compounds III a-c, their aqueous solutions were illuminated with a high pressure mercury lamp (250 W) with a quartz filter, the distance from the light source being 19 cm.

Synthesis of MsCOpA was performed as described [5].

Synthesis of $MsCOp_2A$

0.06 mmol of the trioctylammonium salt of ADP rendered anhydrous by co-evaporation with dry benzene, was dissolved in 0.5 mL dry pyridine, then cooled to 0°C and treated with 0.12 mmol MsCOCl. The reaction mixture was shaken for 2 min at 0°C, then diluted with 1.5 mL of H_2O. The mesitoic acid was extracted with ether (2 mL x 4). The water solution was

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evaporated to 0.5 mL and $MsCOp_2A$ was isolated by paper chromatography in system A.

Synthesis of $MsCOp_3A$ was carried out analogously, using 0.18 mmol of MsCOCl per 0.06 mmol of trioctylammonium salt of ATP.

Synthesis of compounds I a-c

0.012 mmol (180 A_{260} o.u.) of MsCOpA in 0.6 mL of 0.1 M citrate buffer (pH 4.5) was treated with 0.12 mL 1 M aqueous solution of chloroacetaldehyde and the pH of the reaction mixture was adjusted to 4.3-4.5 with 1 N HCl. The mixture was incubated for 5 hr at 37°C and separated by paper chromatography in system B.

Compounds I b,c were synthesized as above.

Synthesis of compounds II a-c

To 0.012 mmol of MsCOpA 1 mL of 0.06 M solution of $NaIO_4$ in 0.05 M acetate buffer (pH 5.5) was added. The reaction mixture was incubated for 2-2.5 hrs at 20°C in the dark. Then 1.2 mL of dioxane was added and the precipitated $NaIO_4$ was removed by filtration. The filtrate was concentrated and chromatographed in system C.

Compounds II b,c were synthesized analogously.

Synthesis of compounds III a-c

An aqueous eluate containing 0.012 mmol of compound IIa was evaporated to dryness and the precipitate was supplemented with 2.1 mL of a previously prepared $2 \cdot 10^{-2}$ M solution of p-azidobenzoylhydrazide in a dioxane - 0.05 M CH_3COONa (6:4) mixture, pH 5.5. The mixture was incubated for 12-14 hr at 4°C in the dark. Then the solution was concentrated and compound IIIa was isolated by preparative electrophoresis and then by paper chromatography in system C.

Compounds III b,c were prepared similarly.

On photolytic decomposition the UV spectra of compounds III a-c have isobestic points at 244 and 292.5 nm and their absorption intensity rapidly decreases during 3-5 min.

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Hydrolysis of $MsCOp_nA$ and compounds I a-c

2 μmol of the given substance were dissolved in 0.3 mL of 0.1 M phosphate buffer (pH 4.5; 5.5; 6.0; 7.0; 7.5; 8.0; 9.3; 10.1) and incubated at 37°C. After 1,2,5 and 15 days, 0.07 mL aliquots were taken and chromatographed in system A with markers. UV absorbing and fluorescing zones were eluted with water and tested spectrophotometrically.

On hydrolysis in 1 N HCl and 1 N NaOH aliquots were taken after 1,2,3,4,6 and 24 hours.

When buffer mixtures 1-3 were used, the hydrolyzates were analyzed 2 days later.

Buffer 1 (pH 7.5): 20 mM potassium morpholinoethanolsulfonate (MES), 2 mM EDTA, 0.25 M sucrose.

Buffer 2 (pH 7.5): 20 mM MES, 2 mM EDTA, 0.25 M sucrose, 4mM $MgSO_4$.

Buffer 3 (pH 7.6): 10 mM Tris-HCl, 10^{-2}M $CaCl_2$, 0.1 M KCl.

Synthesis of 2'(3')-O-mesitoyl-AMP and 2'(3')-O-mesitoyl-ATP

To 4 mg (25 μmol) of mesitoic acid in 0.1 mL of dry DMF was added 16 mg (100 μmol) of 1,1'-carbonyldiimidazole. The anhydrous solution was stirred at room temperature for approximately 10 min and then 7 mg (2 μmol) of AMP in a 0.15 mL DMF-water mixture (2:1) was added quickly. The reaction mixture was stirred at room temperature for 3 h and the product was isolated by paper chromatography in system A. The yield of 2'(3')-O-mesitoyl-AMP was 10%, R_f 0.54.

2'(3')-O-mesitoyl-ATP was prepared in the same way. R_f in system A was 0.28. When subjected to microcolumn chromatography, 2'(3')-O-mesitoyl-AMP and 2'(3')-O-mesitoyl-ATP were eluted at NaCl concentrations of 0.08 M and 0.14 M, respectively.

REFERENCES

1. I.A.Kozlov, M.V.Shalamberidze, I.Yu.Novikova, N.I.Sokolo-

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va, Z.A.Shabarova, Biochem. J. 178, 339 (1979).

2. E.V.Petushkova, V.N.Risnik, S.S.Tretyakova, N.I.Sokolova, Z.A.Shabarova, Biochimia, 45, 726 (1980).
3. R.Krauspe, G.K.Kovaleva, N.N.Guliaev, L.A.Baranova, M.B. Agalarova, E.S.Severin, N.I.Sokolova, Z.A.Shabarova, L.L. Kiselev, Biochimia, 43, 656 (1978).
4. I.A.Madoyan, O.O.Favorova, G.K.Kovaleva, N.I.Sokolova, Z.A.Shabarova, L.L.Kiselev, FEBS Lett., 123, 156 (1981).
5. V.V.Shumyantzeva, N.I.Sokolova, Z.A.Shabarova, Nucleic Acid Res., 3, 903 (1976).
6. V.L.Drutsa, V.F.Zarytova, D.G.Knorre, A.V.Lebedev, N.I. Sokolova, Z.A.Shabarova, Nucleic Acid Res., 5, 185 (1978).
7. N.K.Kochetkov, V.N.Shibaev, A.A.Kost, Dokl.Akad.Nauk SSSR, 205, 100 (1972).
8. S.D.Rose, Biochim.Biophys.Acta, 361, 231 (1974).
9. S.B.Easterbrook, J.C.Wallace, D.B.Keech, Eur.J.Biochem., 62, 125 (1976).
10. G.R.Penzer, Eur.J.Biochem., 34, 297 (1973).
11. J.A.Secrist, J.R.Barrio, N.J.Leonard, G.Weber, Biochemistry, 11, 3499 (1972).
12. R.D.Spencer, G.Weber, G.L.Tolman, J.R.Barrio, N.J.Leonard, Eur.J.Biochem., 45, 425 (1974).
13. S.J.Strickler, R.H.Berg, J.Chem.Phys., 814 (1962).
14. G.L.Tolman, J.R.Barrio, N.J.Leonard, Biochemistry, 13, 4869 (1974).
15. B.V.Tiaglov, S.V.Zenin, E.S.Gromova, G.B.Sergeev, Z.A. Shabarova, M.A.Prokofiev, Molecul.Biol., 8, 595 (1974).
16. B.P.Gottikh, A.A.Krayevsky, N.B.Tarussova, P.P.Purygin, T.L.Tsilevich, Tetrahedron, 26, 4419 (1970).

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