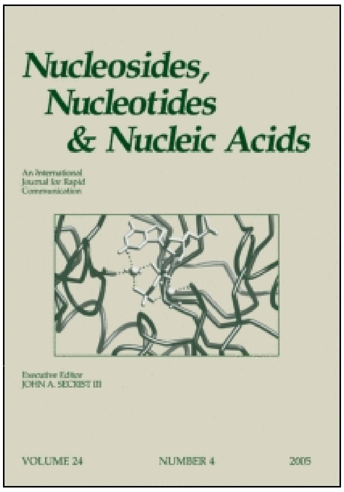

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A ^{31}P NMR STUDY OF THE REACTION OF ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE WITH 2,4,6-TRIISOPROPYLBENZENESULFONYL CHLORIDE

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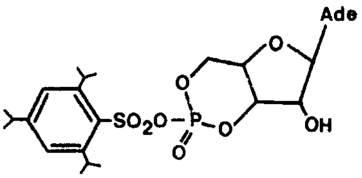
Abstract. The reaction of adenosine 3',5'-cyclic monophosphate (cyclic AMP) with 0.5-2.0 molar equivalents of 2,4,6-triisopropylbenzenesulfonyl (p-toluenesulfonyl or mesitylenesulfonyl) chloride leads to the rapid formation of the three possible phosphorus diastereoisomers [R_pR_p, S_pS_p and $R_pS_p(S_pR_p)$] of cyclic AMP symmetrical anhydride. The diastereoisomers were characterized by ^{31}P NMR. The cyclic AMP sulfonic mixed anhydride could not be detected.

Adenosine 3',5'-cyclic monophosphate (cyclic AMP) derivatives substituted at phosphorus, are valuable probes for binding sites and activation requirements of the enzymes involved in cyclic AMP metabolism.[1] A possible route to such derivatives is the reaction of cyclic AMP with 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl) and subsequent *in situ* treatment of the reactive intermediate with a nucleophile. Nagyváry and coworkers prepared cyclic AMP alkyl esters,[2] Bentrude and Tomasz synthesized cyclic AMP N,N-dimethylamide[3] according to this route. Nagyváry and coworkers considered the cyclic AMP sulfonic mixed anhydride (**1**) to be the reactive intermediate.[2] Bentrude and Tomasz supposed that the initially-formed **1** rapidly converts into the cyclic AMP symmetrical anhydride (**2**), and that **2** reacts with added nucleophile.[3] The latter supposition was based on the well-known instability of phosphodiester sulfonic mixed anhydrides *in the presence of phosphodiester anions,*



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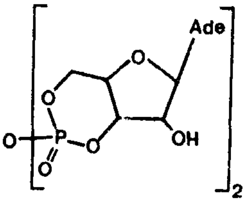

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as a result of which, instead of the mixed anhydrides,[4] the symmetrical pyrophosphates can be detected in these mixtures.[6-12] However, the phosphodiester sulfonic mixed anhydrides are stable enough to be detected in the absence of phosphodiester anions, for example, in the reaction of an activated neutral phosphodiester derivative with sulfonic acid or sulfonic anhydride, as shown by Michalski's group.[10,12] The same group has also pointed out that ^{31}P NMR satisfactorily differentiates between the symmetrical and the sulfonic mixed anhydride of a given phosphodiester -- the mixed anhydride resonances appearing slightly upfield from those of the pyrophosphate.[12] The present work was undertaken with the objective of demonstrating by ^{31}P NMR, whether 1 or 2 (or both) represent reactive intermediate(s) in the above-mentioned synthesis of P-substituted cyclic AMP derivatives.

FIG. 1 shows the proton-decoupled ^{31}P NMR spectrum of a reaction mixture[13] of cyclic AMP with two molar equivalents of TPS-Cl in anhydrous $(MeO)_3PO$ after standing 10 min at room temperature. The spectrum corresponds to that expected for a mixture of the three phosphorus diastereoisomers, $\underline{R}_P\underline{R}_P$, $\underline{S}_P\underline{S}_P$ and $\underline{R}_P\underline{S}_P$($\underline{S}_P\underline{R}_P$) of symmetrical anhydride 2. The two signals at -17.88 and -18.26 ppm may be ascribed to the equivalent phosphorus atoms of $\underline{S}_P\underline{S}_P$-2 and $\underline{R}_P\underline{R}_P$-2, respectively. The four lines at -17.42, -17.61, -19.08 and -19.27 ppm with a symmetric weak : strong : strong : weak intensity distribution show a typical AB pattern and may be assigned

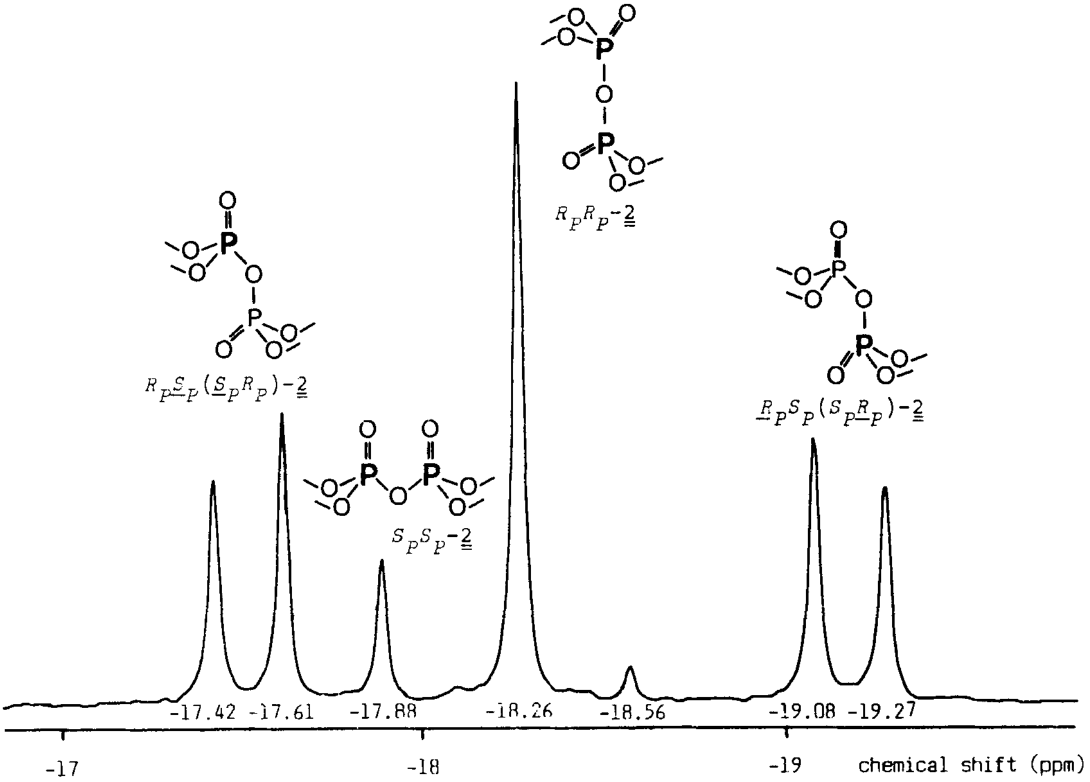

FIG. 1.
The proton-decoupled ^{31}P NMR spectrum of a reaction mixture of cyclic AMP with two molar equivalents of TPS-Cl after standing 10 min at room temperature. The peak at -18.56 ppm (1.6%) is an impurity signal. The broad resonance of $(MeO)_3PO$ about +3.40 ppm and an impurity signal at -0.44 ppm (1.0%) are not shown.

to the two diastereotopic phosphorus atoms of $R_PS_P(S_PR_P)$-**2**. The upfield doublet may be attributed to the phosphorus atom of *R* configuration. On the basis of the ^{31}P chemical shift criterion established for 2-substituted 2-oxo-1,3,2-dioxaphosphorinanes,[14] those diastereomeric phosphorus atoms of **2** should absorb at higher field which have the "other" cyclic AMP residue in the axial position, *i*.*e*. the phosphorus atoms of *R* configuration.

The proton-decoupled ^{31}P NMR spectra of structurally similar acyclic pyrophosphates consist of three distinct signals between -18 and -21 ppm.[9,11-12] It means that $\underline{R}_P\underline{S}_P(\underline{S}_P\underline{R}_P)$ diastereoisomer of these compounds appears as one single signal at room temperature. This signal is expectedly split into two AB type doublets at low temperatures but temperature studies have not been reported. The rigid ring system of $\underline{R}_P\underline{S}_P(\underline{S}_P\underline{R}_P)$-**2** may be responsible for the manifestation of the AB spectrum at room temperature.

The proton-coupled ^{31}P NMR spectrum (FIG. 2) is in agreement with the above structural assignment. The signals ascribed to $\underline{R}_P\underline{R}_P$-**2** and $\underline{S}_P\underline{S}_P$-**2** show complex splitting patterns as expected from the magnetic nonequivalency of the chemically equivalent phosphorus atoms of these diastereoisomers. The proton-coupled subspectrum of $\underline{R}_P\underline{R}_P$-**2** may be considered - though it is a simplification - as the XX' part of an AA'XX' spin system assuming that $^5J_{PH'} = {}^5J_{P'H} \sim 0$ and $^8J_{HH'} \sim 0$. Approximate coupling constants calculated,[15] are: $^2J_{PP'} \sim 20.1$ Hz, $^3J_{PH} = {}^3J_{P'H'} \sim 23.0$ Hz. The proton-coupled subspectrum of $\underline{R}_P\underline{S}_P(\underline{S}_P\underline{R}_P)$-**2** may be analyzed by first order approximation. The upfield doublet is split into doublets of a doublet ($^2J_{PP} = 19.9$ Hz, $^3J_{PH} = 23.0$ Hz), the downfield doublet appears as doublets of doublets of a doublet ($^2J_{PP} = 19.8$ Hz, $^3J_{PH}$s = 7.8 and 12.4 Hz). On the basis of Gorenstein's work on the analogous $\underline{R}_P$ and $\underline{S}_P$-2-aryloxy-2-oxo-trans-5,6-tetramethylene-1,3,2-dioxaphosphorinanes,[14h,14i] these couplings may indicate highly populated chair conformation for the dioxaphosphorinane ring of $\underline{R}_P\underline{S}_P(\underline{S}_P\underline{R}_P)$-**2** that has phosphorus atom of $\underline{R}$ configuration and mixed chair and twist-boat conformations for the dioxaphosphorinane ring that has phosphorus atom of $\underline{S}$ configuration.

The proton-decoupled ^{31}P NMR spectrum did not change when the reaction was performed with half molar equivalent of TPS-Cl for 60 min, or when p-toluenesulfonyl chloride (TS-Cl) or mesitylenesulfonyl chloride (MS-Cl) (one equivalent) was used instead of TPS-Cl. However, as shown in

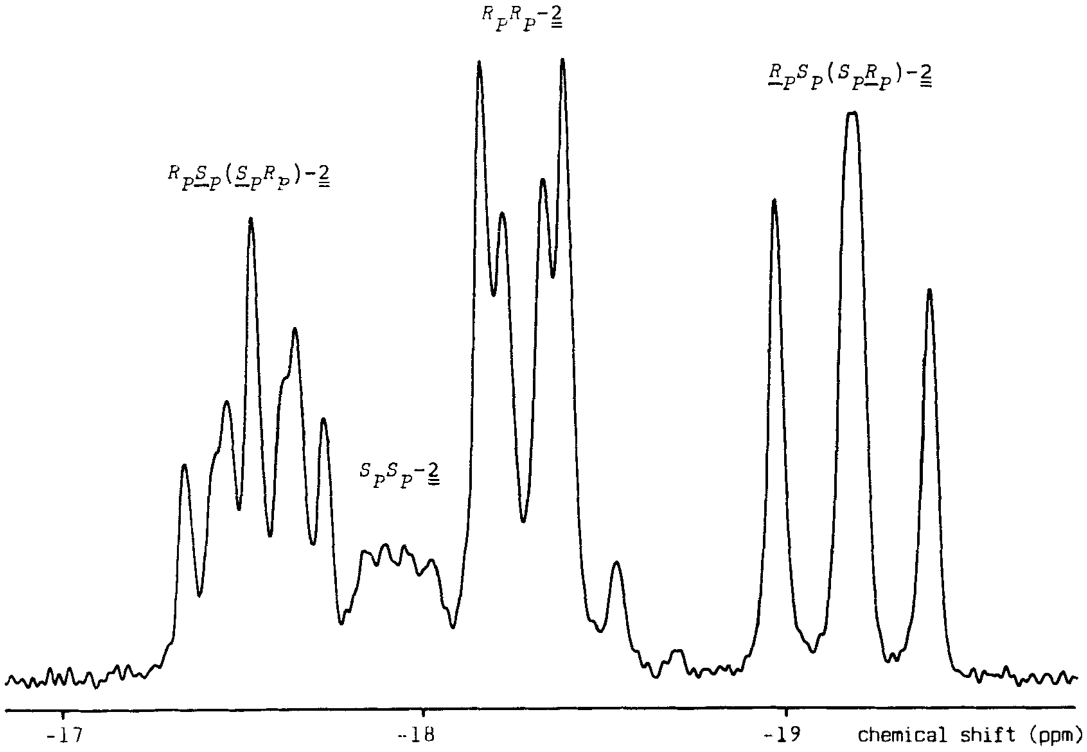

FIG. 2.
The proton-coupled ^{31}P NMR spectrum of cyclic AMP symmetrical anhydride diastereoisomers: R_PR_P-2, S_PS_P-2 and $R_PS_P(S_PR_P)$-2.

TABLE 1.

Percentage distribution of diastereoisomers of 2 formed in the reaction of cyclic AMP with different sulfonyl chlorides in anhydrous $(MeO)_3PO$ at room temperature for 10 min.

Sulfonyl chloride	(molar equiv.)	%[a] R_PR_P	S_PS_P	$R_PS_P(S_PR_P)$
TPS-Cl	2.0	33.2	7.0	59.8
	0.5[b]	69.9	1.1	29.0
TS-Cl	2.0	42.8	5.2	52.0
MS-Cl	1.0	39.7	6.4	53.9

[a] From integrated ^{31}P NMR spectra.
[b] Reaction time 60 min.

TABLE 1, the ratio of diastereoisomers was different, and a part of cyclic AMP [12% (half molar equivalent of TPS-Cl), 14% (TS-Cl) and 45% (MS-Cl)] remained unreacted. The use of DMF or pyridine (the solvents used in Refs. 2 and 3) instead of $(MeO)_3PO$ had no noticeable effect on the ^{31}P NMR spectrum.

Theoretically the two signals at -17.88 and -18.26 ppm might be attributed to mixed anhydride diastereoisomers S_P-**1** and R_P-**1**, respectively. However, on the basis of the identity of the spectra of the reaction mixtures with three *different* sulfonic chlorides, this possibility can undoubtedly be excluded. Phosphoric sulfonic mixed anhydrides containing different sulfonic residues absorb at different fields.[16]

Our results prove that the reaction between cyclic AMP and 0.5-2.0 molar equivalents of TPS-Cl leads to the rapid formation of the symmetrical anhydride **2**, i.e. **2** is the reactive intermediate of the above mentioned synthesis of P-substituted cyclic AMP derivatives. As a consequence of the structure of the reactive intermediate, the theoretical yield of this synthesis is only 50%. Of course, the yield may exceed this value when the cyclic AMP liberated in the reaction with the nucleophile, is repeatedly activated. This is the reason why Nagyváry and coworkers obtained higher than 50% yield.[2]

EXPERIMENTAL

^{31}P NMR spectra were recorded on a Bruker WM-250 FT spectrometer operating at 101.2 MHz. Positive chemical shifts are downfield from external 85% H_3PO_4. Cyclic AMP tri-n-butylammonium salt was prepared according to Ref. 3. TPS-Cl was recrystallized from n-hexane. Solvents were distilled prior to use [$(MeO)_3PO$ through a 80x2 cm insulated Vigreux column at reduced pressure, DMF over P_2O_5] and stored over 0.4 nm molecular sieves.

Reaction of adenosine 3',5'-cyclic monophosphate with 2,4,6-triisopropylbenzenesulfonyl chloride, p-toluenesulfonyl chloride or mesitylenesulfonyl chloride. Cyclic AMP tri-n-butylammonium salt [prepared from cyclic AMP (34.7. mg, 0.1 mmol)] was dissolved in (MeO)$_3$PO (1.0 ml). To this solution a solution of TPS-Cl [(60.6 mg, 0.2 mmol) in (MeO)$_3$PO (2.0 ml) (**A**), or (15.1 mg, 0.05 mmol) in (MeO)$_3$PO (1.0 ml) (**B**)] or TS-Cl [(38.1 mg, 0.2 mmol) in (MeO)$_3$PO (1.0 mL) (**C**)] or MS-Cl [(7.9 μL, 0.1 mmol) in (MeO)$_3$PO (1.0 mL) (**D**)] was added. After standing 10 min (**A**, **C** and **D**) or 60 min (**B**) at room temperature, the ^{31}P NMR spectra were recorded. Reaction **A** was repeated by using DMF instead of (MeO)$_3$PO (**E**), and the ^{31}P NMR spectrum of the reaction mixture was recorded. The ^{31}P NMR spectrum of mixture **A** is shown in FIG. 1. The spectra of mixtures **B**-**E** were identical with the spectrum of mixture **A** except that the cyclic AMP resonance between -2.00 and -2.20 ppm also appeared in mixtures **B**-**D**. Identity of spectra was shown by mixing mixtures **B**-**E** with mixture **A** and recording the spectra of mixtures **A**+**B**, **A**+**C**, **A**+**D** and **A**+**E**. Cyclic AMP was identified by observing the increase of the signal intensity on the addition of an authentic sample to the mixtures. The ^{31}P NMR spectrum of a reaction mixture prepared on exactly the same manner as described in Ref. 2 (2.0 molar equivalents of TPS-Cl in pyridine, 0^{o}C, 2 min), was identical with the spectrum of mixture **A**.

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