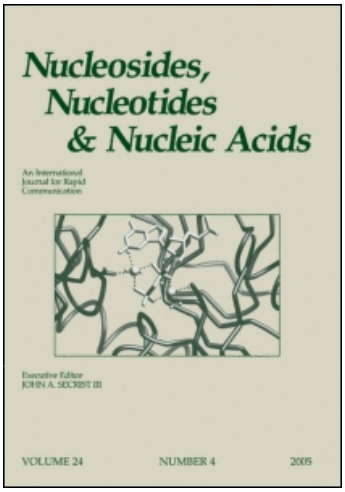

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Preparation, Characterization and Reactivity of Diastereomeric Sulfoxides of 8,2'-S-Anhydroadenosine

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PREPARATION, CHARACTERIZATION AND Reactivity OF DIASTEREOMERIC SULFOXIDES OF 8,2'-S-ANHYDROADENOSINE

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Abstract. The oxidation of 8,2'-S-anhydroadenosine (1a) has been investigated. The major product from the oxidation of 1a using 1-chlorobenzotriazole was the R-sulfoxide. The oxidation of 3',5'-di-O-acetyl-8,2'-S-anhydroadenosine (1b) gave predominately the S-sulfoxide. These sulfoxides were found to be very succeptible to nucleophilic attack at C-8.

Introduction.

Cyclonucleosides or anhydronucleosides are nucleoside analogues in which, in addition to the N-glycoside bond, there is another link from a position on the base to a 2'-, 3'- or 5'-position on the carbohydrate ring. Purine cyclonucleosides have served as intermediates in the synthesis of novel nucleoside antibiotics[1] and find important uses in determining enzyme-substrate conformations[2]. While O[2], 2'-cyclopyrimidines have been employed as protected ribonucleosides for oligoribonucleotide synthesis[3], a corresponding procedure for cyclopurines has not been developed.

One possible approach to the use of anhydropurines in nucleotide synthesis would involve the 8,2'-thioanhydro derivatives. Oxidation of 8,2'-thioanhydroadenosine (1a, SA) to produce the sulfoxide 2, (Scheme I) followed by a Pummerer reaction, would lead to a structure such as 3. Desulfurization of 3 and removal of acyl groups would lead to adenosine. This approach was successfully used by Mizuno in the conversion of 8,5'-S-anhydroxyloadenosine to xyloadenosine[4]. Since SA[5,6] has been incorporated into oligonucleotide chains[7,8], the

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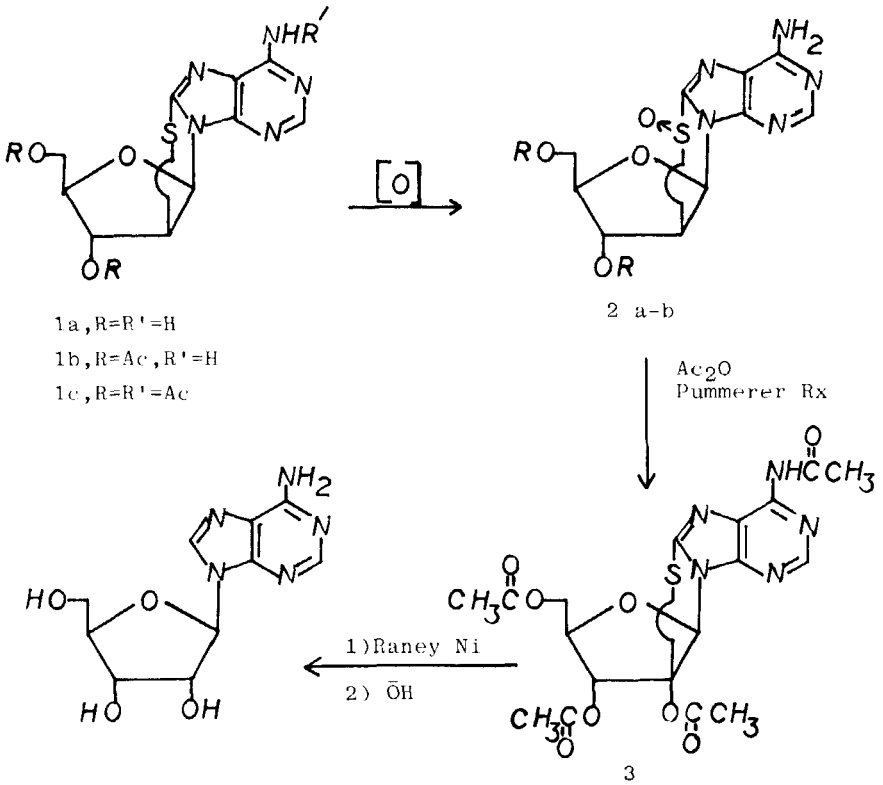


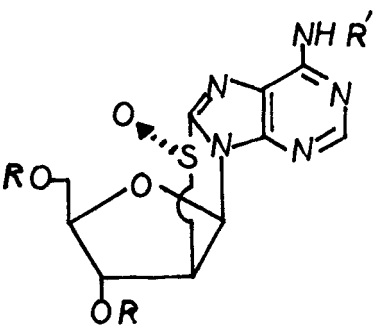
Scheme I

successful development of the reactions of Scheme I would serve as a model for the conversion of thioanhydronucleotides into ribonucleotides.

Results and Discussion

Preparation of Sulfoxides.

Oxidations of SA were carried out using t-butylhypochlorite[9] or 1-chlorobenzotriazole[10] in order to avoid sulfone formation. The best results were obtained with room temperature reactions carried out in methanol. Products were collected after precipitation by pouring the reaction mixture into ether. Using either oxidizing agent, the product (~95% yield) consisted of a mixture of the S- and R-sulfoxides, 4a and 5a respectively (Scheme II). As will be discussed further below, the R-sulfoxide was very unstable and could not be successfully isolated by silica gel chromatography (Table I).



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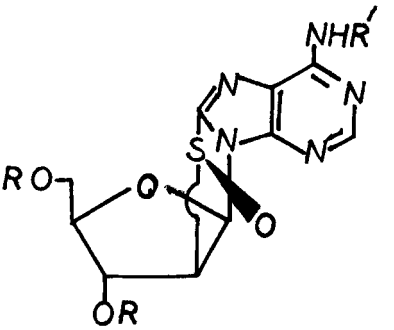

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a, R=R'=H

b, R=Ac, R'=H

c, R=Ac, R'=Cl

d, R=R'=Ac

Scheme II

TABLE I

Chromatographic Properties of Anhydronucleosides

Compound	Paper Chromatography		Thin Layer Chromatography	
	R_f(Solvent B')	R_f(2:1)[a]	R_f(4:1)[a]	R_f(9:1)[a]
1a	0.40	0.42	0.17	
4a	0.24	0.15	0.10	
4a & 5a(1:3)	0.24	0.15,0.29	0.10,0.15	
1b	0.65	-	0.58	0.45
4b	0.54	-	0.37	0.24
5b	0.51	-	0.58	0.40
4c	0.65	-	0.37	0.24
5c	0.65	-	0.37	0.40
1c	0.72	-	0.68	0.53
13	0.58	-	0.63	0.44

[a] Chloroform-ethanol mixture

The sulfoxide products were obtained as the hydrochloride salts. This was supported by the infrared spectrum (Table II) where, in addition to the strong sulfoxide absorption at 1060 cm^{-1}, the amino deformation band at 1660 cm^{-1} in purines had shifted to 1700 cm^{-1}, the purine band at 1610 cm^{-1} was of lowered intensity, and there was no absorption at 1310 cm^{-1}. These changes clearly indicated a pro-

TABLE II

IR Spectra of Anhydronucleosides

Compound	Absorption Band (cm^{-1})		
	Amino Deformation	Sulfoxide Stretch	Carbonyl Stretch
1a	1660		
1b	1660		1760
1c	1610		1740
4a & 5a(1:3)	1700	1060	
4a	1650	1050	
4b	1660	1060	1750
5b	1650	1060	1750
13	1610	1050	1740
4c	1620	1050	1740
5c	1620	1060	1740

tonated purine ring[11]. In addition, a solution of the product in water had an acidic pH. The mass spectrum of the sulfoxide product showed the parent ion at m/e 297 and a peak at m/e 281 caused by loss of oxygen (M-16).

The H' NMR spectrum of the product showed a mixture of two sulfoxides in a ratio of 3 to 1 as determined by the integration of the anomeric proton signals (Table III, exptl). ^{13}C NMR spectra (Table IV) allowed the assignment of configuration. Assignments were made from information obtained from both coupled and decoupled spectra. The C-2' signals for the sulfoxides were shifted downfield relative to SA. However C-3' of the minor sulfoxide product was shifted upfield relative to SA while C-3' of the major sulfoxide was shifted downfield relative to SA. The upfield shift of C-3', due to steric interaction with the sulfoxide oxygen, identifies the minor product as the S-sulfoxide[12].

Further proof of structure was obtained from the diacetates 4b and 5b. Acetylation of the sulfoxide mixture obtained from t-butylhypochlorite oxidation afforded 4b and 5b in 23% and 45% yields respectively. The yields were 23% and 44% respectively from the acetylation of the 1-chlorobenzotriazole oxidation products. In both cases several minor products were obtained which resulted from decomposition of the R-sulfoxide 5a.

TABLE III

90 MHz ^{1}H NMR Spectra of Anhydronucleosides in DMSO-d_6

Compound	H-1'	H-2'	H-3'	H-4'	H-5'	H-2	6-NH$_{(2)}$	CH_3
	δ ppm							
1a	6.49(d) $J_{1'-2'}$=6.3 Hz	4.85(dd) $J_{2'-3'}$=2.5 Hz	4.39(t) $J_{3'-4'}$=2.5 Hz	3.98(m)	3.46(m)	8.02	7.03	-
1b	6.57(d) $J_{1'-2'}$=6.2 Hz	5.16(m)	5.16(m)	4.48(m)	4.10(m)	8.04	7.10	1.90,2.11
4a	6.37(d) $J_{1'-2'}$=6.3 Hz	4.76(dd) $J_{2'-3'}$=4.4 Hz	4.55(t) $J_{3'-4'}$=5.6 Hz	4.08(m)	3.42(m)	8.21	7.70	-
5a[b]	7.12(d) $J_{1'-2'}$=6.3 Hz	4.36(dd)	4.91(b)	4.24(bs)	3.30(m)	8.49	6.94	-
4b	6.52(d) $J_{1'-2'}$=6.2 Hz	5.00(dd) $J_{2'-3'}$=3.3 Hz	5.28(dd) $J_{3'-4'}$=5.1 Hz	4.61(m)	4.07(m)	8.20	7.66	1.86,2.11
5b	7.06(d) $J_{1'-2'}$=6.2 Hz	4.76(dd) $J_{2'-3'}$=3.4 Hz	5.57(t) $J_{3'-4'}$=3.4 Hz	4.62(m)	3.94(m)	8.25	7.77	1.79,2.14
4c	6.61(d) $J_{1'-2'}$=6.2 Hz	5.05(dd) $J_{2'-3'}$=3.2 Hz	5.31(dd) $J_{3'-4'}$=5.0 Hz	4.66(m)	4.07(m)	8.45	8.88	1.87,2.14
1c[a]	6.65(d) $J_{1'-2'}$=6.0 Hz	5.18(m)	5.18(m)	4.47(m)	4.10(m)	8.49	7.05	1.86,2.12 2.19
13	6.65(d) $J_{1'-2'}$=6.2 Hz	5.05(dd) $J_{2'-3'}$=3.1 Hz	5.29(dd) $J_{3'-4'}$=5.4 Hz	4.65(m)	4.09(m)	8.76	7.40	1.84,2.14 2.28

[a] 60 MHz spectrum

[b] Data obtained from a mixture containing 4a and 5a

TABLE IV

^{13}NMR Spectra of Anhydronucleosides in DMSO-d_6

Compound	δ (ppm from TMS)											
						$J_{^{13}C-H}$ (Hz)						
	C-1'	C-2'	C-3'	C-4'	C-5'	C-2	C-4	C-5	C-6	C-8	C=O	CH_3
1a	86.3 179.6	62.3 157.3	77.4 154.3	88.0 152.4	61.1 140.3	151.6	149.1	124.0	154.0	153.1	-	-
4a[a]	85.9 181.2	66.8 149.1	73.7 155.2	88.8 157.0	60.3 140.5	146.8 207.8	146.5 12.7	124.1 0	151.2 12.7	158.3 3.9	-	-
5a[a]	88.8 183.7	72.9 149.3	85.9 151.8	91.3 150.2	61.6 141.0	147.0 207.8	146.4 12.7	123.9 0	151.4 12.7	157.5 3.9	-	-
1b	86.6 179.7	59.9 156.4	80.1 160.2	81.7 153.3	62.9 150.4	151.9 199.2	149.1 9.8	124.1 0	154.1 4.9	152.8 0	169.9,170.4	20.2,20.5
4b	85.8 182.9	70.0 155.5	70.6 158.6	82.9 153.3	62.2 150.0	154.9 199.3	147.6 12.5	124.0 0	156.9 11.7	154.9 3.3	170.0,170.6	20.3,20.6
5b	87.4 181.6	74.3 156.6	81.6 156.0	83.1 153.3	63.9 150.8	154.9 199.3	147.8 12.7	124.9 0	156.9 11.7	153.8 313	169.7,170.7	20.0,20.6

[a]Component of the mixture of 4a-5a, 1:3.

Oxidation of diacetylSA (1b) under carefully controlled conditions led to the S-product 4b in 59% yield, the R-product 5b in only 9% yield and five unknown products in lesser amounts. Consistent results were obtained when 4 equivalents of 1-chorobenzotriazole were added to 1b in methanol at -78 C. Immediately after the addition the dry ice bath was replaced by an ice-water mixture. After 10 min the mixture was applied directly to TLC plates. Each sulfoxide was usually initially contaminated by its N^6-chloroderivative (4c and 5c). When the N^6-chloroderivative of 4c was obtained pure, its 'H NMR spectrum was identical to that of 4b except for the protons on the purine ring. Only one amine proton was present in 4c, and H-2 was shifted relative to 4b. The amino deformation band in the infrared spectrum was shifted from 1620 cm^{-1} for sulfoxides to 1660 cm^{-1} for the chloroamine sulfoxides. Furthermore, the chloramines (4c and 5c) produced a dark yellow solution when dissolved in 80% aqueous acetic acid containing sodium iodide, indicating the presence of a chloramine functionality in the starting material[13]. Chloramines are generally unstable molecules that can be routinely prepared from amines[14], including adenosine[15], using positive halogen oxidizing agents. In the case of 4c and 5c the chlorine is readily replaced by hydrogen under a variety of conditions including dissolution in water, ethanol or DMF, or, more slowly on standing as a solid. Similar conditions have previously been observed to result in the dechlorination of chloramines[14,15].

The sulfoxides 4b and 5b were shown to differ only in configuration at the sulfoxide. The CD spectra of 4b and 5b (Table V) show opposite Cotton effects. The mass spectra of the sulfoxides both showed parent ions at m/e 381 and an M-16 peak at m/3 365. Both sulfoxides showed a strong absorption in the infrared spectrum at 1060 cm^{-1}.

While the 'H NMR spectra of 4b and 5b were distinguishable, absolute assignment of sulfoxide configuration could not be made on the basis of acetylene-like anisotropy of the sulfinyl moiety[16]. However, the assignment could be made from the ^{13}C spectra. The C-2' signal (Table IV) shifted downfield relative to that of 1b in both sulfoxides. However the C-3' signal in the S-sulfoxide (4b) is shifted upfield relative to 1b while C-3' in the R-sulfoxide (5b) is nearly unchanged relative to 1b.

TABLE V

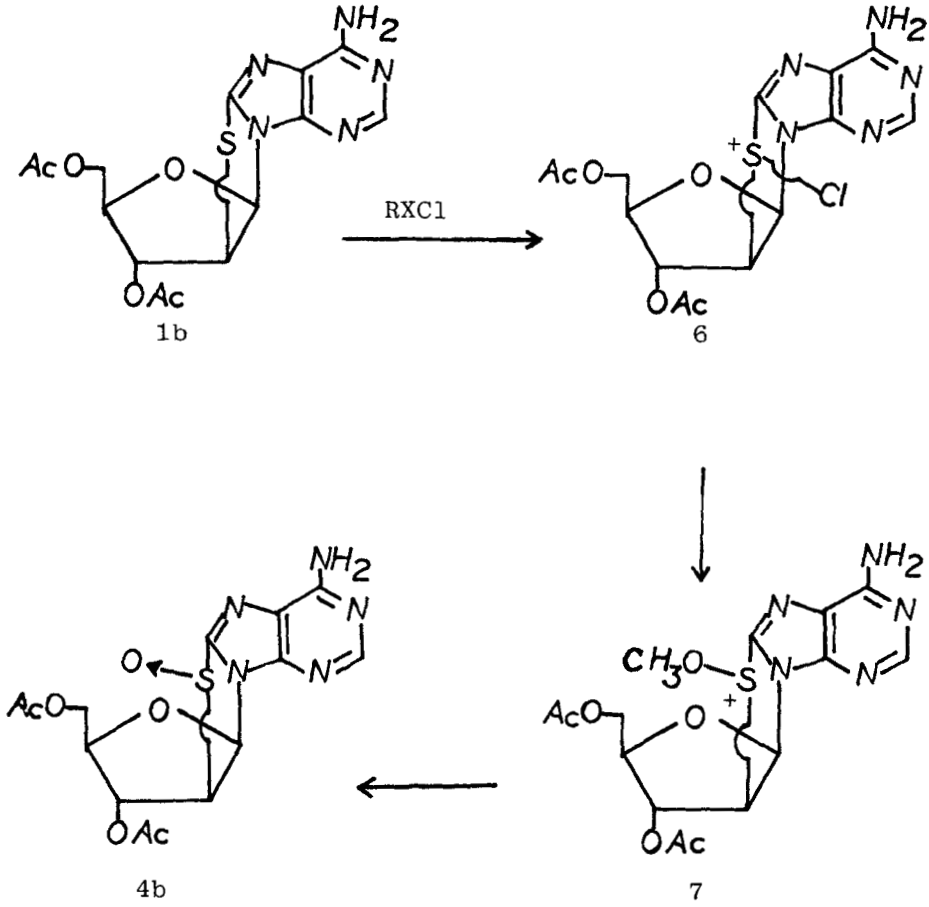
UV and CD Spectra of Anhydronucleosides

Compound	UV in H_2O		CD at pH 3.2	
	$\lambda_{max}(\varepsilon)$	$\lambda_{min}(\varepsilon)$	$\lambda_{peak}(\theta)$	$\lambda_{trough}(\theta)$
1a	276(18200) 220(19000)	238(3500)	280(8200) 255sh(450)	220(1300)
4a	286(10600)	-	285(26000)	245(14000) 220(45000)
4a & 5a(1:3)	284(10400)	239(4000)	245(13000)	280(17000) 225(14000)
1b	276(19100) 220(18600)	238(2800)	280(7700)	220(22000)
4b	287(9800) 219(12200)	239(2300)	285(29000)	245(11000) 220(46000)
5b	289(10700) 222(12100)	241(2300)	250(15000)	285(23000) 230(15000)
4c	293(11900)	250(39100)	290(32000)	240(19000) 215(39000)
5c	296	252	-	-
1c	295(21200) 288(21500) 254sh(7100) 229(19000)[a]	-	-	-
13	287(13300)	243(4200)	285(24000)	250(8000) 220(3000)

[a] In EtOH.

Discussion of Sulfoxide Ratios

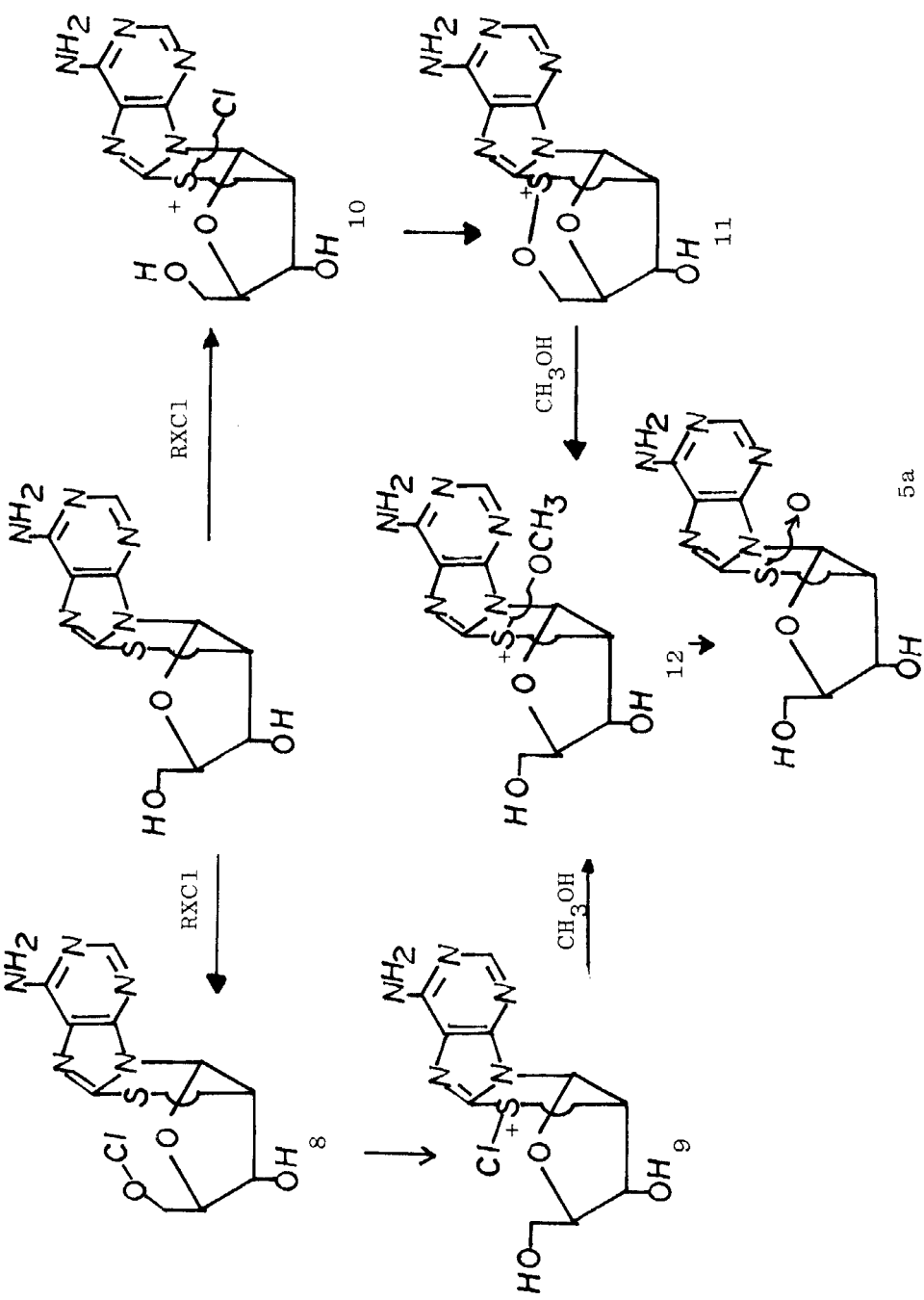
The oxidation of SA (1a) gave a 95% yield of sulfoxides with an R/S ratio of 3:1 while oxidation of 1b produced the S-sulfoxide 4b in 59% yield and only 9% of the R-sulfoxide 5b. This latter result clearly arises from the accepted mechanism (Scheme III) of sulfide oxidation with positive halogen reagents such as 1-chlorobenzotriazole and t-butylhypochlorite[9,17]. The first step is the formation of the chlorosulfonium ion 6 by attack on the least hindered face of 1b.



Scheme III

Subsequent attack from behind the S-Cl bond gives the alkoxysulfonium ion 7. In the absence of base, 7 breaks down to give the S-sulfoxide 4b. The minor product 5b is formed from the reverse attack at each step in the mechanism.

The apparently anomalous results of SA oxidation can be explained by involving the unprotected 5'-hydroxyl group in an intramolecular mechanism. One possibility (Scheme IV) incorporates formation of the 5'-hypochlorite 8 (1-chlorobenzotriazole[18] and tertbutylhypochlorite[19] have been used to oxidize alcohols) which then relays the chlorine to the sulfur from behind to give chlorosulfonium ion 9. Continuation of the reaction in the standard way provides the R-sulfoxide. A similar process has been found to occur in the formation of p-chloroanisole



Scheme IV

using sodium hypochlorite in the presence of a cyclodextrin[20]. A relay of chlorine from an amido nitrogen to a sulfide has also been suggested to account for the stereospecific formation of the penicillin sulfoxide diastereomer having the more sterically accessible sulfoxide oxygen[21].

Another likely possibility is that intramolecular attack in the chlorosulfonium ion 10 (Scheme IV) gives the cyclic alkoxysulfonium ion 11. Subsequent attack by methanol from the less sterically hindered side would lead to formation of 5a. A cyclic alkoxysulfonium ion has been isolated which was created via anchimeric assistance of a hydroxyl group similar to that shown in Scheme IV[22]. A similar mechanism was operative in the oxidation of a sulfoxide to a sulfone[23].

Sulfide oxidations passing through chlorosulfonium ions in which backside attack is sterically hindered have been reported to lead to undesirable by-products[10,17c]. This probably explains the more facile and cleaner oxidation of 1a relative to 1b. The conversion of either 9 or 10 into 12 would be expected to proceed faster than the conversion of 6 into 7.

Ikehara has reported the stereoselective synthesis of 5a from SA in high yield using t-butylhypochlorite or performic acid as oxidizing agent[24]. While the sulfoxide is referred to as the S-diastereomer it is clearly depicted as the R-sulfoxide[24]. It is described as originating by attack of the oxidizing agent on the sulfur atom from the more accessible "front" side. The Cotton effects in the CD spectrum of the product[24] are similar to those reported here for the R-sulfoxide. We found that no oxidation occurred at -78 C using t-butylhypochlorite when reactions were rapidly quenched. However, reaction occurs rapidly on raising the temperature above -78 C. Oxidation of SA with performic acid using the described conditions[24] did not lead to reaction while use of a larger amount of performic acid produced a complex mixture.

Stability of Sulfoxides

The sulfoxides that were prepared, particularly 5a, were very susceptible to decomposition giving many derivatives. A complex mixture resulted when silica gel thick layer chromatography was used in an attempt to separate 4a and 5a which had been isolated as a mixture of their hydrochloride salts. While treatment of the mixture of

sulfoxides with acetic anhydride/pyridine gave a near quantitative conversion of 4a to its diacetate 4b, only about a 65% conversion of 5a to 5b could be obtained. These observations were found to result from using conditions that did not maintain the degree of acidity necessary to stabilize the R-sulfoxide.

A solution of a mixture of sulfoxide salts in D_2O having a pH of less than 2 was found to be unchanged after 3 days as shown by UV and ^{13}C NMR spectroscopy. However, when dissolved in a pH 3.2 buffer for 2 days, decomposition occurred as shown by a decrease in the λ_{max} (H_2O) from 284 to 280 nm. In aqueous solutions varying in pH from 5.5 to 6.9, the mixture of sulfoxide salts decomposed rapidly to produce a complex mixture with a constant λ_{max} (H_2O) 263 nm. Paper chromatography of these mixtures led to the separation of five products. One of these products resembled the starting material in that it moved at $R_F^{B'}$ 0.24 on paper chromatography and had λ_{max} (H_2O) 286 nm. Comparison of R_F values on TLC and ^1H NMR and CD spectra revealed that this product was exclusively the minor component of the sulfoxide mixture, i.e. 4a. This material was found to be stable in water and accounted for 21% of the total UV absorbance of the pH 6.9 decomposition mixture, in good agreement with the composition of the original sulfoxide mixture (25% 4a and 75% 5a). Thus the R-sulfoxide was stable in acid but not at neutral pH while the S-sulfoxide was stable under both conditions.

Ikehara[24] has reported that the sulfoxide of 8,3'-S-anhydroadenosine is stable at neutral pH but decomposes in dilute alkali. The products were tentatively identified as arising from an intramolecular attack of the 5'-hydroxyl on C-8 resulting in 8,5'-O-anhydroadenosine derivatives. These are presumed to arise via a reactive sulfenic acid intermediate[24]. This also appears to be the case in the R-sulfoxide derived from SA since all of the decomposition products had $\lambda_{max}(H_2O)$ at 261 nm, identical to that of 8,5'-O-anhydroadenosines[25]. At pH 9.4 the S-sulfoxide (4a) decomposed more slowly than the R-sulfoxide (5a) and gave products having a broad absorption centered at 265 nm. The acetylated sulfoxides 4b and 5b decomposed at pH 9.4 giving products whose UV spectra resembled that of 8-hydroxyadenosine with $\lambda_{max}(H_2O)$ at 270 nm. This suggests that in the acetylated sulfoxides, decomposition results from hydroxide or water attack at C-8.

Attempted Pummerer Reactions

The Pummerer conditions that were applied to the sulfoxides 4 and 5 consisted of heating the sulfoxide with a carboxylic acid anhydride at 80 °C, usually in the presence of the sodium salt of the carboxylic acid[26]. TLC and paper chromatography indicated that the use of the sodium salt of the acid had little affect on the product distribution but did facilitate examination of reactions by chromatography. Products of the reactions were largely independent of whether the unacetylated (4a and 5a) or acetylated sulfoxides (4b and 5b) were used as starting materials. Since 4b and 5b were easier to obtain pure, most experiments were conducted on the acetylated sulfoxides.

The S-sulfoxide (4b) produced a single product after treatment with acetic anhydride for 20 min at 80°C. Spectroscopic analysis showed that the product was the $N^6,O^{3'},O^{5'}$-triacetylsulfoxide 4d and not the desired product 3. The CD spectrum of 4d was identical to that of 4b and the sulfoxide band was still present at 1050 cm^{-1} in the infrared spectrum. These conditions convert 1b into 1c. The reaction period was then extended leading to a complex mixture after 1 day.

In the case of the R-sulfoxide, 5b, a complex product mixture was obtained after only 10 min. TLC analysis showed 10 significant products in the mixture. When the product mixture obtained after heating 4a and 5a in acetic anhydride for two hours was treated with ammonium hydroxide, the major product was N^6-acetyl-8-hydroxyadenine.

The Pummerer conditions were varied over a range of conditions including the use of other anhydrides (e.g. benzoic anhydride). However, none of the conditions attempted led to a product which could be attributed to a normal Pummerer mechanism. In nearly all cases a complex mixture of products was obtained. From the UV spectral characteristics of these products, they appear to arise from attack at C-8 during the Pummerer reaction. Clearly, this route does not offer a promising alternative to ribonucleotide synthesis.

Experimental

Descending paper chromatography was performed using 55 cm sheets of Whatman 3 MM paper. The solvent system, B', was prepared on a volume basis and was the organic phase of n-butanol-ethanol-water (4:1:5). TLC was carried out by the ascending technique using 6.7 cm

strips of Brinkman Polygram SIL G/UV 254 silica gel-coated sheets. Thick layer chromatography was carried out on 20 cm x 20 cm glass plates coated with a 1 mm thick layer of Camag DSF-5 silica gel. Nucleosides and their derivatives were visualized on papers and silica gel plates using an UV light source (Mineralite, output at 254 nm). Products were eluted from papers by water and from thick layer plates by organic solvents.

UV spectra were recorded on a Cary 17 spectrophotometer using EtOH and neutral aqueous solutions. Circular dichroism spectra were obtained on a Jasco ORD/UV-5 instrument using pH 3.2 solutions. IR spectra were obtained on a Unicam SP 1000 or a Perkin-Elmer 521 Spectrophotometer using KBr disks for sample preparation. Mass spectra were obtained on an AEI-MS-902 mass spectrometer at 70 eV using a direct insertion probe. ^{13}C (22.63 MHz) and ^{1}H NMR spectra were recorded on a Bruker WH-90 FT instrument. 60 MHz ^{1}H NMR spectra were recorded on a Varian T-60-A spectrometer. Melting points were determined on a Fisher-Johns melting point apparatus and are reported uncorrected. Elemental analyses were performed by Canadian Microanalytical Service Ltd., Vancouver, B.C. The sample submitted was prepared by crystallization followed by heating in a drying apparatus over phosphorus pentoxide. The presence of a chloramine functionality in a molecule was indicated by formation of a darkly colored solution upon addition of the compound to a 4% solution of KI in 80% aqueous acetic acid[14].

Reagent grade pyridine was distilled from p-toluenesulfonyl chloride, redistilled from CaH, and stored over Linde 4A molecular sieves. Reagent grade DMF was distilled from CaH and stored over 4A molecular sieves. Spectrograde MeOH was dried by storage over 4A molecular sieves. Reagent grade acetic anhydride was distilled from phthalic anhydride and stored in the dark. All EtOH was the 95% solvent. All other organic solvents were reagent grade and were used without further purification. The buffers used for sulfoxide stability studies were: 0.1 M potassium dihydrogen phosphate adjusted to pH 3.2 by addition of 0.1 N HCl; 0.1 M potassium dihydrogen phosphate adjusted to pH 6.9 by addition of 0.1 N NaOH; 0.05 M sodium bicarbonate adjusted to pH 9.4 by addition of 0.1 N NaOH.

Following literature procedures, 1-chlorobenzotriazole[17] was prepared from benzotriazole (Aldrich) and 5% sodium hypochlorite. Tert-

butylhypochlorite[27] was prepared from t-BuOH and 5% sodium hypochlorite. Adenosine was purchased from Sigma Chemical Co. 8,2'-S-Anhydroadenosine (SA, 1a, m.p. 190° dec) was prepared in 49% yield from adenosine by following the literature procedure[6]. Its physical properties are described in tables in the Experimental.

Preparation of the mixture of the hydrochloride salts of the sulfoxides of 8,2'-S-anhydroadenosine (4a and 5a)

a) With 1-chlorobenzotriazole

To a mixture of 200 mg (0.71 mmole) 8,2'-S-anydroadenosine in methanol (4 ml) under a dry nitrogen atmosphere at room temperature was added 220 mg (1.43 mmole) of 1-chlorobenzotriazole. After 15 min the orange solution was added to 40 ml of anhydrous ethyl ether. The precipitate was collected by centrifugation and washed twice with 40 ml of anhydrous ethyl ether to give 225 mg (95%) of the mixture of the sulfoxides as their hydrochloride salts, m.p. 220°, dec.; mass spectrum m/e 297 (M^+), 281 (M^+-O). Integration of the anomeric proton resonances in the 'H NMR spectrum (Table III) of the product revealed that the product was composed of 75% 5a and 25% 4a. Chromatographic data is listed in Table I, UV and CD spectral properties are recorded in Table V, IR spectral properties are recorded in Table II and the ^{13}C NMR spectrum of each component of the mixture is described in Table IV.

b) With t-butyl hypochlorite

The reaction was carried out as above but with 0.15 ml (1.26 mmole) of t-butyl hypochlorite substituted for 1-chlorobenzotriazole. The yield was 226 mg (95%) of a mixture of sulfoxides of the same composition as above. Yields in a) or b) decreased by 10-20% when the operations were not performed under a dry nitrogen atmosphere.

Preparation of 3',5'-di-O-acetyl-8,2'-S-anhydroadenosine (1b)

To a mixture of 500 mg (1.80 mmole) of 8,2'-S-anhydroadenosine in 4 ml of pyridine was added 4 ml of acetic anhydride. After stirring at room temperature for 10 min, 5 ml of ethanol was added to the reaction mixture. The solution was then applied to 6 silica gel thick layer plates which were developed first with ether and then with chloroform-

ethanol (9:1). The band at R_f 0.45 was eluted with chloroform-ethanol (2:1) and on evaporation of the solvent, 586 mg (90%) of 1b was obtained. The product was recrystallized from ethanol as white crystals, m.p. 220° dec; mass spectrum m/e 365 (M^+). The band at R_f 0.53 gave 23 mg (3%) of N^6,3',5'-triacetyl-8,2'-S-anhydroadenosine[8] (1c) m.p. 179° dec. Other physical properties of the products are presented in Tables I - V.

Preparation of the sulfoxides of 1b (4b and 5b)

a) Oxidation of 1b

To a mixture of 250 mg (0.68 mmole) of 1b in methanol (4 ml) cooled to -78° by a dry ice-acetone bath was added 440 mg (2.87 mmole) of 1-chlorobenzotriazole. Immediately after addition an ice bath was substituted for cooling and ten minutes later the yellow suspension was applied onto 3 silica gel thick layer plates. The plates were developed first with ether and then with chloroform-ethanol (9:1). The band at R_f 0.24 containing a mixture of 4b and its chloramine derivative 4c and the band at R_f 0.40 containing a mixture of 5b and its chloramine derivative 5c were eluted with ethanol. The solutions were evaporated and each residue was dissolved in DMF. One day later the solutions were evaporated to give 153 mg (59%) of 4b, m.p. 150° dec.; mass spectrum m/e 381 (M^+), 365 (M^+-O); and 24 mg (9%) of 5b, m.p. 150° dec.; mass spectrum m/e 381 (M^+), 365 (M^+-O). Elemental analysis was performed on 4b (recrystallized from ethanol): Calcd. for $C_{14}H_{15}N_5O_6S$: C, 44.10; H, 3.97; N, 18.37. Found: C, 44.07; H, 3.73; N, 18.24. The chromatographic properties (Table I), IR spectra (Table II), UV and CD spectra (Table V, ^1H NMR spectra (Table III) and ^{13}C NMR spectra (Table IV), both sulfoxides are described.

In some reactions the products with R_f 0.24 and R_f 0.40 that were isolated from thick layer plates did not contain 4b or 5b. In these cases, the two products were identified as the N-chlorosulfoxides 4c and 5c. Their physical properties are given in Tables I, II and V. The presence of a chloramine functionality in both of these compounds was indicated by a positive chloramine test[14].

b) Acetylation of the mixture of 75% 5a and 25% 4a

i) A solution of 150 mg (0.45 mmole) of the unacetylated sulfoxide mixture obtained from oxidation with 1-chlorobenzotriazole in pyridine

(1 ml) and acetic anhydride (1 ml) was stirred at room temperature. After 10 min the solution was applied onto 2 thick layer plates which were developed first with ether and then twice with chloroform-ethanol (9:1). Elution of the product with R_f 0.40 (chloroform-ethanol, 9:1) gave 75 mg (44%) of sulfoxide identical to 5b obtained above. Analogously elution of the product with R_f 0.24 gave 39 mg (23%) of sulfoxide identical to 4b obtained above.

ii) Identical treatment as above of the unacetylated sulfoxide mixture obtained from oxidation with tert-butylhypochlorite gave 77 mg (45%) of 5b and 40 mg (23%) of 4b.

Decomposition of sulfoxides

Decompositions were carried out by dissolving 15 mg of sulfoxide in 10 ml of an aqueous buffer. Workups involved evaporating the water, washing the predominantly inorganic salt residue with DMF, concentrating the DMF washings and applying the concentrate onto paper sheets which were developed with Solvent B'.

a) Stability of the unacetylated sulfoxide mixture at pH 6.9

Dissolution for 2 days gave products with $R_f^{B'}$ 0.40, 0.15, 0.09 and 0.05 having $\lambda_{max}^{H_2O}$ 261 or 262 nm. A product was also obtained with $R_f^{B'}$ 0.24 and $\lambda_{max}^{H_2O}$ 286 nm whose UV absorbance accounted for 21% of the total UV absorbance. The latter product was identified as 4a (m.p. 170° dec). Physical properties of 4a are listed in Tables I - V.

b) Stability of the unacetylated sulfoxide mixture at pH 9.4

Dissolution for 2 days gave the same products as above. However 4a accounted for 14% of the total UV absorbance.

c) Stability of 4a at pH 9.4

Dissolution for 4 days gave products with $R_f^{B'}$ 0.47, $\lambda_{max}^{H_2O}$ 266; $R_f^{B'}$ 0.33, $\lambda_{max}^{H_2O}$ 267; $R_f^{B'}$ 0.10, $\lambda_{max}^{H_2O}$ 267; and $R_f^{B'}$ 0.05, $\lambda_{max}^{H_2O}$ 264 nm. Starting material with $\lambda_{max}^{H_2O}$ 286 nm was also present at $R_f^{B'}$ 0.24 and represented about half of all the material.

d) Stability of 4b at pH 9.4

Dissolution for 4 days gave products with $R_f^{B'}$ 0.38, 0.18 and 0.07 having $\lambda_{max}^{H_2O}$ 270 nm. Starting material with $\lambda_{max}^{H_2O}$ 286 nm was also present at $R_f^{B'}$ 0.54 and represented about half of all the material.

e) Stability of 5b at pH 9.4

Dissolution for 3 days gave the same three decomposition products as in (d). No starting material was present.

Attempted Pummerer reactions on sulfoxides

Transformations were attempted by reacting the sulfoxide with an acid anhydride (2 ml of acetic anhydride or 1 g or benzoic anhydride per 100 mg sulfoxide), usually in the presence of the relevant sodium carboxylate (same amount by weight as sulfoxide), at 80°C. Reactions were worked up at varying times by application of the reaction mixture onto paper sheets or silica gel thick layer plates.

a) Preparation of the S-sulfoxide of N^6,2',3'-triacetyl-8,2'-S-Anhydroadenosine (4d)

A mixture of 25 mg of 4b and sodium acetate in acetic anhydride was reacted for 20 min. Subsequent workup either on paper sheets developed with Solvent B' or on a thick layer plate developed first with ether and then with chloroform-ethanol (1:1), gave one product corresponding to 4d m.p. 155° dec.; mass spectrum m/e 381 (M^+-C_2H_2O), 365 (M^+-$C_2H_2O_2$). Other physical properties are presented in Tables I - III and V.

b) Attempted Pummerer Reaction on the R-sulfoxide of 2',3'-diacetyl-8,2'-S-anhydroadenosine (5b)

After 10 min, an acetic anhydride-sodium acetate reaction on 150 mg of 5b was worked up on 2 thick layer plates. Development of the plates first with ether and then with chloroform-ethanol (9:1), enabled the isolation of 10 products with λ_{max}^{EtOH} 287 nm. The UV spectra were qualitatively identical to that of N^6-acetyl-8-hydroxyadenosine[25]. A similar complex mixture was obtained from a 22 hr reaction on 4b.

c) Reactions with benzoic anhydride-benzoic acetate

Reactions were performed on 4b, 5b and on the unacetylated sulfoxide mixture. In each case chromatography revealed the formation of a mixture of numerous products.

d) Base hydrolysis of a reaction mixture

A 200 mg (0.60 mmole) mixture of 75% 5a and 25% 4a was treated with sodium acetate and acetic anhydride under Pummerer conditions for 2 hr. Subsequently, ethanol was added, the solution was evaporated and the residue was dissolved in pyridine (2 ml) and concentrated ammonium hydroxide (4 ml). After stirring for 48 hr at room temperature the solution was evaporated, the residue was taken up in ethanol, the mixture was centrifuged and the supernatant was applied onto paper chromatograms which were developed with Solvent B'. The band at R_f 0.49 gave 30 mg (26%) N^6-acetyl-8-hydroxyadenine, m.p. 160° dec.; R_f 0.14 (ether; R_f 0.38 (ethyl acetate); UV $\lambda_{max}^{H_2O}$ 287 nm, $\lambda_{max}^{H_2O}$ 243 nm; IR, 1710 cm^{-1}, broad (carbonyl), 1620 cm^{-1} (N-H); mass spectrum, m/3 193 (M^+), 175 (M^+-CH_3), 151 ($M^+-C_2H_2O$).

Preparation of N^6-acetyl-3',5'-di-O-acetyl-8,2'-S-anhydroadenosine (1c)

A mixture of 100 mg (0.27 mmole) of 1b and 100 mg of sodium acetate in acetic anhydride (2 ml) was stirred at 80°C for 30 min. The reaction mixture was then worked up on a thick layer plate which was developed with chloroform-ethanol, (1:1). The band with R_f 0.53 gave 107 mg (96%) of 1c[8]. Physical properties are given in Tables I, II, III and V.

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