it may serve as a probe of transition-state geometry in solution.

(4) Useful information on the relative importance of participating groups, preferred ring sizes, and relative radical stabilities can be obtained.

Experimental Section

Pyridyl-1-propanols **1–3** were commercial samples (Aldrich Chemical Co.) and were redistilled before use. The acetate **4** was prepared by acetylation of the alcohol with acetyl chloride in the presence of pyridine at −10 to 10°, and the product was fractionally distilled, bp 59–62° (0.08 mm). Acetates **5** and **6** were prepared by the same method, but in the absence of pyridine, to give products, bp 113–114° (2.5 mm) and 42° (0.75 mm), respectively. Tosylates **7–9** were prepared by Wiberg's method[28] using as reactant a suspension of powdered KOH in ether; products were extracted and chromatographed on alumina but could not be crystallized. Alcohols **10** and **11** were prepared from the corresponding picolines and paraformaldehyde;[29,30a] the products had bp 87–89° (2 mm) [lit.[29] bp 114–116° (9 mm)] and 121–122° (2 mm) [lit.[30a] 151–152° (13–14 mm)], respectively. 2-Phenethyl tosylate (**12**) and *p*-anisylethyl tosylate (**14**) were prepared by the method of Wiberg.[28] Nosylates **13**, **15**, and **17** were prepared by adding an ether solution of the corresponding alcohols to an ether solution of methyllithium (1 molar equiv) and adding an equivalent amount of *p*-nitrobenzenesulfonyl chloride.[4] Alcohol **18** was prepared by the sequence phenylacetonitrile → *p*-nitrophenylacetonitrile, mp 116° (lit.[30b] mp 116–117°), → *p*-nitrophenylacetic acid, mp 151–153° (lit.[31] mp 151–152°), → 2-(*p*-nitrophenyl)-ethanol. The corresponding tosylate (**16**) was prepared by Wiberg's method.[28] The preparation of the azulylpropanols and tosylates is detailed elsewhere[4,24] (the methylazulenes were converted to the carbanions and allowed to react with ethylene oxide to form the alcohols). 2-(2-Pyridyl)ethyl tosylate was a gift from Dr. G. Jones, University of Keele. The purity of all compounds was checked by nmr and in the few cases among the tosylates where impurities were present these did not contribute to the peaks of interest in the mass spectra.

Mass spectra were obtained by direct insertion for the tosylates and nosylates and by use of the heated introduction system in other cases. Spectra were obtained at 70 eV, 100 μA, and 8 kV on an AEI MS9 mass spectrometer. Ionization and appearance potentials were measured against benzene as internal standard using the semilog plot technique.

Registry No.—**1**, 2859-68-9; **2**, 2859-67-8; **3**, 2629-72-3; **4**, 38456-23-4; **5**, 38456-24-5; **6**, 38456-25-6; **7**, 38456-26-7; **8**, 38456-27-8; **9**, 38456-28-9; **10**, 103-74-2; **11**, 5344-27-4; **19**, 38456-31-4; **20**, 38456-32-5; **21**, 38305-10-1; **22**, 38456-34-7; **23**, 35046-09-4.

Acknowledgments.—We gratefully acknowledge support of this research through grants to R. N. M. from the National Science Foundation (GP-7818, GP-10691) and for funds to purchase the MS-9 mass spectrometer, and to the Phillips Petroleum Company for a fellowship to H. E. P. (1968–1969).

(28) K. B. Wiberg, B. R. Lowry, and T. H. Colby, *J. Amer. Chem. Soc.*, **83**, 3908 (1961).
(29) A. Ladenburg, *Justus Liebigs Ann. Chem.*, **301**, 117 (1898).
(30) (a) G. R. Robertson, "Organic Syntheses," Collect. Vol. I, Wiley, New York, N. Y., 1932, p 396; (b) *ibid.*, p 406.
(31) J. Meisenheimer, *Justus Liebigs Ann. Chem.*, **420**, 199 (1920).

The Study and Characterization of Nucleosides by Mass Spectrometry. III. Comparison between the Mass Spectra of Trimethylsilyl Derivatives of Purine 2′- and 3′-Linked Anhydro, Thioanhydro, and Aminoanhydro Nucleosides

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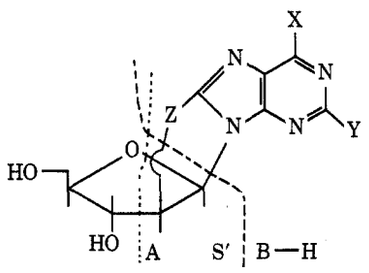
Received November 14, 1972

The mass spectra of trimethylsilyl derivatives of several purine O, S, and NH linked anhydro nucleosides were studied in order to determine the positional effects of 8,2′ *vs.* 8,3′ linkage of base to sugar. Principal ions in the spectra were common to both series of anhydro nucleosides but variations in intensity could be used to distinguish between them. These variations could be related to the anticipated ease of formation of the basic ion type from the skeleton of the molecule.

Cyclonucleosides are important analogs of natural nucleosides. They are characterized by having, in addition to the *N*-glycoside linkage, a covalent linkage, either directly or *via* bridging atoms, between the 2′, 3′, or 5′ carbons of the sugar and a carbon or nitrogen atom (other than the nitrogen of the glycoside bond) of the purine or pyrimidine ring. Anhydro nucleosides are cyclonucleosides in which the extra covalent linkage is *via* bridging atoms. Acid or base hydrolysis of the anhydro linkage normally leads to ribo, arabino, or xylo nucleosides.[1] Nucleophilic displacement of the anhydro linkage leads to nucleosides modified on the sugar moiety[1–3] and sometimes on the base moiety.[1,4–6] Chemical conversion of anhydro nucleosides has led to the synthesis of modified nucleosides such as the antitumor drug arabinocytidine[7,8] and the antibiotic cordycepin.[6] They have been used as model substrates for studies of enzyme activity[9,10] and as intermediates in the chemical synthesis of nucleotides.[11–13] Recently, the synthesis and properties of the dinucleoside monophosphate A^spA^s and its conversion to dApdA have been described.[14] In conjunction with our synthetic objectives it was necessary to develop methods of identifying small quantities of specific anhydro nucleosides and to estimate their purity. We have found mass

(1) J. J. Fox, *Pure Appl. Chem.*, **18**, 223 (1969).
(2) M. Imazawa, T. Ueda, and T. Ukita, *Tetrahedron Lett.*, 4807 (1970).
(3) J. F. Codington, I. L. Doerr, and J. J. Fox, *J. Org. Chem.*, **29**, 558 (1964).
(4) M. Ikehara and K. Muneyama, *Chem. Pharm. Bull.*, **18**, 1196 (1970).
(5) T. Ueda and S. Shibuya, *ibid.*, **18**, 1076 (1970).
(6) M. Ikehara, *Accounts Chem. Res.*, **2**, 47 (1969).
(7) T. Kanai, T. Kojima, O. Maruyama, and M. Ichino, *Chem. Pharm. Bull.*, **18**, 2569 (1970).
(8) K. Kikugawa and M. Ichino, *Tetrahedron Lett.*, 867 (1970).
(9) K. K. Ogilvie, L. A. Slotin, and P. Rheault, *Biochem. Biophys. Res. Commun.*, **45**, 297 (1971).
(10) K. K. Ogilvie and P. Rheault, unpublished results.
(11) K. K. Ogilvie and D. Iwacha, *Can. J. Chem.*, **48**, 862 (1970).
(12) M. Ikehara, S. Uesugi, and M. Yasumoto, *J. Amer. Chem. Soc.*, **92**, 4735 (1970).
(13) K. L. Nagpal and M. M. Dhar, *Tetrahedron Lett.*, 47 (1968).
(14) (a) S. Uesugi, M. Yasumoto, M. Ikehara, K. N. Fang, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **94**, 5480 (1972). (b) A^spA^s represented the dinucleoside monophosphate of 8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)adenine.

spectrometry to be an invaluable and powerful aid in these studies. For purine anhydro nucleosides a major difficulty lies in the distinction between the 8,2′- and 8,3′-linked isomers **1** and **2**.



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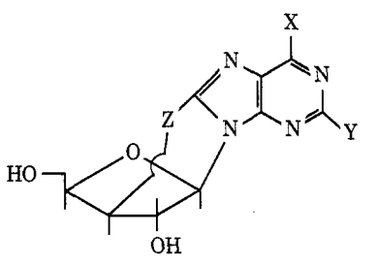


2

The isomers do not separate by paper chromatography or electrophoresis. Since gas chromatography of trimethylsilyl (TMS) derivatives of natural purine nucleosides has been achieved,[15] a gc–mass spectral method appears to hold future promise for identifying anhydro nucleosides. In this paper we report mass spectra obtained for TMS derivatives of several purine anhydro nucleosides with the objective of identifying basic ion types, emphasizing trends in relative intensities, and providing data which can be used to identify isomers or for analytical purposes.

Results and Discussion

Table I lists the purine anhydro nucleosides (and an abbreviation for each) presently available to us, together with their melting points, and also sample temperatures required for mass spectral analysis of the free compounds (when attempted) and of the TMS derivatives. Partial mass spectra of some O, S, and NH bridged 8,2′-, 8,3′- and 8,5′-anhydroadenosines,[16,17] certain of their *N*-methyl analogs,[17,18] and 8,2′-thioanhydroinosines[19] have been reported, as well as a molecular ion for 8,2′-SAnG.[20] No systematic interpretation of the spectra has been given. The mass spectra of the 8,2′- and 8,3′-linked isomers are either very similar,[16] or they show unexpected fragmentations[18] which reduce the possibility of distinguishing structural differences between them by mass spectrometry. Additionally, it has been found that the spectra of 2,2′- and 2,3′-anhydrouridines are very similar.[21] These observations probably arise from the polar nature of the molecules and the consequent high temperatures necessary to cause vaporization of the samples. (See Table I for temperatures required in our instrument for the free anhydro nucleosides.) These differences should be reduced by the preparation of volatile derivatives. We have found trimethylsilylation to be a satisfactory derivatization procedure. The method is simple and the mass spectra of these derivatives show abundant molecular ions. In addition, the TMS-d_9 derivatives are valuable aids in spectral interpretation. The deuterium shifts, combined with the availability of the various bases, largely compensate for the nonavailability of a high-resolution mass spectrometer. Under mild conditions (see Exprimental Section), one hydrogen of each OH and NH_2 group of sugar and base moieties was replaced by TMS. For compounds 8,2′-NHAnA and 8,2′-NHAnG the hydrogen of the bridging NH group was replaced at room temperature, but for 8,2′-NHAnI warming of the reaction mixture was required. (Acetylation is sometimes used to produce volatile derivatives. We note that the trimethylsilyl derivatives are probably preferable for compounds described here. We have found[22] that the di-*O*-acetyl derivatives of 2′- and 3′-linked anhydrouridines have indistinguishable mass spectra, possibly because of a thermally or electron-impact induced molecular rearrangement. On the other hand, the corresponding di-*O*-TMS derivatives have readily distinguishable spectra.[21,22])

The mass spectra we obtained for the free anhydroadenosines 8,2′-OAnA, 8,2′-SAnA, and 8,2′-NHAnA agreed well with those reported in the literature and will not be further discussed. The relative intensities of structurally significant ions for the TMS derivatives are shown in Tables II and III for the 2′- and 3′-linked compounds, respectively.[23] In many cases *m/e* 73 (Me_3Si^+) was the biggest peak, but, since the derivatives have different numbers and types of TMS-derived groups, it seemed preferable to ignore this ion when assigning the base peak. Thus its relative intensity is usually greater than 100 in Tables II and III. When this is done, the molecular ion often becomes the base peak. These spectra show many of the basic ion types observed in the mass spectra of TMS derivatives of pyrimidine cyclonucleosides[21,24,25] and natural purine nucleosides,[26] and in addition two additional ion types labelled below as A^+ and $(A + 13)^+$. In contrast to the previous spectra, the present spectra show much less extensive fragmentation and though characteristic ions in the spectra are of lower abundance they are of sufficient intensity to provide structural information. Except for trivial processes, metastable ions were generally absent from the spectra. Individual ion types will be discussed separately.

M^+ and $(M - CH_3)^+$.—Both ions are of high abundance in all spectra. Thus it is to be expected that the molecular ion will be easily recognized for TMS derivatives of compounds of these structure types. In

(15) A. M. Lawson, R. N. Stillwell, M. M. Tacker, K. Tsuboyama, and J. A. McCloskey, *J. Amer. Chem. Soc.*, **93**, 1014 (1971).
(16) M. Ikeda, Y. Tamura, and M. Ikehara, *J. Heterocycl. Chem.*, **7**, 1377 (1970).
(17) M. Kaneko, B. Shimizu, and M. Ikehara, *Tetrahedron Lett.*, 3113 (1971).
(18) M. Ikehara and H. Morisawa, *Chem. Pharm. Bull.*, **19**, 2593 (1971).
(19) M. Ikehara and M. Muraoka, *ibid.*, **20**, 550 (1972).
(20) M. Kaneko, M. Kimura, and B. Shimizu, *ibid.*, **20**, 635 (1972).
(21) S. Tsuboyama and J. A. McCloskey, *J. Org. Chem.*, **37**, 166 (1972).
(22) D. C. K. Lin, K. K. Ogilvie, and J. B. Westmore, unpublished results.
(23) Complete mass spectra may be obtained from one of the authors (J. B. W.) on request, and have also been submitted to the Mass Spectrometry Data Center, Aldermaston.
(24) J. B. Westmore, D. Lin, K. K. Ogilvie, H. Wayborn, and J. Berestiansky, *Org. Mass Spectrom.*, **6**, 1243 (1972).
(25) J. B. Westmore, D. Lin, and K. K. Ogilvie, *ibid.*, in press.
(26) J. A. McCloskey, A. M. Lawson, K. Tsuboyama, P. M. Krueger, and R. N. Stillwell, *J. Amer. Chem. Soc.*, **90**, 4182 (1968).

TABLE I.—COMPOUNDS STUDIED, MELTING POINTS, AND SAMPLE TEMPERATURES REQUIRED FOR MASS SPECTRAL ANALYSIS[d]

Compd	Abbreviation	Registry no.	X	Y	Z	Mp, °C	Sample temperature— Free compd	TMS deriv
8,2′-Anhydroadenosine	8,2′-OAnA	13089-44-6	NH_2	H	O	170 dec	170	47
8,2′-Thioanhydroadenosine	8,2′-SAnA	16667-76-8	NH_2	H	S	159–162	190	52
8,2′-Aminoanhydroadenosine	8,2′-NHAnA	33962-27-5	NH_2	H	NH	260 dec	215	80
8,2′-Anhydroinosine	8,2′-OAnI	38659-93-7	OH	H	O	232 dec		134
8,2′-Thioanhydroinosine	8,2′-SAnI	38659-94-8	OH	H	S	217 dec	dec[a]	115
8,2′-Aminoanhydroinosine	8,2′-NHAnI	38659-95-9	OH	H	NH	210 dec		44,[b] 134[c]
8,2′-Anhydroguanosine	8,2′-OAnG	38659-96-0	OH	NH_2	O	210 dec		109
8,2′-Thioanhydroguanosine	8,2′-SAnG	38659-97-1	OH	NH_2	S	256 dec	dec[a]	86
8,2′-Aminoanhydroguanosine	8,2′-NHAnG	38659-98-2	OH	NH_2	NH	203 dec		109
8,2′-Thioanhydroxanthosine	8,2′-SAnX	38659-99-3	OH	OH	S	173 dec	dec[a]	115
8,3′-Anhydroadenosine	8,3′-OAnA	28234-87-9	NH_2	H	O	266–267		40
8,3′-Thioanhydroadenosine	8,3′-SAnA	16667-78-0	NH_2	H	S	166–168		75
8,3′-Anhydroinosine	8,3′-OAnI	38660-01-4	OH	H	O	229 dec		62
8,3′-Thioanhydroinosine	8,3′-SAnI	38660-03-6	OH	H	S	218 dec		35
8,3′-Thioanhydroguanosine	8,3′-SAnG	38660-04-7	OH	NH_2	S	183 dec		131

[a] No spectrum could be obtained; upper temperature reached, 300°. [b] Tetrakis TMS derivative. [c] Tris TMS derivative. [d] Refer to structures 1 and 2 for locations of groups X, Y, and Z.

TABLE II.[b]—RELATIVE INTENSITIES OF SELECTED IONS IN THE MASS SPECTRA OF TMS DERIVATIVES OF PURINE 8,2′-ANHYDRO NUCLEOSIDES

Ion or *m/e*	OAnA(TMS)$_3$	SAnA(TMS)$_3$	NHAnA(TMS)$_4$	OAnI(TMS)$_3$	SAnI(TMS)$_3$	NHAnI(TMS)$_3$	NHAnI(TMS)$_4$	OAnG(TMS)$_4$	SAnG(TMS)$_4$	NHAnG(TMS)$_5$	SAnX(TMS)$_4$
M^+	481/100	497/100	552/100	482/35	498/47	481/46	553/66	569/100	585/100	640/100	586/100
$(M - CH_3)^+$	466/37	482/35	537/27	467/20	483/16	466/14	538/13	554/35	570/28	625/22	571/48
$(A + 30 + TMS)^+$	350/18	366/4	421	351/26	367/16	350	422	438/7	454/<1	509	455
$(A + TMS)^+$	320/3	336/3	391/1	321/10	337/18	320/8	392/4	408/3	424/2	479	425/6
$(A + 31)^+$	278/2	294	349	279/11	295	278	350	366	382/2	437	383
$(A + 13)^+$	260/29	276/6	331/4	261/16	277/11	260/10	332	348/7	364/1	419	365/4
$(A + H)^+$	248/8	264/15	319/13	249/20	265/43	248/28	320/28	336/7	352/5	407	353/23
A^+	247	263/3	318/9	248/19	264/10	247/16	319/16	335	351	406	352
$(B + HTMS)^+$	296/7	312/1	367	297/13	313/3	296/3	368	384/3	400	455	401/2
$(B + TMS)^+$	295/11	311/3	366	296/25	312/8	295/7	367	383/5	399	454	400/3
$(B + HTMS - CH_3)^+$	281/4	297	352	282/6	298	281	353/14	369/2	385	440	386/1
$(B + TMS - CH_3)^+$	280/15	296	351/1	281/13	297	280	352/7	368/4	384	439	385/2
$(B + 2H)^+$	224/5	240/5	295/3	225/13	241/8	224	296/11	312/3	328/1	383/27	329/8
$(B + H)^+$	223/10	239/16	294/8	224/9	240/17	223	295/26	311/7	327/3	382/82	328/22
B^+	222/8	238/19	293	223/16	239/18	222	294	310/5	326/2	381	327/9
$(B - H)^+$	221/5	237/19	292	222/10	238/48	221	293	309/4	325/3	380	326/22
$(S' - H)^+ \equiv 259$	14	13	7	69	53	16	51	9	1	6	13
$(S' - 2H)^+ \equiv 258$	5	2		13		5	7	4	<1	9	2
$(S' - 17)^+ \equiv 243$	7	3	3	14			11	8	1	16	3
$(S' - 43)^+ \equiv 217$	9	21	28	33	66	100[a]	100[a]	25	3	20	20
189	8	12	7	18	32	22	13	5	3		10
169	19	6	2	16	23	15	20	13	2	42	8
147	15	12	22	57	120	25	39	28	8	73	74
103	54	35	30	100[a]	100[a]	92	87	24	9	13	39
75	16	11	13	220	135	82	53	82	5	191	28
73	156	112	157	305	495	208	303	84	44	170	198
$\%\Sigma_{50}^{M}$	9.73	11.91	12.91	1.39	2.00	2.32	1.98	7.94	22.14	3.72	8.54

[a] Used as base peak when M^+ is not base peak. [b] The data presented are *m/e*/rel intensity (upper part of table); rel intensity (lower part of table).

TABLE III[a]

RELATIVE INTENSITIES OF SELECTED IONS IN THE MASS SPECTRA OF TMS DERIVATIVES OF PURINE 8,3′-ANHYDRO NUCLEOSIDES

Ion or *m/e*	OAnA(TMS)$_3$	OAnI(TMS)$_3$	SAnA(TMS)$_3$	SAnI(TMS)$_3$	SAnG(TMS)$_4$
M^+	481/100	482/100	497/100	498/100	585/100
$(M - CH_3)^+$	466/26	467/68	482/47	483/39	570/38
$(A + 30 + TMS)^+$	350/45	351/92	366/8	367/35	454/6
$(A + TMS)^+$	320/1	321/7	336	337/5	424
$(A + 31)^+$	278/3	279	294/9	295/5	382/8
$(A + 13)^+$	260/62	261/40	276/3	277/8	364
$(A + H)^+$	248/4	249/11	264	265	352/4
A^+	247/2	248/3	263	264	351/2
$(B + 2H)^+$	224/4	225/19	240/5	241/15	328/7
$(B + H)^+$	223/9	224/12	239/9	240/9	327/11
B^+	222/16	223/29	238/16	239/26	326/6
$(B - H)^+$	221/4	222/13	237/5	238/13	325
$(S' - H)^+ \equiv 259$	6	39	3	12	5
$(S' - 2H)^+ \equiv 258$	4	61	4	31	4
$(S' - 17)^+ \equiv 243$	6	28	9	30	15
$(S' - 43)^+ \equiv 217$	2	18		3	8
189	2	6			
169	13	47	5	17	9
147	10	22	6	14	32
103	19	46	22	23	16
75	21	37	15	55	105
73	156	254	89	181	131
$\%\Sigma^{M}_{50}$	10.52	4.52	11.89	4.94	5.29

[a] The data presented are *m/e*/rel intensity (upper part of table); rel intensity (lower part of table).

TABLE IV[a]

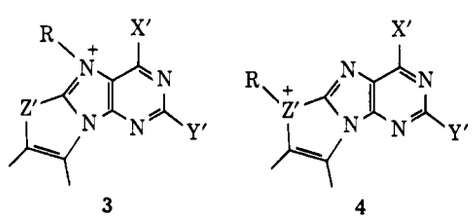
COMPARISON OF RELATIVE INTENSITIES OF SELECTED IONS IN THE MASS SPECTRA OF TMS DERIVATIVES OF PURINE 8,2′- AND 8,3′-ANHYDRO NUCLEOSIDES

Ion	Anticipated magnitude	OAnA(TMS)$_3$	OAnI(TMS)$_3$	SAnA(TMS)$_3$	SAnI(TMS)$_3$	SAnG(TMS)$_4$
$(A + H)^+$	2′ > 3′	8/4	20/11	15/—	43/—	5/4
$(A + TMS)^+$	2′ > 3′	3/1	10/7	3/—	18/5	2/—
A^+	2′ > 3′	—/2	19/3	3/—	10/—	—/2
$(A + 31)^+$	3′ > 2′	2/3	11/—	—/9	—/5	2/8
$(A + 30 + TMS)^+$	3′ > 2′	18/45	26/92	4/8	16/35	1/6
$(A + 13)^+$	3′ > 2′	29/62	16/40	6/3	11/8	1/—
$(S' - H)^+$	2′ > 3′	14/6	69/39	13/3	53/12	1/5
$(S' - 2H)^+$	3′ > 2′	5/4	13/61	2/4	—/31	1/4
$(S' - 17)^+$	3′ > 2′	7/6	14/28	3/9	—/30	1/15
$(S' - 43)^+$	2′ > 3′	9/2	33/18	21/—	66/3	3/8

[a] The data given are rel intensity for 2′-linked isomer/rel intensity for 3′-linked isomer.

doubtful cases, comparison of spectra with those of TMS-d_9 derivatives would remove possible ambiguities in determining whether the highest observed value of *m/e* is due to M^+ or $(M - CH_3)^+$. We note that the extent of fragmentation of the molecular ion, as emphasized by the percentage of the total ion current above *m/e* 50 carried by the molecular ion, *i.e.*, the $\%\Sigma^{M}_{50}$ values given in Tables II and III, is significantly greater for the anhydroinosines than for the other compounds.

(A + H)$^+$ and (A + TMS)$^+$.—These ions are characteristic of 2′-linked anhydro nucleosides of both pyrimidine and purine types. Structural analogs have been proposed previously[21,24,25] and invoke hydrogen or TMS transfer to the fused based plus anhydro-ring moiety to produce the well-stabilized ions **3** and **4**.



3 **4**

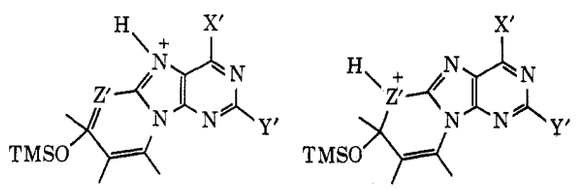
R = H or TMS
X′, Y′, Z′ = TMS derived function of X, Y, Z

The hydrogen transferred comes partially from the TMS groups and partially from the ribose skeleton, the ratio depending upon the nature of the base moiety and of the heteroatom of the anhydro linkage. Comparison of the spectra of the TMS and TMS-d_9 derivatives gives the following approximate values for the percentage of hydrogen transferred from the TMS groups under the instrumental conditions given in the Experimental Section: 8,2′-OAnA (50%), 8,2′-SAnA (50%), 8,2′-NHAnA (50%), 8,2′-SAnI (40%), 8,2′-NHAnI (50%), 8,2′-SAnG (20%).

Ions of the same mass as $(A + H)^+$ and $(A + TMS)^+$ are also present in the mass spectra of the 3′-linked isomers (Table III). Formation of these ions from the 3′-linked compounds presumably requires a ring

contraction and their formation should represent a less favorable fragmentation process. This expectation is supported by the data of Table IV, which directly compares the intensities of these ions for those nucleotides for which both isomers are available.

(A + 31)$^+$ and (A + 30 + TMS)$^+$.—Structural analogs of these ions have been observed in the mass spectra of pyrimidine[21,24,25] and purine[16,17,19] 2′- and 3′-anhydro nucleosides and TMS derivatives of pyrimidine[21,24,25] 2′- and 3′-anhydro nucleosides. For the 2′-linked compounds we have previously proposed[24] two requirements for the formation of these ions: (i) ring expansion to incorporate the $C_{3'}$ atom into a six-membered ring, and (ii) migration of hydrogen to the base moiety or heteroatom of the anhydro linkage to give the well-stabilized ions **5** and **6**.



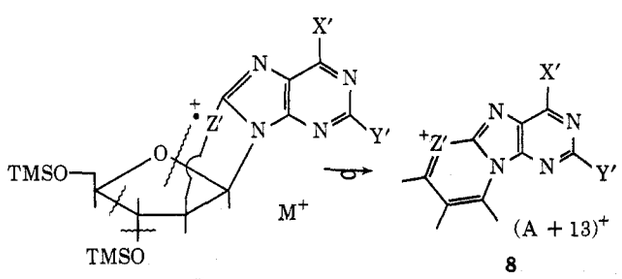
5 **6**

X′, Y′, Z′ = TMS derived function of X, Y, Z

For the 2′-linked compounds these ions are of generally low abundance (Table II) and have their highest intensities for the O-linked compounds. This implies that the proposed ring expansion required for these ions occurs more readily when O rather than S or NH is present in the anhydro linkage.

For the 3′-linked compounds the (A + 31)$^+$ and (A + 30 + TMS)$^+$ ions can be formed from the molecular ion without the necessity for ring expansion. We anticipate that their intensities should be higher for the 3′- than for the 2′-linked isomers. With one exception the data of Table IV support this view.

(A + 13)$^+$.—Comparison of the spectra of the TMS and TMS-d_9 derivatives of compounds containing the different bases identifies an ion which contains the base moiety with its TMS substituent(s) and the heteroatom of the anhydro linkage, and which can only be reasonably represented as (A + 13)$^+$. For the 2′-linked compounds the only logical route to its formation therefore involves fission of the $C_{3'}$–$C_{4'}$ bond and incorporation of the $C_{3'}$ and $H_{3'}$ atoms into the ion product.



7 **8**

A ring expansion is invoked which appears to occur more readily for the O-linked than for the NH- or S-linked compounds (Table II). This trend parallels that found for the formation of the (A + 31)$^+$ and (A + 30 + TMS)$^+$ ions. Because a ring expansion is not required we anticipated that this ion would have enhanced intensities for the 3′-linked compounds, but the data of Table IV are inconclusive, though in the expected sense for those ions of high intensity. Structural analogs of this ion have not been previously discussed, though examination of reported spectra reveals its presence at (M − 77)$^+$ for several free purine anhydro nucleosides.[16–19] In the case of 8,3′-anhydro-8-oxy-9β-D-xylofuranosyl-N^6-dimethyladenine this peak may have been misinterpreted.[18]

A$^+$.—This odd-electron ion is presumably stable because of the extended π system of the base. It has not been observed for pyrimidine anhydro nucleosides and is of generally low intensity here, except for some of the 2′-linked anhydroinosines. As anticipated, it is usually more abundant for the 2′- than for the 3′-linked isomers. Analogs of this ion can be found at (M − 90)$^+$ in reported spectra of purine anhydro nucleosides.[16–19]

(B − H)$^+$, B$^+$, (B + H)$^+$, (B + 2H)$^+$.—Comparison of the relative intensities of the ions in this group reveals that hydrogen transfer to the base moiety is not so extensive as for the pyrimidine analogs.[24,25] In particular, (B − H)$^+$ is sometimes prominent in this group, where it is usually insignificant for the pyrimidine compounds.

(B + TMS)$^+$, (B + HTMS)$^+$, (B + TMS − CH$_3$)$^+$, and (B + HTMS − CH$_3$)$^+$.—These low-intensity ions, whose compositions are supported by appropriate mass shifts in the spectra of the TMS-d_9 derivatives, have been observed in the spectra of TMS derivatives of purine nucleosides[26] and nucleotides,[27] but have not been previously reported for TMS derivatives of anhydro nucleosides.

(S′ − H)$^+$, (S′ − 2H)$^+$, and (S′ − 17)$^+$.—These ions, which are characteristic of the sugar moiety, occur at *m/e* 259, 258, and 243, respectively, and their mechanistic origins have been previously discussed.[21,24,25] Although the reason is not clear, the intensity of *m/e* 258 is greatly enhanced for 2,3′-anhydrouridine with respect to 2,2′-anhydrouridine,[21] as is the intensity of (S′ − 17)$^+$, which can be formed from (S′ − 2H)$^+$ by loss of a methyl group. Table IV shows that this observation generally holds for the purine anhydro nucleosides as well.

(S′ − 43)$^+$.—The structure of this ion, *m/e* 217, has been discussed previously.[21,24,27–29] In agreement with the requirement that it contain three skeletal carbon atoms its intensity is generally greater in the spectra of the 2′-linked than the 3′-lined anhydro nucleosides (Table IV) in spite of the known tendency for trimethylsilyl group migration.[21,24,26,28–33]

Other Ions.—The remaining prominent ions in most spectra, *i.e.*, *m/e* 189, 169, 147, 103, 75, and 73, are

(27) A. M. Lawson, R. N. Stillwell, M. M. Tacker, K. Tsuboyama, and J. A. McCloskey, *J. Amer. Chem. Soc.*, **93**, 1014 (1971).
(28) D. C. DeJongh, T. Radford, J. D. Hribar, S. Hanessian, M. Bieber, G. Dawson, and C. C. Sweeley, *ibid.*, **91**, 1728 (1969).
(29) M. Zinbo and W. R. Sherman, *ibid.*, **92**, 2105 (1970).
(30) G. Peterson, *Tetrahedron*, **26**, 3413 (1970).
(31) S. Karady and S. H. Pines, *ibid.*, **26**, 4527 (1970).
(32) E. White, S. Tsuboyama, and J. A. McCloskey, *J. Amer. Chem. Soc.*, **93**, 6340 (1971).
(33) G. H. Draffan, R. N. Stillwell, and J. A. McCloskey, *Org. Mass Spectrom.*, **1**, 669 (1968).

either ribose- or TMS-derived fragments and have been discussed previously.[21,24,25]

Conclusion

In Table IV are listed for comparison the relative intensities of those ions expected to distinguish between 2′- and 3′-linked isomers. The general trends can be used to differentiate between the isomers, but, because of exceptions in the relative intensities in a few cases, caution must be exercised and all the ions listed in Table IV should be considered. The data can be used to identify isomers if a gas chromatographic separation can be achieved and to enable predictions to be made on the general form of the mass spectra of isomers not yet prepared. However, the positive identification of a single unknown isomer from the mass spectral data alone would remain uncertain.

Experimental Section

The mass spectra were recorded on a Hitachi RMU-6D single-focusing mass spectrometer using published procedures,[24] at an electron energy of 50 V and a nominal 900-V ion-accelerating energy. Trimethylsilyl derivatives were prepared[24] by treating 0.5 mg of a sample with 100 μl of BSTFA and 15 μl of TMCS (Pierce Chemical Co.). Complete trimethylsilylation (*i.e.*, one hydrogen of each OH, NH, or NH_2 group of sugar and base moieties was replaced by a TMS group[34]) was achieved overnight at room temperature in all cases except for 8,2′-NHAnI. Three TMS groups were incorporated at room temperature but four were incorporated by heating to 60° for 30 min. For the preparation of the TMS-d_9 derivatives BSA-d_{18} and TMCS-d_9 (Merck Sharpe and Dohme, Montreal) were used.

Syntheses of the compounds have been previously described: 8,2′-OAnA,[35,36] 8,2′-SAnA,[36-39] 8,2′-NHAnA,[17,39] 8,2′-OAnI,[40] 8,2′-SAnI,[19,20,38,39] 8,2′-NHAnI,[39] 8,2′-SAnG,[20,38,39,41] 8,2′-NHAnG,[39] 8,2′-SAnX,[20,40] 8,3′-OAnA,[35] 8,3′-OAnI,[40] 8,3′-SAnA,[37] 8,3′-SAnI,[19,40] 8,3′-SAnG.[42]

Acknowledgment.—We thank the National Research Council of Canada for financial support of this work.

(34) A. E. Pierce, "Silylation of Organic Compounds," Pierce Chemical Co., Rockford, Ill., 1968.
(35) M. Ikehara and M. Kaneko, *Chem. Pharm. Bull.*, **18**, 2401 (1970).
(36) M. Ikehara and S. Tezuka, *Tetrahedron Lett.*, 1169 (1972).
(37) M. Ikehara and M. Kaneko, *Tetrahedron*, **26**, 4251 (1970).
(38) K. K. Ogilvie, L. A. Slotin, J. B. Westmore, and D. C. K. Lin, *Can. J. Chem.*, **50**, 2249 (1972).
(39) K. K. Ogilvie, L. A. Slotin, J. B. Westmore, and D. C. K. Lin, *J. Heterocycl. Chem.*, **9**, 1179 (1972).
(40) M. Ikehara, *Chem. Pharm. Bull.*, **8**, 367 (1960).
(41) K. K. Ogilvie, L. A. Slotin, J. B. Westmore, and D. C. K. Lin, *Can. J. Chem.*, **50**, 1100 (1972).
(42) K. K. Ogilvie, L. A. Slotin, D. C. K. Lin, and J. B. Westmore, *ibid.*, **50**, 3276 (1972).

Noncoordinating Buffers. I. Synthesis and Characterization of Water-Soluble Derivatives of 2,6-Di-*tert*-butylpyridine

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Received October 11, 1972

Several derivatives of 2,6-di-*tert*-butylpyridine, containing alkyl, trimethylammonium [$-N(CH_3)_3^+$], or dimethylammonium [$-NH(CH_3)_2^+$] substituents, have been prepared and characterized. The mechanism of their synthesis, their water solubilities, pK_a's, and possible utilization as noncoordinating buffers and nonnucleophilic bases are briefly discussed.

Studies of reactions of metal ions in aqueous solution are limited by the fact that all common Lewis bases that may be used for buffering action will also coordinate to metals, making it impossible to investigate the behavior of uncomplexed metal ions in buffered media. In a search for water-soluble noncoordinating buffers, *i.e.*, weak bases that may donate their electron pair to a proton but not to a metal ion, several dimethylaminopyridines and quaternary ammonium pyridines with 2- and 6-*tert*-butyl substituents were prepared. The pyridine nitrogen of these compounds can coordinate to a proton, but is too sterically shielded to coordinate to Lewis acids even as small as CH_3^+ or BF_3.[1] This ensures that the title compounds will not coordinate to metal ions and that they will also have very low nucleophilic activity. It is now well known that the commonly used buffer 2,6-lutidine is not sufficiently shielded to prevent its coordination to metal ions.[2]

Results and Discussion

Synthesis.—The reaction of an alkyllithium compound with pyridine provides a direct method for the introduction of alkyl substituents ortho to the pyridine nitrogen. Since the original work of Ziegler and Zeiser[3] this reaction has been extensively utilized and studied, the intermediacy of a *N*-lithio 1,2-dihydropyridine derivative having been established.[4-6] In the presence of excess *tert*-butyllithium, pyridine is converted to 2,4,6-tri-*tert*-butylpyridine in one synthetic step.[7] Employing this one-step procedure, we have synthesized several *tert*-butylated pyridines (see Table I). Presumably these reactions proceed in a stepwise matter, there being enough *tert*-butyllithium present at the decomposition of the first dihydro derivative(s) so that subsequent reaction can occur.

In addition to alkylating pyridine bases in the above manner, an alkyllithium may also metalate either the

(1) H. C. Brown and B. Kanner, *J. Amer. Chem. Soc.*, **88**, 986 (1966).
(2) (a) J. R. Allen, D. H. Brown, R. H. Nuttal, and D. W. A. Sharp, *J. Inorg. Nucl. Chem.*, **26**, 1895 (1964); **27**, 1305 (1965). (b) 2,6-Lutidine coordinates to the pentaammineruthenium(II) center in dilute aqueous solution: Professor H. Taube, Stanford University, private communication (1972).
(3) K. Ziegler and H. Zeiser, *Ber.*, **63**, 1847 (1930).
(4) R. Foster and C. A. Fyfe, *Tetrahedron*, **25**, 1489 (1969).
(5) R. A. Abramovitch and G. A. Poulton, *Chem. Commun.*, 274 (1967).
(6) C. S. Giam and J. L. Stout, *ibid.*, 142 (1969).
(7) F. V. Scalzi and N. F. Golob, *J. Org. Chem.*, **36**, 2541 (1971).