Chemical Ionization Mass Spectrometry of Nucleosides. Mechanisms of Ion Formation and Estimations of Proton Affinity

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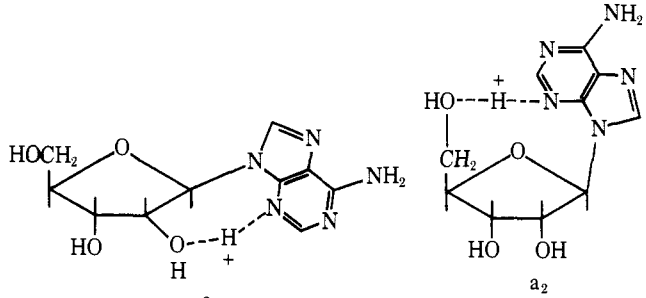
Abstract: Chemical ionization mass spectra of a number of nucleosides, their analogs, and purine and pyrimidine bases have been studied using CH_4, i-C_4H_{10}, NH_3, $MeNH_2$, Me_2NH, and Me_3N reagent gases at approximately 0.4 Torr pressure. In addition to protonated molecular species, the principal ion product from nucleosides was base (b) + 2H and, in lesser amounts, b + 30 and b + 44 in analogy to electron ionization spectra. Based in part on isotopic labeling, the bH_2 species was concluded to arise preferentially by initial base protonation followed by transfer of hydrogen from O-2′ with cleavage of the glycosidic bond. Proton affinity limits of 13 bases and 8 nucleosides were estimated by the mass spectrometric bracketing technique based on occurrence of proton transfer from reagent gases of known proton affinity. Orders of basicity obtained for the common bases and nucleosides were cytosine, adenine, guanine > hypoxanthine, purine > uracil, thymine; and cytidine, adenosine > uridine, thymidine. Nucleosides were found to be more basic in the gas phase than the corresponding base, a reversal of the order in aqueous solution.

In the past few years, chemical ionization mass spectrometry[1] has undergone remarkable growth in terms of analytical and structural applications[2] and has clearly been established as a technique which is more complementary to, rather than competitive with, electron ionization mass spectrometry. In addition to the widely recognized characteristic of enhanced molecular weight-related ion abundance, chemical ionization is potentially useful for organic structural studies because of the possibility for functional group selectivity,[3] and measurement of proton affinity (e.g., ref 4 and 5). In view of previous studies of the electron ionization mass spectra of nucleosides and their successful use in the determination of nucleoside structure,[6,7] we have carried out a systematic investigation of the chemical ionization mass spectra of nucleosides. Reagent gases which exhibit a range of proton affinities were used, with a goal of establishing the characteristics and mechanisms of the reactions involved and measuring the proton-affinity limits of free nucleosides in the gas phase. Earlier preliminary communications have dealt with the general features of nucleoside chemical ionization spectra,[8] and with comparison of gas and condensed phase hydrolysis of the nucleoside glycosidic bond after protonation.[9]

Discussion of Mass Spectra

Reagent gases which were studied were CH_4, i-C_4H_{10}, NH_3, Me_2NH, and several of their isotopically labeled analogs. A typical spectrum is represented by that of adenosine determined with NH_3 reagent gas, shown in Figure 1. The ion current is distributed between two principal species, the protonated molecule (MH) and the base fragment (b) plus two hydrogens. This general appearance in which most base-containing ions and other ions of low mass are of low abundance is also found in the corresponding CH_4,[8,10] H_2O,[11] and D_2O,[11,12] reagent gas spectra of adenosine, as well as the spectrum of 2′-deoxyadenosine.[13] A similar pattern is exhibited by chemical ionization spectra produced from other gases and nucleosides, as indicated in Table I. The relative abundance of MH is directly related to the difference in proton affinities between reagent gas and nucleoside and can therefore be controlled by suitable choice of reagent gas. This trend is demonstrated by the MH abundance data from adenosine shown in Table II, with the exception of the close but reversed values from NH_3 and $MeNH_2$. As the basicity of reagent gas increases, the protonation reaction becomes less exothermic, with the result that less energy is available for decomposition of MH. Numerous opportunities for intramolecular proton sharing in the molecular ion exist, e.g., a_1 and a_2, but, unlike the simpler case with aliphatic diols, diamines, and dithiols,[16] the effect upon MH abundance cannot be easily predicted. As pointed out by Weinkam,[17] not only the enthalpy of protonation but also the steric configuration of the protonated species is influential in determining the extent and routes of fragmentation.



a_1 a_2

In cases in which the proton affinity of the nucleoside is less than that of the reagent gas, proton transfer will not occur but the molecular mass can be readily deduced from the collision-stabilized adduct which forms instead: MCH_3, MC_2H_5, and MC_3H_5 from CH_4; MNH_4 from NH_3; MC_3H_7 and MC_4H_9 from i-C_4H_{10}; and MH_2NMe_2 from $HNMe_2$. As shown in Table I, both adduct and protonated molecular species are formed in many cases, thus further assisting in correct identification of the molecular mass. The ratio MH/M·adduct was observed to change inversely with temperature with any given nucleoside, as expected,[18] and is therefore in general not reproducible in the sense of electron ionization spectra.

In most instances the molecular mass of a nucleoside can be satisfactorily established from its electron ionization spectrum since molecular ion relative intensities generally fall in the range 5–15%, and the mass value of M can usually be established indirectly from fragment ions. However, in some cases such as many purine nucleosides,[19] or dihydrouridine,[7,19] the molecular ion may be absent, and so chemical ionization may offer a distinct advantage. One

Table I. Selected Ions from the Chemical Ionization Mass Spectra of Nucleosides

Nucleoside	Reagent gas	(m/e) Rel abundance[a] MH	bH_2	c[b]	d	MR[c]	Other ions
Adenosine	CH_4	(268) 92	(136) 100	(164) 27	(178) 5.0	9.5/4.0	(135) 8.3, (150) 3.5, (176) 2.0
	Me_2NH	(268) 100	(136) 10	(164)[d]	(178)[d]	*d*	
1-Methyladenosine[e]	CH_4	(282) 52	(150) 100	(178) 26	(192) 5.0	3.0/*d*	(149) 44, (164) 6.0, (281) 6.5
	NH_3	(282) 6.0	(150) 100	(178) 1.5	(192) 1.5	*d*	(135) 5.0, (136) 4.5
	Me_2NH	(282) 100	(150) 80	(178)[d]	(192)[d]	*d*	
N^6-Methyladenosine	CH_4	(282) 100	(150) 100	(178) 47	(192) 14	18.5/4.5	(149) 30, (164) 16, (296) 8.5
	NH_3	(282) 100	(150) 64	(178) 3.0	(192) 5.0	*d*	(136) 5.5, (149) 4.0
	Me_2NH	(282) 100	(150) 9.0	(178)[d]	(192) 1.0	*d*	(195) 4.0
N^6,N^6-Dimethyladenosine	CH^4	(296) 100	(164) 52	(192) 20	(206) 4.0	15/3.5	(134) 4.0, (178) 5.5, (295) 8.0
	NH_3	(296) 100	(164) 20	(192) 2.0	(206) 2.0	*d*	
	Me_2NH	(296) 100	(164) 14	(192) 2.5	(206) 3.0	*d*	
N^6,N^6-Dimethyl-3'-amino-3'-deoxyadenosine	CH_4	(295) 100	(164) 96	(192) 35	(206) 11	12/5.3	(134) 8.2, (178) 4.0, (222) 5.0
	NH_3	(295) 100	(164) 45	(192) 3.5	(206) 2.5	*d*	
	Me_2NH	(295) 100	(164) 7.5	(192)[d]	(206)[d]	*d*	
N^6-(3-Methyl-2-butenyl)-adenosine	NH_3	(336) 100	(204) 76	(232) 3.5	(246) 8.0	*d*	(136) 34, (268) 19, (338) 12
	Me_2NH	(336) 100	(204) 2.3	(332)[d]	(246)[d]	*d*	(268) 3.0
2'-*O*-Methyladenosine	CH_4	(282) 100	(136) 41	(164) 16	(192) 7.0	14/4.0	(146) 3.5, (251) 3.5, (280) 5.5
	Me_2NH	(282) 100	(136)[d]	(164)[d]	(192)[d]	*d*	(181) 2.5
4'-Thioadenosine	CH_4	(284) 23	(136) 100	(180) 5.0	(178) 7.0	2.0/1.0	(150) 6.0, (164) 20, (253) 3.5
	NH_3	(284) 13	(136) 100	(180)[d]	(178) 7.0	*d*	
	Me_2NH	(284) 100	(136) 2.7	(180)[d]	(178)[d]	*d*	(181) 1.5
2'Deoxy-α-adenosine	CH_4	(252) 44	(136) 100	(164) 22	(162) 6.8	1.5/1.0	(99) 7.8, (117) 6.0, (150) 4.0
	NH_3	(252) 100	(136) 20	(164)[d]	(162) 3.5	*d*	
	Me_2NH	(252) 100	(136) 2.3	(164)[d]	(162)[d]	*d*	(181) 4.0
2'-Deoxy-β-adenosine	CH_4	(252) 31	(136) 100	(164) 23	(162) 5.8	2.5/2.0	(99) 7.5, (117) 5.0, (150) 3.0
	NH_3	(252) 100	(136) 20	(164)[d]	(162) 2.8	*d*	
	Me_2NH	(252) 100	(136) 3.3	(164)[d]	(162)[d]	*d*	(181) 3.5
3'-Deoxyadenosine	Me_2NH	(252) 100	(136)[d]	(164)[d]	(178)[d]	*d*	(181) 1.5
Cytidine[e]	NH_3	(244) 9.5	(112) 100	(140) 2.5	(154) 1.0	*d*	(151) 3.0
	Me_2NH	(244) 42.5	(112) 100	(140)[d]	(154) 1.0	(289) 4.5	(126) 3.7
α-Cytidine[e]	CH_4	(244) 5.0	(112) 100	(140) 9.5	(154) 2.0	*d*	(151) 6.0
	NH_3	(244) 15	(112) 100	(140)[d]	(154) 3.5	*d*	
	Me_2NH	(244) 26	(112) 8.8	(140)[d]	(154) 3.0	(289) 35	(157) 100
Uridine	NH_3	(245) 100	(113) 9.0	(141)[d]	(155) 1.7	(252) 65	(130) 11, (150) 4.5
	Me_2NH	(245)[d]	(113)[d]	(141)[d]	(155)[d]	(290) 100	
α-Uridine	CH_4	(245) 5.0	(113) 100	(141) 8.8	(155) 3.0	*d*	(127) 4.5, (133) 8.2, (153) 3.5
	NH_3	(245) 100	(113) 7.0	(141)[d]	(155)[d]	(262) 23	(130) 4.5, (150) 2.5
	Me_2NH	(245)[d]	(113)[d]	(141)[d]	(155)[d]	(290) 100	
α-5,6-Dihydrouridine	CH_4	(247) 65	(115) 28	(143)[d]	(157) 100	14/3.5	(72) 45, (140) 52, (230) 41
	NH_3	(247) 100	(115) 1.5	(143) 2.5	(157) 12	(264) 22	(72) 14, (89) 19
	Me_2NH	(247) 100	(115) 4.0	(143)[d]	(157)[d]	(292) 100	
β-5,6-Dihydrouridine	NH_3	(247) 100	(115) 4.0	(143)[d]	(157) 9.0	(264) 9.5	(89) 3.5, (132) 4.5, (140) 1.5
	Me_2NH	(247)[d]	(115)[d]	(143)[d]	(157)[d]	(292) 100	
2'-*O*-Methyluridine	CH_4	(259) 9	(113) 100	(141) 18.5	(169) 5.0	*d*	(147) 23, (153) 4.2, (227) 1.5
	NH_3	(259) 100	(113) 12	(141)[d]	(269) 1.5	(266) 47	(130) 4.5, (146) 4.0, (164) 5.5
	Me_2NH	(259)[d]	(113)[d]	(141)[d]	(169)[d]	(304) 100	
α-Pseudouridine	CH_4	(245) 100	(113) 3.0	(141) 7.0	(155) 78	14/*d*	(179) 13, (209) 51, (227) 5.5
	NH_3	(245) 100	(113)[d]	(141)[d]	(155) 20	(262) 20	(172) 34, (185) 5.5, (202) 7.0
	Me_2NH	(245)[d]	(113)[d]	(141)[d]	(155)[d]	(290) 100	(200) 3.0, (254) 5.0
β-Pseudouridine	CH_4	(245) 64	(113) 28	(141) 19	(155) 100	10/2.0	(127) 11, (179) 20, (209) 54
	NH_3	(245) 100	(113)[d]	(141) 2.5	(155) 33	(262) 12	(172) 39, (185) 7.5, (209) 4.5
Thymidine	NH_3	(243) 100	(127) 26	(155)[d]	(153)[d]	(260) 4.2	(117) 5.0, (134) 2.0, (144) 2.5
	Me_2NH	(243)[d]	(127)[d]	(155)[d]	(153)[d]	(288) 100	

Table I (*Continued*)

Nucleoside	Reagent gas	(*m/e*) Rel abundance[a] MH	bH_2	c[b]	d	MR[c]	Other ions
5,6-Dihydrothymidine	CH_4	(245) 100	(129) 44	(157) 18	(155) 14	5.0/3.0	(99) 27, (117) 96, (227) 14
	NH_3	(245) 100	(129) 6.0	(157)[d]	(155) 2.5	(262) 6.0	(99) 1.5, (117) 2.0, (146) 8.5
	Me_2NH	(245)[d]	(129)[d]	(157)[d]	(155)[d]	(290) 100	

[a] Reagent gas ions omitted. [b] Ion c may occur at the same nominal mass as b + H + C_2H_5 in CH_4 reagent-gas spectra. [c] Adduct ions: CH_4 reagent gas, R = C_2H_5/C_3H_5, *m/e* omitted; NH_3 reagent gas, R = NH_4; Me_2NH reagent gas, R = Me_2NH. [d] A <1% relative intensity. [e] Partial pyrolysis during vaporization.

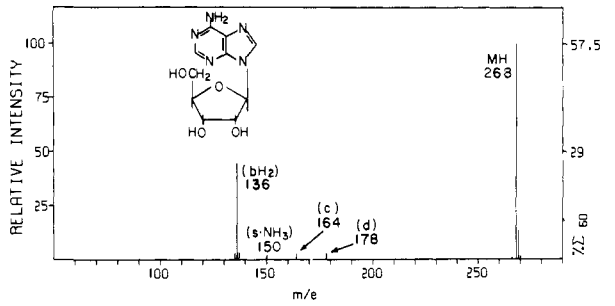


Figure 1. Chemical ionization (NH_3) mass spectrum of adenosine.

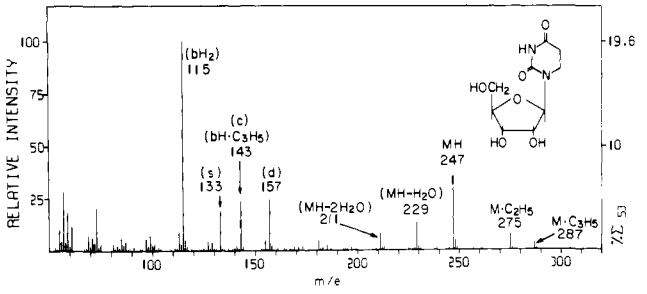


Figure 2. Chemical ionization (CH_4) mass spectrum of 5,6-dihydrouridine.

Table II. MH Abundance from Adenosine

Reagent gas	Proton affinity, kcal/mol	MH, $\%\Sigma_{50}$[a]
CH_4	126[b]	34
i-C_4H_{10}	195[c]	36
NH_3	207[b]	66
$MeNH_2$	216.3[d]	60
Me_2NH	222.4[d]	92
Me_3N	226.6[d]	95

[a] Calculated excluding molecular ion adducts, e.g., M·C_2H_5. [b] Reference 14. [c] J. L. Beauchamp and M. C. Caserio, *J. Am. Chem. Soc.*, 94, 2638 (1972). [d] Reference 15.

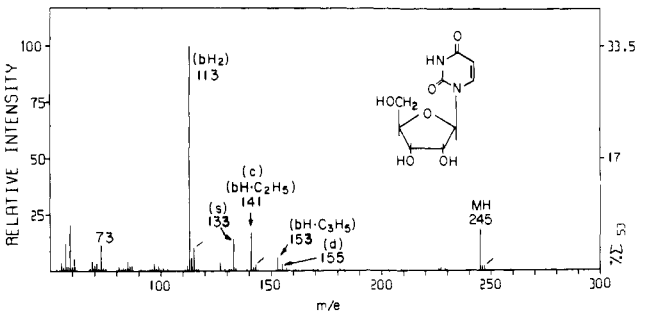
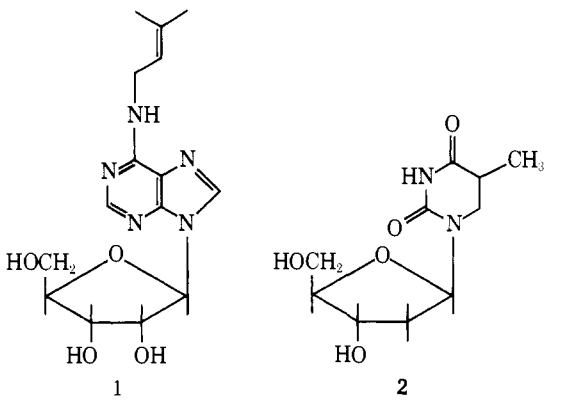


Figure 3. Chemical ionization (CH_4) mass spectrum of uridine. Marks denote peaks whose relative intensities increase with time.

such example is offered by the methane chemical ionization spectrum of dihydrouridine, Figure 2, which exhibits MH of *m/e* 247 and typical adduct ions of *m/e* 275 and 287. The corresponding electron ionization spectrum shows no molecular ion and exhibits an unusual lack of reproducibility.[7]

An unusual feature associated with the chemical ionization spectra of nucleosides was the appearance of peaks 2 amu above the expected value of MH in the case of pyrimidine nucleosides such as uridine (Figure 3) and in N^6-(3-methyl-2-butenyl)adenosine (**1**, Figure 4). Analogous peaks are associated with all base-containing ions but not those which represent the sugar. The sole exceptions to the appearance of +2 peaks in the pyrimidine series were α- and β-5,6-dihydrouridine and 5,6-dihydrothymidine (**2**), which strongly suggests the 5,6-double bond to be the site of additional saturation. In the case of **1**, the additional peaks are not associated with ions resulting from loss of the side chain,[7] MH − C_5H_8 (*m/e* 268) and base + 2H − C_5H_8 (*m/e* 136), therefore indicating the isopentenyl group to be the reactive site. The relative intensities of the anomalous peaks were found to slowly increase with time, starting slightly above the theoretical value of the second isotope peak of the principal ion immediately after sample insertion, then rising to 5–10% of the principal peak intensity during the first 1–2 min of sample vaporization. The effect was observed from all reagent gases studied with the exception of isobutane. Attempts to trace the source of added hydrogen by using deuterium-labeled reagent gases were unsuccessful because of exchange reactions involving labile hydrogens (discussed in the following section). The reaction was determined to be ion related as a result of an experiment with N^6-(3-methyl-2-butenyl)adenosine (**1**) in which the mass spectrometer filament was switched on and off during sample vaporization. Each time the filament was off the MH_3/MH ratio would return to the minimal starting value and would increase only when the filament remained on. Although the mechanism of the saturation reaction is not completely understood, the appearance of +2 ions does



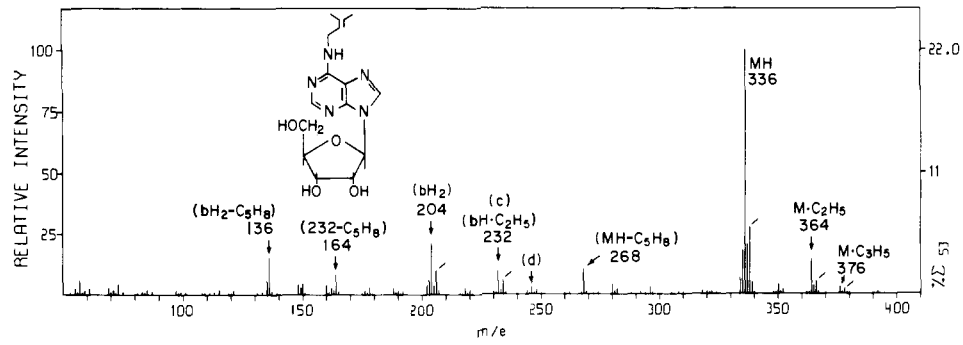
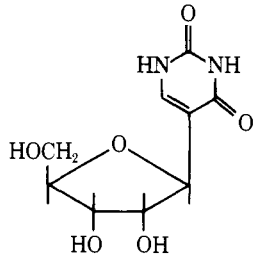


Figure 4. Chemical ionization (CH_4) mass spectrum of N^6-(3-methyl-2-butenyl)adenosine. Marks denote peaks whose relative intensities increase with time.

not otherwise interfere with the interpretation of the spectrum and can be used empirically to verify the identity of base-containing ions.

Fragment ion products from chemical ionization correspond in part to those formed by electron impact, but by different mechanisms. The most abundant species was found in most cases to correspond to the base fragment (b) plus two hydrogens (e.g., m/e 136 in Figure 1). As a significant exception, the abundance of bH_2 was lower in the spectra of the C-nucleoside pseudouridine (**3**) and its α ano-



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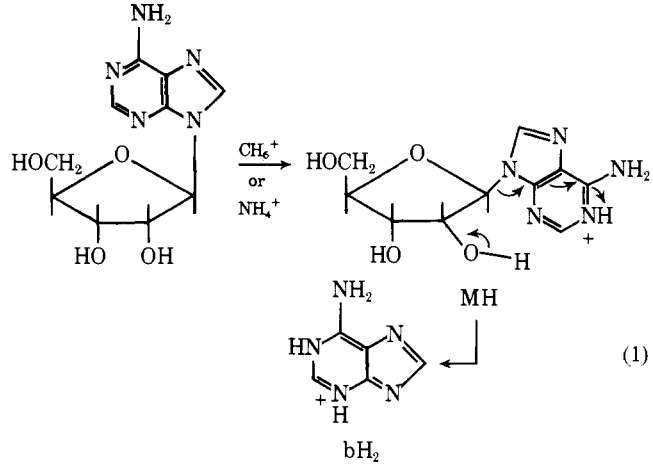
mer (see Table I), reflecting the analogous behavior upon electron ionization[20-22] which was attributed in part to higher stability of the C-C glycosidic bond.[22]

The abundance of bH_2 was observed to increase slightly as a function of time when NH_3 reagent gas was used but was much less pronounced with the more basic methylamine and dimethylamine. This effect, previously noted,[9] is evidently due to ammonolysis to produce the free base, followed by protonation to form bH_2. The principal ion population of bH_2 from electron ionization arises from double hydrogen transfer from labile hydrogens in the sugar.[23,24] In order to establish the origins of both hydrogens in the case of chemical ionization, a number of nucleosides were examined using the labeled reagent gases, i-C_4D_{10}, CD_4, and ND_3. In the case of ND_3, exchange of labile hydrogens occurred as expected from the work of Hunt,[25] producing MD species from adenosine of m/e 274 and bD_2 of m/e 140. The latter ion shows that at least one hydroxyl hydrogen rather than skeletal hydrogen is transferred to the base in the formation of bH_2 but leaves the origin of the second hydrogen unknown. When CD_4 reagent gas was used, the spectra showed extensive but incomplete deuterium exchange both in the base and sugar, making quantitative measurements of isotopic content of bH_2 or other fragment ions difficult. A typical labeling pattern from adenosine and CD_4 was 38% d_1, 38% d_2, 20% d_3, and 4% d_4.[8] Similar results were obtained with a steroid model, cholestan-3β,5α-diol-6-one. Since the reagent gas adduct ions showed a labeling pattern virtually identical with that of MH and bH_2, a chemical rather than ionic exchange reaction is apparently involved. Although drying of the reagent gas had no apparent effect on the extent of exchange, the formation of HDO and D_2O by ion-molecule reactions between CD_5^+ and H_2O might occur which would then undergo exchange with the labile hydrogens from the nucleoside.

When using i-C_4D_{10}, it was found that the extent of exchange could be greatly reduced by decreasing the ratio of reagent gas to sample. The favorable result from this latter condition is shown by the isobutane spectra in Figure 6. From a comparison of the molecular ion and bH_2 regions of the spectrum, it is evident that the principal base species contains one hydrogen (deuterium) from the reagent gas and one from the sugar. The most reasonable mechanism of formation of bH_2 therefore involves protonation by the reagent ion followed by transfer of a sugar hydroxyl hydrogen with cleavage of the glycosidic bond. Earlier electron ionization studies established that O-2′ was the preferred but not sole source of hydrogen transferred to the base.[24] We therefore conclude that the reaction shown in eq 1 is fol-



(1)

lowed by the main population of decomposing protonated molecules, although other routes may also be operative. In contrast to solution, the sites of nucleoside protonation in the gas phase are not known, but the arbitrary representation of N-1 protonation in eq 1 is not required for the overall mechanistic rationale which is implied.

Two additional less abundant base-containing ions which are common to most of the spectra examined correspond in mass to b + 30 (ion c) and b + 44 (ion d). These ions were shown to be structurally analogous to their electron ionization counterparts by measurement of exact mass (using **3**, 4′-thioadenosine (**4**), 2′-methyladenosine (**5**), and N^6,N^6-dimethyladenosine (**6**) and by mass shifts resulting from heteroatom replacement (**4** → ion c′) and substituent labeling (**5**,**6**)). Although less abundant than their electron ionization counterparts, ions c and d are diagnostically important for establishing the sites and nature of sugar modification in

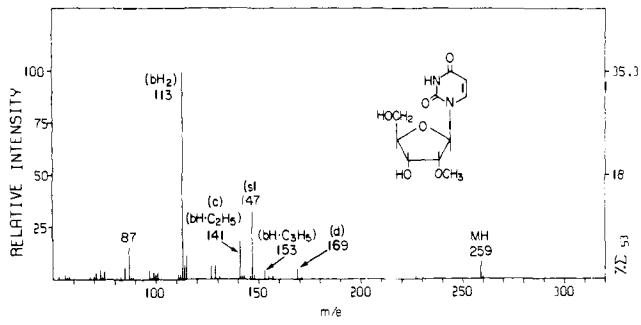
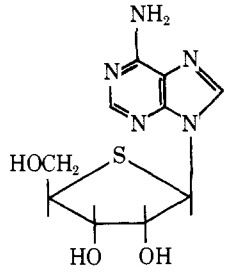


Figure 5. Chemical ionization (CH_4) mass spectrum of 2′-*O*-methyluridine.



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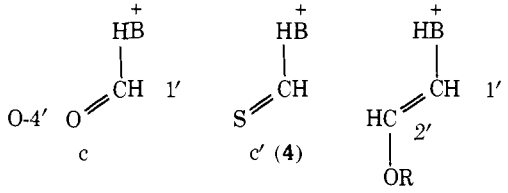
a nucleoside of unknown structure. In the case of CH_4 reagent gas, ion c falls at the same nominal mass as the adduct ion b + H + C_2H_5 (discussed in a later section). Measurement of exact mass in various spectra and comparison of the spectra of adenosine and its 4′-thio analog (**4**) showed

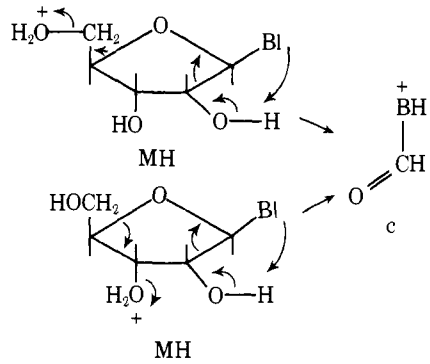


c c′ (**4**) d, R = H, OCH_3

that both ions are generally present, but their relative abundances will vary with experimental conditions and the structure of the base. The shift of ion d and its use to establish methylation at O-2′ is illustrated by comparison of the spectra of uridine (Figure 3, *m/e* 155) and 2′-*O*-methyluridine (Figure 5, *m/e* 169).

The use of *i*-C_4D_{10} reagent gas led to the unexpected observation that, in the case of adenosine (Figure 6), ions c and d do not contain reagent gas hydrogen, unlike bH_2. The possibility of formation by direct electron ionization was excluded, because their relative abundances are (1) not proportional to other peaks expected from electron ionization and (2) not enhanced by decreasing the reagent gas pressure. It is recognized that *m/e* 268 (MH) in Figure 6b probably arises from self-protonation of adenosine (because of abnormally high sample/reagent gas ratio to suppress exchange, as previously discussed), which could in principle contribute to the unlabeled c and d species. However, such contributions are considered unimportant, because the ion current contributions for c and d in Figure 6a are quantitatively accounted for in Figure 6b. There is furthermore no reason to assume *a priori* that *m/e* 268 in Figure 6b will decompose to yield c and d, because it is formed by a different mechanism than *m/e* 269. The proton affinity of the sugar moiety is below 207 kcal/mol as evidenced by the failure of ribose,[26] glucose, and related sugars[27] to accept a proton from NH_4^+ reagent ion. The conclusion that ions c and d from isobutane reagent gas are derived from sugar-protonated MH species is therefore supported by the observation (Table I) that c and d are greatly suppressed in the spectra produced by amine reagent gases, whose proton affinities are too high to permit sugar protonation.

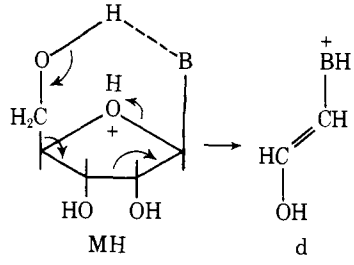
The formation of ion c is envisioned as occurring from protonation at either O-3′ or O-5′, with sugar fragmentation initiated by expulsion of a neutral molecule of H_2O. The transfer of hydrogen from O-2′ to the electron-rich base is sterically feasible and is analogous to the mechanism



MH c MH

of formation of ions c and d on electron impact.[24] If decomposition occurs from proton-shared forms of MH such as structure a_2, the proton is expected to remain with the more basic purine or pyrimidine moiety, leading to bH_2 rather than the ions c or d.

The production of ion d is most readily rationalized in terms of protonation at O-4′; e.g., it is interesting to note a certain degree of similarity between the mechanisms of formation of ions c and d in chemical and electron ionization.



MH d

In both cases, a sugar hydrogen is abstracted by the base, and the ion products have nominally the same structure. In the case of electron ionization, however, the initial reaction is triggered by the odd-electron radical-ion base, and fragmentation of the sugar proceeds by a series of homolytic bond cleavages.

Other base-containing fragment ions are produced in substantially lower abundance compared with electron ionization, and so the latter technique is generally better suited for the determination of some structural features. Examples in the present study of reactions which are of major diagnostic value in electron ionization are the loss of CH_2NH[28,29] from the bH ion in the methane spectrum of N^6,N^6-dimethyladenosine (*m/e* 163 → 134; see Table I) and loss of C_5H_8[7] from bH_2 of **1** (Figure 4). Identities of the products are supported by appropriate metastable transitions and, in the former case, by measurement of exact mass.

Adduct ions containing the base fragment plus hydrogen are formed in some cases in analogy to molecular ion adducts. Examples are evident in the isobutane spectrum of adenosine (Figure 6a) [*m/e* 174 (bH·C_3H_3), 176 (bH·C_3H_5), 178 (bH·C_3H_7; doublet with ion d as shown in Figure 6b), 192 (bH·C_5H_9)] and in the methane spectrum of thymidine (Figure 7) [*m/e* 141 (bH·CH_3), 155 (bH·C_2H_5; doublet with ion c), and 167 (bH·C_3H_5)]. Although these

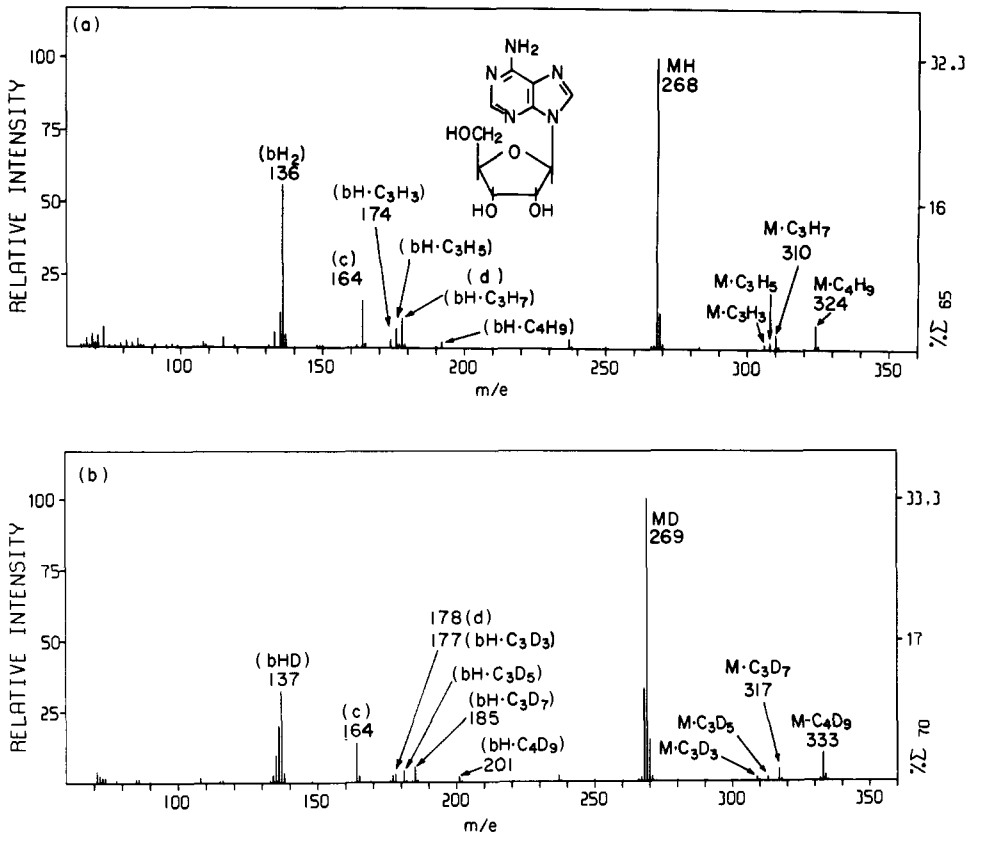
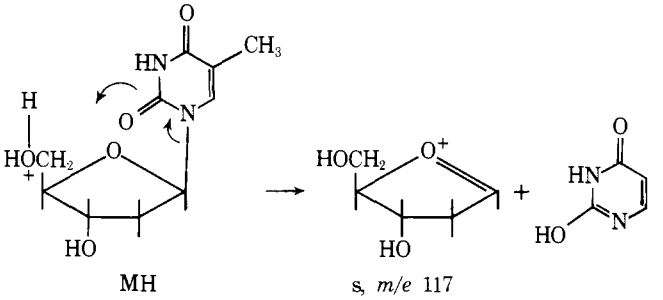


Figure 6. Chemical ionization mass spectra of adenosine from (a) i-C_4H_{10} and (b) i-C_4D_{10} reagent gases.

ions could in principle be formed by collision between reagent ions and neutral base molecules formed by fragmentation of MH, an alternative pathway is by decomposition of the corresponding molecular ion adduct. Metastable ion defocusing[30] experiments using adenosine with CH_4 reagent gas confirmed that at least a portion of the $bH{\cdot}C_2H_5$ and $bH{\cdot}CH_3$ species arises directly from $M{\cdot}C_2H_5$ and $M{\cdot}CH_3$ precursors.

Chemical ionization leads to preferential protonation of the base rather than the sugar with distribution between the two populations depending upon the basicity of the gas used. This leads to a preponderance of base-containing ions and relatively fewer sugar ions than in the case of electron ionization. Sugar ions (s) are exhibited in significant abundance in the spectra of pyrimidine nucleosides such as uridine (Figure 3) and thymidine (Figure 7). In the latter case, further decomposition of ion s by expulsion of one and two molecules of water is observed, similar to the electron ionization reactions.[31] A reasonable mechanism can be formulated in which sugar protonation leads to cleavage of the glycosidic bond, with elimination of a neutral molecule of the base (MH → s). Other mechanisms involving protonation at different sites of the sugar or base are also possible, although the latter case is considered less likely as a precursor to ion s. Recognition of methylation at O-2′, the most common form of sugar modification in ribonucleic acid, is based on the presence of m/e 147 as shown in Figure 5. However, the characteristic s-H ion, which in electron ionization spectra differentiates O-2′ from other sites of alkylation,[24] is absent in chemical ionization spectra. In some instances, ions corresponding to sugar adducts are observed, but their origins are not known. For example, the ammonia spectrum of adenosine (Figure 1) exhibits a peak of m/e 150, equivalent in mass to $s{\cdot}NH_3$, whose identity was confirmed by a 1-amu shift in the spectrum produced by $^{15}NH_3$ reagent gas. The formation of $s{\cdot}NH_3$ (or s-H + NH_4) may be analogous to that of ion s shown above, but derived by elimination of a neutral base molecule from the MNH_4 adduct.



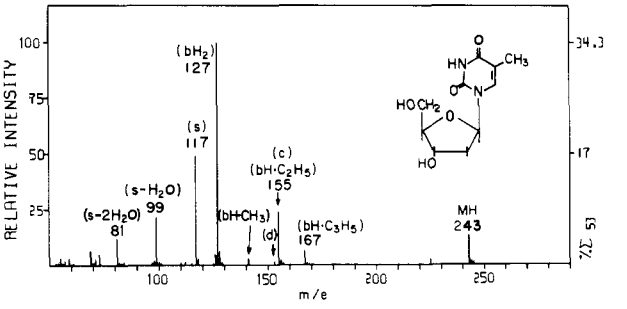


Figure 7. Chemical ionization (CH_4) mass spectrum of thymidine.

Measurements of Proton Affinity

The proton affinity (PA) of a molecule M is defined as the negative of the enthalpy for the protonation reaction shown as eq 2. The availability of accurate thermochemical

$$H^+ + M \longrightarrow MH^+ \qquad (2)$$

Table III. Proton-Affinity Limits for Nucleosides and Purine and Pyrimidine Bases

Nucleosides	Bases (IP)[a]
207–216.3 kcal/mol	
Thymidine	2-Hydroxypyrimidine
Uridine	Thymine (9.4)
5,6-Dihydrouridine	Uracil (9.8)
2',3'-*O*-Isopropylideneuridine	6-Chloropurine
216.3–222.4 kcal/mol	
	Dithiouracil
	Purine (9.7)
	Hypoxanthine (9.2)
222.4–226.6 kcal/mol	
	Cytosine (8.9)
	6-Methylpurine
	Adenine (8.9)
	Guanine
>226.6 kcal/mol	
Cytidine	9-Cyclopentyladenine
9-β-D-Ribofuranosylpurine	8-Methylguanine
Adenosine	
2',3'-*O*-Isopropylideneguanosine	

[a] Ionization potential in eV; values taken from C. Lifschitz, E. D. Bergmann, and B. Pullman, *Tetrahedron Lett.*, 4583 (1967).

data for gas phase protonation[32,33] when compared with solution data has recently led to a clearer understanding of the intrinsic properties of isolated molecules[33-37] and hence of the effects of solvation.[15,38] In the most interesting and important example, the anomalous order of amine basicities ($NH_3 < RNH_2 < R_2NH > R_3N$) in aqueous solution was shown to result largely from hydrogen-bonding interactions,[15,33,38] after Munson[39] and others[36,37] showed that the gas-phase basicity follows the order $NH_3 < RNH_2 < R_2NH < R_3N$ expected from alkyl inductive effects. As a result, a complete analysis of the thermodynamic properties of amines was made and related to various structural and solvation effects.[15,33,40]

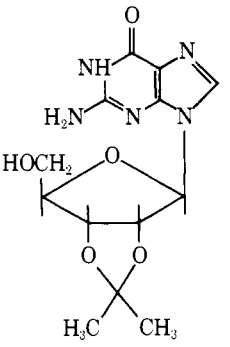
Measurements of absolute proton affinity values have been made by appearance potential determinations or an empirical method involving correlation of translational energies of products from ion–molecule reactions.[14] Relative proton affinities can be measured by ion cyclotron resonance techniques,[41] with accuracies as high as ±0.2 kcal/mol.[15,32] By contrast, the technique used in the present study, high-pressure mass spectrometry, while capable of high accuracy,[37] is generally less useful for the accurate assessment of thermodynamic properties than the latter method but has certain experimental and other advantages. The mass spectrometric method[14,36,39,42-45] is based on the observation that ion–molecule reactions proceed with negligible activation energy so the reaction shown as eq 3 will pro-

$$RH^+ + M \rightleftharpoons R + MH^+ \qquad (3)$$

ceed to the right only if PA(M) > PA(R), under the usually valid assumption that RH^+ is collisionally deactivated at the pressures employed. By using several gases, the proton affinity can be established within a range limited by the differences in reagent-gas proton affinity, and with an accuracy, usually taken as 2–5 kcal/mol,[32] which is related to the accuracy of the reagent-gas values. For molecules which exhibit proton affinities greater than NH_3 (207 kcal/mol), the selection of gases is at present relatively restricted. The four amines NH_3, $MeNH_2$, Me_2NH, and Me_3N are useful, because their PA values cover a relatively broad range, and they are sufficiently volatile for use as conventional gases.

Limiting proton-affinity values for nucleosides and bases which were established using NH_3, $MeNH_2$, Me_2NH, and Me_3N reagent gases as PA standards are given in Table III. Within the limits of resolution attainable, the proton-affinity values of the major nucleosides and bases follow the solution orders of basicity based on pK values:[46] cytidine, 9-β-D-ribofuranosylpurine, adenosine > uridine, thymidine and cytosine, adenine, guanine > purine, hypoxanthine > thymine, uracil. However, as discussed below, nucleosides in the gas phase were found to be more basic than their bases, a reversal of the order in aqueous solution. With the possible exception of purine, the proton-affinity orders of the purine and pyrimidine bases follow an inverse relationship with ionization potential (Table II), in agreement with earlier observations.[47,48] It must be noted, however, that the six ionization potentials represented in Table II fall within a range of only 0.9 eV, and so their order may be readily influenced by small errors in measurement.

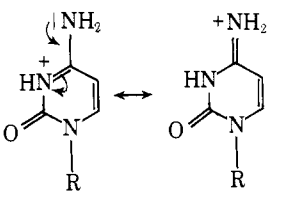
In the nucleoside series, uridine and its analogs are separated by at least 10 kcal/mol from the more basic nucleosides, whose proton affinities are above that of the trimethylamine standard. Since guanosine is too polar for vaporization without thermal decomposition, its 2',3'-*O*-isopropylidene derivative (**7**) was examined. It is assumed that the



proton affinity of the isopropylidene derivative is approximately the same as that of the free nucleoside. Proton sharing (e.g., structure a_2) is known to have a strong positive influence on the proton affinity in amines, but differences (a_2 or a_1) between guanosine and **7** are expected to be minimal. As limited confirmation of the inert effect of the isopropylidene group, the analogous derivative of uridine was examined and found to fall in the same range as uridine as shown in Table III.

Although cytidine differs from uridine only by replacement of OH by NH_2, it was found to be sufficiently basic to fall in the same range as the purine nucleosides. The effect of amino substitution is also shown by comparison of the corresponding base cytosine with 2-hydroxypyrimidine, whose PA is ~6–20 kcal/mol lower, and purine vs. adenine and guanine which fall in adjacent groups (Table III). These results follow those of Aue and Webb who found the PA of 2-aminopyridine to be 4.2 kcal/mol higher than that of pyridine.[49] Assuming protonation on the pyrimidine ring, the influence of N^4 of cytosine is readily visualized as ion e, which reflects the more powerful electron-donating ability of N^4 compared with that of O^4 (i.e., uridine or uracil) as recently demonstrated by electron ionization experiments.[50]

Induction–polarization effects resulting from alkylation



e

R = H, ribosyl

of the base (uridine–thymidine, uracil–thymine, purine–6-methylpurine, adenine–9-cyclopentyladenine, and guanine–8-methylguanine) are evident in most cases in Table III, the exception being the uracil compounds which fall within the same set of limiting PA values. In the latter cases, the differences in PA cannot be resolved because of the width of the PA brackets but, in any case, methylation at C-5 would be expected to lead to minimal stabilization if protonation occurs at O^2 or O^4 rather than in the ring. Alkylation of the purine nucleus in each case was found to increase the proton affinity to the next higher range. The effects of increased proton affinity resulting from methylation have been studied with qualitatively the same results using simpler models, notably the aliphatic amines,[33,35-37] pyridine vs. 4-methylpyridine (difference 3–5 kcal/mol),[34,49] and pyridine vs. the six 2-, 3- and 4-methyl- and -ethylpyridines (3–5 kcal/mol).[49]

Following the trend shown by methylation, a similar influence can be noted by examining the effect of ribosylation of the base upon its proton affinity. As shown in Table III, the nucleosides in every case were found to be more basic than the corresponding purine or pyrimidine base. This effect is opposite to that exhibited in aqueous solution based on pK values.[46] This effect is attributed in part to the relatively greater decrease in basicity suffered by nucleoside when hydrogen bound in solution, as well as to changes in polarizability upon substitution by the large polar ribose moiety. However, a considerable effect may be exerted by proton sharing as illustrated by structures a_1 and a_2. Aue et al.[51] and Yamdagni and Kebarle[52] have reported differences in proton affinities (i.e., enthalpies of cyclization) between aliphatic α-amines and α,ω-diamines of up to 20 kcal/mol, depending on ring-strain factors. In the present case, proton sharing would occur between two groups of very different basicity (heterocyclic base vs. sugar hydroxyl) and, although space-filling CPK models predict relatively little strain in a_1 or a_2, the quantitative effects of proton sharing in nucleosides cannot be presently determined. We note, however, that Aue and his collaborators have shown that intramolecular hydrogen bonding results in proton-affinity enhancement of as much as 12.5 kcal/mol when OH is added to an aliphatic amine.[53]

Replacement of oxygen by sulfur was examined by comparison of uracil and 2,4-dithiouracil and was observed to result in PA enhancement (Table III) in accordance with earlier studies, for example, the comparisons CH_3OH (182 kcal/mol) – CH_3SH (185)[14] and CH_3OCH_3 (186) – CH_3SCH_3 (197).[41] By contrast, the inductive effect generated by chlorine in 6-chloropurine was found to decrease the proton affinity of the purine nucleus to below 216 kcal/mol. Similarly, Aue and Webb have observed a decrease of 6.3, 5.1, or 3.2 kcal/mol in the PA of pyridine when substituted by chlorine at C-2, -3 or -4, respectively.[49]

The estimation of proton affinities by high-pressure mass spectrometry is advantageous for work involving complex molecules of low volatility, such as the nucleosides whose melting points are often above 200°. As recently pointed out by Munson,[45] the establishment of proton-affinity values may prove useful for organic structural applications. This is particularly important, because the data can be produced simultaneously with a mass spectrum with its ancillary advantages, such as measurement of exact mass. In addition, a knowledge of proton affinities, both for functional groups and polyfunctional molecules, enhances the utility of chemical ionization mass spectrometry with regard to reagent gas selectivity,[3] a potentially important aspect of the chemical ionization technique. The amine reagent gases[54] are presently the most useful for this purpose, but further development of this field is expected to produce a wider variety of gases which exhibit suitable physical and chemical properties.

Experimental Section

Sources of Materials. Most nucleosides and bases were commercial products. Less common compounds were obtained from the following sources: Heterocyclic Chemical Corp., Harrisonville, Mo., 9-cyclopentyladenine; P-L Biochemicals, Milwaukee, Wis., 2,4-dithiopyrimidine (2,4-dithiouracil); Cancer Chemotherapy National Service Center, National Institutes of Health, 8-methylguanine, 2′,5′-dideoxyadenosine, 4′-thioadenosine, N^6-(3-methyl-2-butenyl)adenosine; Dr. Leon Goodman, Stanford Research Institute, Menlo Park, Calif., 2′-deoxy-α-adenosine; Dr. M. Honjo, Takeda Chemical Co., Osaka, Japan, 2′-O-methyluridine. 5′-Deoxyadenosine and 2′-O-methyladenosine were prepared earlier in this laboratory by Dr. S. J. Shaw.[24]

CH_4 (99.97%), i-C_4H_{10} (99.96%), NH_3 (>99.9%), $MeNH_2$ (98.0%), Me_2NH (99.0%), and Me_3N (99.0%) were purchased from Matheson Gas Products, La Porte, Texas; CD_4 (isotopic purity, 99 atom %), ND_3 (99 atom %), and i-C_4D_{10} (98 atom %) from Merck Sharp & Dohme of Canada, Montreal, Canada; $^{15}NH_3$ (99 atom %) from Bio-Rad Laboratories, Richmond, Calif.

Instrumentation and Procedures. Mass spectra were acquired using a CEC 21-110B instrument which was previously modified for high-pressure operation,[55,56] under the following conditions: accelerating potential 8 kV, ionizing electron energy usually 200 eV and occasionally 70 eV, repeller field 0–30 V/cm. Reagent gas pressure in the ionization chamber was within the limits 0.3–0.5 Torr, as measured with a Baratron MKS capacitance manometer, and was maintained at a constant level during each run. The analyzer pressure was maintained at 4–6 × 10^{-7} Torr. All samples (0.5–3 μg) were introduced by direct probe at the minimum ion source temperature required for vaporization, which covered the range 100–200° for purine and pyrimidine bases and 180–290° for nucleosides.

Abundances of the principal fragment ions from the mass spectra of reagent gases were as follows: CH_4 (0.5 Torr), m/e 15 (36% rel intensity), 16 (25), 17 (100), 27 (12), 29 (75), 41 (8); i-C_4H_{10} (0.3 Torr), m/e 39 (3), 43 (3), 56 (4), 57 (100); NH_3 (0.3 Torr), m/e 18 (100), 35 (4); CH_3NH_2 (0.3 Torr), m/e 30 (5), 32 (100), 46 (4), 63 (7); $(CH_3)_2NH$ (0.3 Torr), m/e 44 (6), 46 (100), 58 (9), 91 (12); $(CH_3)_3N$ (0.3 Torr), m/e 32 (3), 44 (10), 46 (2), 59 (4), 60 (100), 61 (25), 62 (81), 63 (3).

The origin of the m/e 58 peak in the spectrum of Me_2NH is not known; some nucleoside spectra showed an analogous peak at M + 58. No significant changes in the ratios of major ions such as MH or M·adduct in the chemical ionization spectra of nucleosides and bases were evident over the pressure range used but were observed in some cases below 0.1–0.15 Torr. Compounds for which proton-affinity values had been previously established in other laboratories were examined and found to fall within the limits dictated by the reagent gases used (NH_3, $MeNH_2$, Me_2NH, Me_3N). For example, acetamide (210.4 kcal/mol),[52] 207–216.3 kcal/mol; N-methylaniline (222.1 kcal/mol),[52] 216.3–222.4 kcal/mol; cyclohexylamine (222.7 kcal/mol),[57] 222.4–226.6 kcal/mol.

Measurements of exact mass were made using photographic recording with mixtures of perdeuterioalkanes as internal standards,[55] at resolving power of 15–18,000.

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Kinetic Isotope Effect in the Homolytic Abstraction of Benzylic Hydrogen by *tert*-Butoxy Radical

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Abstract: The kinetic isotope effect for the abstraction of hydrogen (deuterium) from toluene by *tert*-butoxy radical derived from *tert*-butyl hypochlorite has been determined as a function of temperature. The determinations were made under conditions eliminating chlorine atom chains. The curved Arrhenius-type plot that is observed is discussed in terms of tunneling.

Chlorination of hydrocarbons by *tert*-butyl hypochlorite is known to proceed via a homolytic chain reaction.[2] For the light-initiated reaction, the sequence shown in eq 1-3 is

$$(CH_3)_3COCl \xrightarrow{h\nu} (CH_3)_3CO\cdot + Cl\cdot \quad (1)$$

$$t\text{-BuO}\cdot + RH \longrightarrow t\text{-BuOH} + R\cdot \quad (2)$$

$$R\cdot + t\text{-BuOCl} \longrightarrow RCl + t\text{-BuO}\cdot \quad (3)$$

generally accepted.[2-5]

Extensive data exist on the relative reactivities of a number of organic substrates toward *tert*-butyl hypochlorite. Discrepancies exist in the work of various investigators.[5-10] These difficulties have been resolved by the demonstration of the incursion of chlorine atom abstraction competitive with reaction 2.[5,6,10] Chlorine atom chains have also led to difficulties in interpreting the kinetics of the reaction.[3,4,11,12]

In extending our investigation of the chlorination of toluene,[8,13] we have examined the kinetic isotope effect with toluene-α-d_3. Previous data suggest that chlorine atom abstraction is significant in results that have been reported. Kennedy and Ingold[9] report k_H/k_D as 2.4 at 40°, and Lee and Teo[10] report a value of 2.5 at the same temperature. These results were obtained by direct competition between the deuterated and nondeuterated toluenes. The k_H/k_D value[5] of 5.0-5.5 at 40° obtained indirectly from the *tert*-butyl alcohol:acetone ratio is substantially larger.

We have determined the value of k_H/k_D for the *tert*-butyl hypochlorite reaction using conditions were chlorine atom chains are excluded. This paper describes these deter-