Table I. Ions Observed in the Thermal Desorption of a Mixture of Tetramethyl- and Tetraethylammonium Iodide Salts

m/e	species	relative abundance, %
	Intact Cations	
130	$(C_2H_5)_4N^+$	9.0
116	$(C_2H_5)_3N^+CH_3$	0.9
102	$(C_2H_5)_2N^+(CH_3)_2$	0.6
88	$C_2H_5N^+(CH_3)_3$	0.7
74	$(CH_3)_4N^+$	48.4
	Major Decomposition Ions	
100	$(C_2H_5)_2$-N^+=C_2H_4	95.0
86	$C_2H_5(CH_3)N^+$=CH-CH_3 $(C_2H_5)_2N^+$=CH_2	40.0
72	$C_2H_5(H)N^+$=CH-CH_3 $C_2H_5(CH_3)N^+$=CH_2	17.7
58	$(CH_3)_2N^+$=CH_2	100.0

a mixture of quaternary ammonium salts. Table I shows a portion of the spectra obtained by heating a filament coated with a solution containing approximately equal amounts of tetramethyl- and tetraethylammonium iodide. The major ions observed from this filament are the same as those shown in Figures 1-3 and are the result of the direct desorption or pyrolysis-desorption process. There are, however, a number of low-intensity ions that may be the result of a dequaternization/quaternization process of the sort described by Vincze and Gefon.[8] While interesting, the process is not a major one, which will be a distinct advantage in the analysis of mixtures or quaternary ammonium salts.

The temperatures at which the R_4N^+ ions desorb are lower than those at which K^+ or Na^+ are observed, as shown in Figures 1 and 2. While the Langmuir-Saha equation might be used to predict this observation, its use in this fashion is not strictly correct, since it predicts the ratio of desorbed ions to desorbed neutrals. Rather the process is more realistically expressed as (for sodium ions)

$$Na^+Cl^-(s) \rightarrow Na^+(g) + Cl^-(g)$$

and involves ionic bond rupture rather than ionization. The energy required for this process is the lattice energy, defined by

$$U_{lattice} = \Delta H_f(Na^+, g) + \Delta H_f(Cl^-, g) - \Delta H_f(NaCl, s)$$

If standard heats of formation for gaseous ions[9] and crystalline NaCl are used,[10] the process for thermal desorption of Na^+ and Cl^- requires an energy of 184 kcal/mol (8.0 eV). For KCl, using similar tables, the energy required is 167 kcal/mol (7.2 eV). Heats of formation for $(CH_3)_4N^+$ and lattice energies for $(CH_3)_4NCl$ are not available, but one may place an upper limit on this value by considering the lattice energy of NH_4Cl, reported as 153 kcal/mol (6.6 eV).[11] Since substitution of methyl groups to form $(CH_3)_4NCl$ would increase the ionic radius of the cation, increase the ionic bond length, and decrease the ionic strength, the production of $(CH_3)_4N^+$ through bond rupture would be expected to require somewhat less energy than the production of NH_4^+. While use of crystal lattice energies and heats of formation in a Born-Haber cycle, where the actual physical states of the species on a heated surface are not completely known, may not be completely justified, the general scheme substantiates the observation of quaternary ions at lower temperatures than potassium and sodium ions. Röllgen[3] has observed a related phenomenon in FD, in which desorption of quaternary ammonium ions and alkali attachment to polar molecules both occur at lower field strengths than the field ionization of molecules having low ionization potentials.

(8) A. Vincze and L. Gefen, *Israel J. Chem.*, **17**, 236 (1978).
(9) H. N. Rosenstock, K. Droxl, F. W. Steiner, and J. T. Herron, *J. Phys. Chem. Ref. Data*, **6**, (1977).
(10) "Handbook of Chemistry and Physics", Vol. 54, R. C. Weast, Ed., CRC Press, Cleveland, 1973.
(11) I. M. Klotz, "Chemical Thermodynamics", W. A. Benjamin, New York, 1964, p 74.

It is generally expected that heating quaternary ammonium salts in the vacuum region of a mass spectrometer results in a decomposition process in which losses of alkyl groups produces tertiary amines which are volatilized as *neutrals* prior to ionization by electron impact or chemical ionization. This major thermal process occurs in this study as well but is not observable with the electron beam off. Rather, only those reactions, which produce stable ionic products retaining a tetravalent nitrogen, result in desorption of *ions*, which can be observed without an electron beam, when formed inside the ion source. Stoll and Röllgen have also recently reported the thermal desorption of quaternary ammonium ions[12] but, unlike this present work, have not observed the surface reactions leading to the desorption of stable cationic fragments.

Not only does this study demonstrate the ability to desorb stable cations of organic compounds without the aid of auxiliary ionization techniques, but it also demonstrates a method by which these and related materials may be ionized by using rather standard mass spectrometry instrumentation. Because electrostatic fields are unnecessary, it is likely that anions are produced simultaneously with the cations, so that organic anions might also be subject to analysis by this simple process. Finally, this work suggests the role of thermal processes in other ionization techniques, such as laser, field, and plasma desorption, and as such represents an important contribution to the understanding of these mechanisms.

Acknowledgment. This work was funded in part by grants from the National Institutes of Health, GM-21248, and the National Science Foundation, CHE-78-18396, and was conducted at the National Institutes of Health facility in Bethesda, MD.

(12) R. Stoll and F. W. Röllgen, *J. Chem. Soc., Chem. Commun.*, 789 (1980).

A Novel Photoreaction of Thymidine with Lysine. Photoinduced Migration of Thymine from DNA to Lysine[1]

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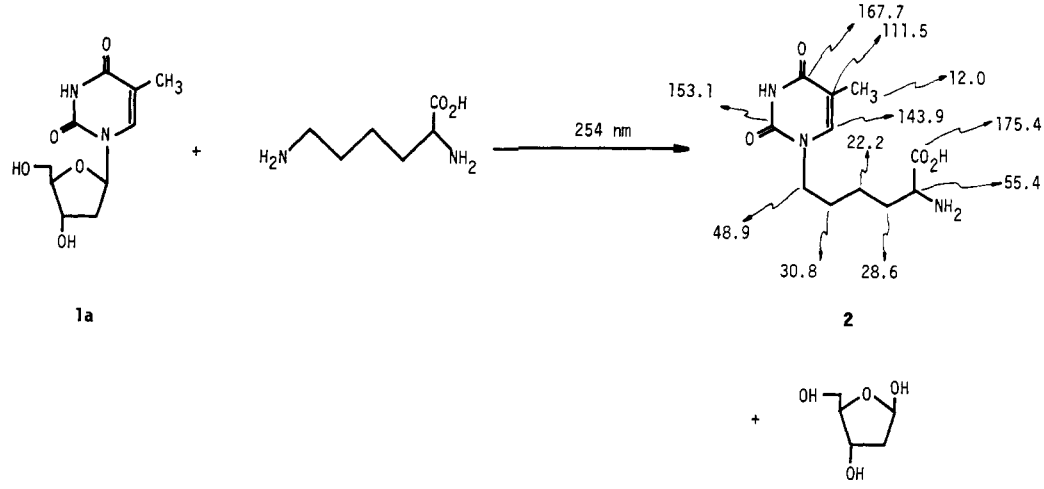
Cross-linking of proteins to DNA is one of the important processes that can take place when bacterial or mammalian cells are irradiated with UV light.[2] Investigation of this type of cross-linking is particularly useful in understanding the nature of radiation-induced damage in cells.[2] Direct photocross-linking technique, without the aid of other reagents, also provides an important method for studying specific protein-nucleic acid associations.[3] Knowledge of the photoreactions of relevant model systems could be of significant value in achieving an understanding of UV-induced cross-links.[4] Shetlar and co-workers recently reported that irradiation of thymine-labeled DNA and lysine in aqueous solvent followed by acid hydrolysis produces a photoproduct that behaves like thymine-lysine adduct,[5] while the

(1) Photoinduced Reactions. 130.
(2) (a) Smith, K. C., Ed. "Aging, Carcinogenesis and Radiation Biology; The Role of Nucleic Acid Additions"; Plenum Press: New York, 1976. (b) Smith, K. C. *Photochem. Photobiol. Nucleic Acids* **1976**, *2*, 187.
(3) Schimmel, P. R. *Acc. Chem. Res.* **1977**, *10*, 411.
(4) (a) Varghese, A. J., ref 2a, p 207. (b) Gorelic, L. S.; Lisagor, P.; Yang, N. C. *Photochem. Photobiol.* **1972**, *16*, 465. (c) Fisher, G. J.; Varghese, A. J.; Johns, H. E. *Ibid.* **1974**, *20*, 109. (d) Shetlar, M. D. *Ibid.* **1980**, *32*, 587. (e) Toulme, F.; Hélène, C. *Ibid.* **1980**, *32*, 679. (f) Saito, I.; Ito, S.; Matsuura, T. *J. Am. Chem. Soc.* **1978**, *100*, 2901. (g) Ito, S.; Saito, I.; Nakata, A.; Matsuura, T. *Photochem. Photobiol.* **1980**, *32*, 683. (h) Ito, S.; Saito, I.; Matsuura, T. *J. Am. Chem. Soc.* **1980**, *102*, 7535.
(5) Schetlar, M. D.; Schott, H. N.; Martinson, H. G.; Lin, E. T. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 88.

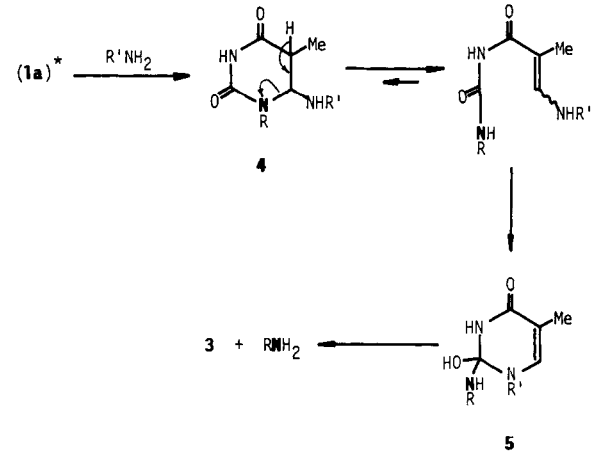
0002-7863/81/1503-1598$01.25/0

Scheme I



1a 2

Scheme II

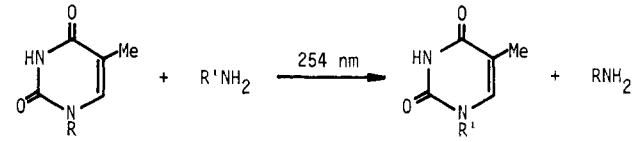


4 5

structure of the photoproduct has remained to be characterized. Such photochemical linkage of lysine to thymine in DNA is of potential significance in the cross-linking of DNA to histones, since lysine is one of the major constituents of various histone fractions.[6] We report here that photoexcited thymidine readily reacts with lysine to produce N(1)-substituted thymine, **2**, and 2-deoxy-D-ribose, not the cross-coupled product, as exemplified in Scheme I. We also observed that the same photoproduct **2** is obtained directly from the UV irradiation of DNA and lysine without acid hydrolysis. These observations indicate that irradiation of DNA in the presence of lysine induces the selective migration of thymine base from DNA to ϵ-amino group of lysine.

A solution (pH 9.4) of thymidine (**1a**, 4 mM) and free L-lysine (13 mM) in unbuffered double-distilled water was irradiated with low-pressure mercury lamp (10 W) through Vycor filter (>250 nm) under nitrogen atmosphere. The resulting solution was lyophilized to dryness, and the residue was subjected to preparative TLC[7] to give a ninhydrin positive photoproduct which was further purified by preparative high-performance LC.[8] The structure of the photoproduct was assigned as **2** on the basis of spectral data.[9] Assignment of the ^{13}C NMR signals are shown in Scheme I.[10] The yield of the photoproduct **2** was 18% on the basis of consumed thymidine. The existence of 2-deoxy-D-ribose in the photolysate was confirmed by means of TLC analysis.[11]

Photoexcited thymidine (**1a**) and 1-alkylthymines (**1b,c**) react with primary alkylamines in a similar fashion to produce N-(1)-substituted thymines (**3**). For example, irradiation of a



1a, R = deoxyribosyl
1b, R = Et
1c, R = *n*-heptyl

3a, R' = *n*-butyl
3b, R' = cyclohexyl
3c, R' = hydroxyethyl
3d, R' = *tert*-butyl
3e, R' = 1-adamantyl

solution (pH 9.5) of 1-ethylthymine (**1b**, 5 mM) and *n*-butylamine (15 mM) in distilled water gave 1-*n*-butylthymine[12] (**3a**, 54% based on consumed **1b**). Irradiation of thymidine (**1a**, 1 mM) and *n*-butylamine (3 mM) in aqueous solution (pH 9.5) produced **3a** (56%) with the quantum yield of 4.1×10^{-4}.[13] Similarly, photoreaction of **1a** with cyclohexylamine, 2-aminoethanol, *tert*-butylamine, and 1-aminoadamantane provided **3b** (50%), **3c** (45%), **3d** (43%), and **3e** (35%), respectively, providing a simple method for the introduction of functional groups at the N(1) position of thymine.[14]

The solution pH plays a crucial role in these bimolecular photoreactions. At acidic or neutral pH, irradiation of **1a** and *n*-butylamine did not give **3a**, whereas the quantum yield for the formation of **3a** increased with increasing pH in alkaline pH region (pH 7.5–10.5).[15] On irradiation of 1-*n*-heptylthymine (**1c**) and *n*-butylamine, both **3a** and *n*-heptylamine were obtained in approximately the same yield, indicating that the N(1) nitrogen is

(6) Johns, E. W. "Histones and Nucleohistones"; Phillips, D. M. P., Ed.; Plenum Press: New York, 1974; p 66.

(7) R_f 0.30 on silica gel TLC (*tert*-butanol–ethyl acetate–28% ammonia–H_2O, 4:3:1:2).

(8) Reverse-phase Nucleosil 7 C_{18} column; methanol–H_2O (1:9).

(9) All new compounds exhibited consistent spectral data and elemental analyses. Selected spectral data follow. **2**: mp 165–168 °C; ^1H NMR (D_2O) δ 1.52–2.36 (m, 6 H), 2.18 (d, 3 H, J = 1 Hz), 4.04 (t, 1 H, J = 6 Hz), 4.09 (t, 2 H, J = 7 Hz), 7.80 (q, 3 H, J = 1 Hz); UV (H_2O) (log ϵ) 207 nm (4.29), 271 (4.35). **6**: ^1H NMR ($CDCl_3$) δ 0.96 (d, 6 H, J = 7 Hz), 1.62 (m, 1 H), 1.80 (m, 2 H), 1.92 (d, 3 H, J = 1 Hz), 5.30 (t, 1 H, J = 8 Hz), 6.08 (br s, 1 H, NH), 7.16 (br s, 1 H, NH), 7.40 (q, 1 H, J = 1 Hz), 10.00 (br, 1 H, NH).

(10) Assignments are based on multiplicities in the off-resonance decoupled spectrum and chemical shifts in model compounds.

(11) The spot (R_f 0.53) was detected on a silica gel TLC (*tert*-butanol–AcOH–H_2O, 4:1:1) by spraying a solution of diphenylamine–aniline–80% phosphoric acid in aqueous acetone: McNally, S.; Overend, W. G. *J. Chromatogr.* **1966**, *21*, 160.

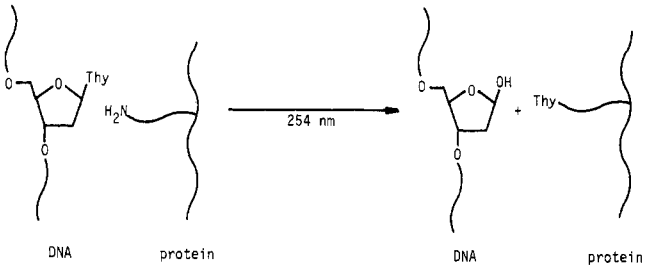
(12) Murdock, K. C.; Angier, R. B. *J. Org. Chem.* **1962**, *27*, 3317.

(13) Quantum-yield measurement was carried out at 20 °C in a merry-go-round apparatus using 5-bromouracil as an actinometer: Campbell, J. M.; SchulteFrohlinde, D.; von Sonntag, C. *Photochem. Photobiol.* **1974**, *20*, 465.

(14) The structures of the photoproducts were confirmed by independent syntheses according to the known procedure: Cusak, N. J.; Hildick, H.; Robinson, D. H.; Rung, P. H.; Shaw, G. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1720.

(15) It seems possible that a partially ionized form of **1a** (pK_a 9.8) is responsible for the photoreaction. In fact, N(3)-protected thymines such as 1,3-dimethylthymine did not react with primary amines under the conditions.

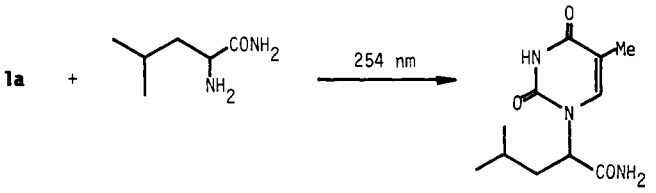
Scheme III



extruded from **1c** as *n*-heptylamine, with the incorporation of the nitrogen of *n*-butylamine into the N(1) position of the photoproduct **3a**. In contrast to the direct irradiations, acetone-sensitized irradiation of **1a** with *n*-butylamine in alkaline aqueous solution never produced **3a** but gave a mixture of thymidine photodimers exclusively,[16] nor was the formation of **3a** observed when **1a** was irradiated in acetonitrile containing *n*-butylamine.

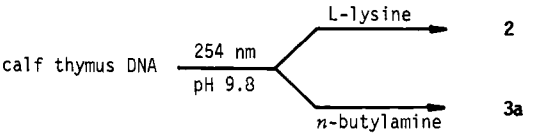
On the basis of these observations, the plausible mechanism shown in Scheme II is proposed. Nucleophilic attack of the amino group on C_6 of the photoexcited **1a**, probably the singlet state, would occur first to give 6-alkylamino-5,6-dihydrothymidine **4**.[4g,17] Ring opening of **4** under basic conditions followed by subsequent intramolecular cyclization would then furnish **5**.[18] An alternative mechanism involving a photohydrate of **1a** as the precursor of **4** might be ruled out by the fact that reaction of thymidine photohydrates, prepared by the known method,[19] with *n*-butylamine did not give **3a** under the basic conditions.

Under conditions where **1a** reacts smoothly with lysine, irradiation of other nucleosides such as adenosine, guanosine, cytidine, and uridine in the presence of lysine did not produce any ninhydrin positive photoproduct, whereas photoreaction of ribothymidine with lysine gave **2** (40%). These results indicate that the photoadduct formation with lysine is specific for thymidine. Irradiation of **1a** with other amino acids such as leucine, glycine, aspertic acid, and arginine did not give the corresponding photoadduct under the conditions. However, irradiation of **1a** with their amide derivatives provided the corresponding photoadducts. For example, photoreaction of **1a** (1 mM) with free L-leucine amide (3 mM) in water (pH 8.8) proceeded sluggishly to give **6**[9] (24%).



6

In order to ascertain whether this type of photoreaction occurs between DNA and lysine, we have examined the photoreaction of DNA with lysine. A solution (pH 9.8) of calf thymus DNA (5 mg, P-L Biochemicals) and free L-lysine (50 mg) in double-distilled water (20 mL) was irradiated with low-pressure mercury lamp (Vycor filter) at 10 °C under similar conditions. After passing through a membrane filter to remove DNA, the solution was concentrated and the residue was subjected to preparative TLC[7] as described above. High-performance LC[8] of the extract showed a peak corresponding to **2**. Collection of the high-performance LC peak gave a photoproduct whose chromatographic behaviors and UV spectrum are identical with those of **2**. Thus, we were able to confirm that the same photoproduct **2** is indeed formed in the photoreaction of DNA with lysine. The yield of **2** estimated by high-performance LC was ca. 1% on the basis of thymine contained in DNA. Likewise, irradiation of DNA (2 mg)



with *n*-butylamine (10 mg) in distilled water (10 mL, pH 9.8) followed by a similar workup provided **3a** (2.5% based on thymine), whereas irradiation of heat-denaturated single-strand DNA in the presence of *n*-butylamine produced **3a** (9%) more efficiently under the identical conditions.[20]

The foregoing photoreaction of DNA in the presence of lysine or alkylamines can induce a specific cleavage of thymine moieties from DNA chains without any acid hydrolysis, thus enabling utilization of this reaction as a new method for specific modification of DNA. It seems likely that a similar reaction can take place between photoexcited DNA and lysine residues in a protein. In such cases the thymine moiety in DNA might migrate to a neighboring lysine residue of the protein as illustrated in Scheme III. The present observations also suggest that this type of photoreaction may play an important role in UV-induced damage on nucleic acids in cells. We are continuing to explore the scope and mechanism of this novel photoreaction.[21]

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education of Japan and the Yamada Science Foundation.

(16) (a) Fisher, G. F.; Johns, H. E. *Photochem. Photobiol. Nucleic Acids* **1975**, *1*, 169. (b) Ben-Hur, E.; Elad, D.; Ben-Ishai, R. *Biochim. Biophys. Acta* **1967**, *149*, 355.

(17) (a) Summers, W. A.; Enwall, C.; Burr, J. G.; Letsinger, R. L. *Photochem. Photobiol.* **1973**, *17*, 295. (b) Schetlar, M. D. *Ibid.* **1976**, *24*, 315 and references therein.

(18) For analogous reactions, see: ref 14 and (a) Broom, A. D.; Anderson, G. L. *J. Org. Chem.* **1977**, *42*, 4159. (b) Hirota, K.; Kitade, Y.; Senda, S.; Halat, M. J.; Watanabe, K. A.; Fox, J. J. *J. Am. Chem. Soc.* **1979**, *101*, 4423. (c) Sasaki, T.; Minamoto, K.; Suzuki, T.; Yamashita, S. *Tetrahedron* **1980**, *36*, 865.

(19) Cadet, J.; Ducolomb, R.; Teoule, R. *Tetrahedron* **1977**, *33*, 1603.

(20) Control experiments showed that 254-nm light and *n*-butylamine are indispensable for the formation of **3a**.

(21) **Note Added in Proof.** An alternative pathway involving nucleophilic attack of the amino group on C_2 of the photoexcited **1a** cannot be ruled out at this time. Characterization of the intermediate formed at low-temperature irradiation will be reported in a forthcoming paper.

Tricyclo[4.2.2.2^{2,5}]dodeca-1,5-diene

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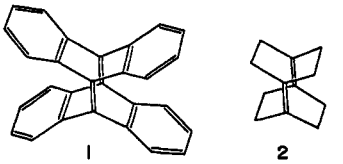
Received May 27, 1980

The properties of compounds such as **1**,[1] in which two nonconjugated double bonds are forced by their geometry to interact with each other, have been of considerable interest.[2] We wish to report a simple preparation for the parent hydrocarbon, tricyclo[4.2.2.2^{2,5}]dodeca-1,5-diene (**2**).



1 **2**

Deuterium-labeled $\Delta^{1(4)}$-bicyclo[2.2.0]hexene (**3-d_4**) was prepared by the elctrochemical reduction of 1-chloro-4-bromo-

(1) Viavattene, R. L.; Greene, F. D.; Cheung, L. D.; Majeste, R.; Trefonas, L. M. *J. Am. Chem. Soc.* **1974**, *96*, 4342.

(2) Hoffmann, R. *Acc. Chem. Res.* **1971**, *4*, 1. Wittsel, K.; McGlynn, S. P. *Chem. Rev.* **1977**, *77*. 745. Bishof, P.; Hashmall, J. H.; Heilbronner, E.; Hornung, V. *Helv. Chim. Acta* **1969**, *52*, 1745.

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