Table I

mixture of 19-chiral and [4-^{14}C]-3β-hydroxyandrost-5-en-17-one		% conversion to estradiol		recovered, $\times(10^{-3}$ dpm)		TCOOH/THO	steric mode of 19-hydroxylation
		[19-T,D,H]-androgen[d]	[4-^{14}C]-androgen	TCOOH	THO		
19*R*[a]	1	4.86	5.69	133	92	1.45	retention
	2	4.75	6.42	130	89	1.47	retention
	3	4.70	5.93	127	90	1.41	retention
19*RS*[b]	1	4.83	5.70	101	108	0.94	
	2	5.02	6.46	100	103	0.97	
	3	4.95	6.34	99	108	0.92	
19*S*[c]	1	4.59	5.82	87	128	0.68	retention
	2	4.66	5.40	88	126	0.70	retention
	3	4.70	5.64	88	128	0.69	retention

[a] Control recovery *T*: [1,2,6,7-^{3}H]-estradiol (12×10^3 dpm, 3.5 μmol), H^{14}COOH (42×10^3 dpm, 0.47 μmol), ^{3}HOH (45×10^3 dpm, 1.39 μmol), glucose-6-phosphate (75 μmol), NADP$^+$ (19-^{3}H,^{2}H,^{1}H) (4.54×10^6 dpm, 100 μg) and 4-^{14}C, (2.03×10^6 dpm, 33 μg) in 5 mL of tris buffer incubated (1 h) then placental microsomes (equivalent to 21 g of wet tissue, 45 mg of protein) were added, followed by addition of 4.25% phosphoric acid (1 mL). All experimental counts in the table are corrected for the following recoveries: estradiol, 75.9% (average of 75.6%, 78.6%, 73.5%); H^{14}COOH, 93.4% (average of 93.8%, 91.3%, 95.1%), ^{3}HOH, 76.5% (average of 76.5%, 76.7%, 76.2%). [b] Control recovery II: [1,2,6,7-^{3}H]-estradiol (12×10^3 dpm, 3.5 μmol), H^{14}COOH (42×10^3 dpm, 0.47 μmol), ^{3}HOH (45×10^3 dpm, 1.39 μmol), glucose-6-phosphate (75 μmol, NADP$^+$ (45 μmol), glucose-6-phosphate dehydrogenase (5 μkat), placental microsomes (equiv. to 21 mg wet tissue, 45 mg of protein) in 5 mL of tris buffer. Reaction was incubated (1 h) and terminated with 4.25% phosphoric acid (1 mL). All experimental counts corrected for the following recoveries: estradiol, 74.7% (average of 72.2%, 75.8%, 76.1%); H^{14}COOH 94.0% (average of 94.0%, 93.7%, 94.2%); ^{3}HOH, 82.5% (average of 81.6%, 83.8%, 82.1%). Control recovery I was also run (in triplicate) and gave the following average recoveries: estradiol, 74.7%; H^{14}COOH, 94.4%; ^{3}HOH, 82.5%. [c] Control as I: Results corrected for H^{14}COOH recovery 93.7% and for HTO recovery 79.7%. [d] Calculated on the basis of total distillable tritium corrected for recovery.

covered via partitioning with 1.25 M NaOH. The obtained estrogens were purified by HPLC and counted.[22] Control recovery experiments (in triplicate) for each step were carried out and the results were corrected accordingly (Table I).

It is apparent that incubation of (19*RS*)-3β-hydroxyandrost-5-en-17-one (F = 50) with human placental aromatase resulted in the formation of estrogens with the concomitant release of tritium, which was distributed about *equally* between the formic acid and water. In the aromatization of (19*R*)-androgen **1** (F = 63), the produced *formic acid* contained the *major portion* of the released tritium while a significantly smaller amount was in the water. In contrast, the aromatization of the (19*S*)-androgen (F = 33) gave *formic acid* that contained *less* tritium than the water (Table I).

It should be noticed that the results for each of the three substrates (Table I) are reproducible within a narrow range. Several lines of evidence support the hypothesis of the operation of an isotope effect ($k_H > k_D > k_T$). The aromatization of the 19*RS* substrate gave formic acid and water containing nearly *equal* amounts of tritium. Additionally *unequal* amounts of tritiated formic acid and tritiated water were produced in the aromatization of 19*R* and 19*S* substrates. The distribution of the isotope in products of the latter experiments was reciprocal. As previously discussed these results are expected in the operation of a normal kinetic isotope effect in the initial hydroxylation.

The hypothesis of the functioning of an isotope effect in the first hydroxylation is also supported by the observation that in all nine experiments the internal standard [4-^{14}C]-3β-hydroxyandrost-5-en-17-one was aromatized to a larger extent (ca. 20%) than the 19*R*, 19*S*, and 19*RS* substrates.

The exact reciprocity of the results for 19*R* and 19*S* androgens (Table I) shows that the "second" hydroxylation proceeds without a detectable isotope effect.[23]

(22) The estrogens recovered from the incubations of 19*S* and 19*RS* substrates were reduced with $NaBH_4$, and the resulting estradiols were purified by HPLC. In parallel to the 19*RS* incubation, six control recovery experiments were carried out.

(23) The average tritium ratios of products derived from (19*R*)- and (19*S*)-androgens are exactly reciprocal, $(TCOOH)/(THO)_{19R} = (THO)/(TCOOH)_{19S}$. The aromatization of *the two pairs of C-19 alcohols* proceeds via a "second" C-19 hydroxylation requiring the abstraction of a 19-*pro-R* hydrogen atom. There are no subsequent steps involving a C-19 hydrogen-bond cleavage. Since different hydrogen isotopes (HDT) are displaced in the second hydroxylation of 19-alcohols and considering that the TCOOH/THO ratios from aromatization of 19*R* and 19*S* substrates are exactly reciprocal, it follows that the second hydroxylation proceeds without a detectable isotope effect.

These results are fully consistent with the view that the "first" C-19 hydroxylation by human placental aromatase proceeds with retention, namely, that the incoming hydroxyl assumes the orientation of the displaced hydrogen (or isotopic hydrogen) atom. It may, therefore, be concluded that the steric mode of hydroxylation at the C-19 primary carbon, by placental aromatase proceeds in a manner analogous to the hydroxylation at primary carbon atoms by rat liver microsomes[7,8] and *Ps. oleovorans*.[6]

Acknowledgment. This work was supported by NIH grant HD 14906. We are indebted to Drs. C. C. Chang (postdoctoral fellow (1980–1981)) and K. H. Busch (postdoctoral fellow (1982–1983)) for the initial studies of Kuhn–Roth oxidation and chirality determination of the derived acetic acids. We are indebted to Dr. Thomas B. Pokoly and the staff of the Department of Obstetrics and Gynecology of the Memorial Hospital, Worcester, MA, for providing human placentae.

Isolation and Characterization of a Thymine–Lysine Adduct in UV-Irradiated Nuclei. The Role of Thymine–Lysine Photoaddition in Photo-Cross-Linking of Proteins to DNA[1]

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Photobiologists are intrigued by the possibility that cross-linking of proteins to DNA is an important mode of UV-induced damage in biological systems.[2] Molecular biologists interested in structural relations in nucleic acid–protein systems, e.g., chromatin, are examining the feasibility of using photo-cross-linking as a probe for determining contact points between DNA and histones in nucleosome.[3,4] Despite many investigations, photochemical re-

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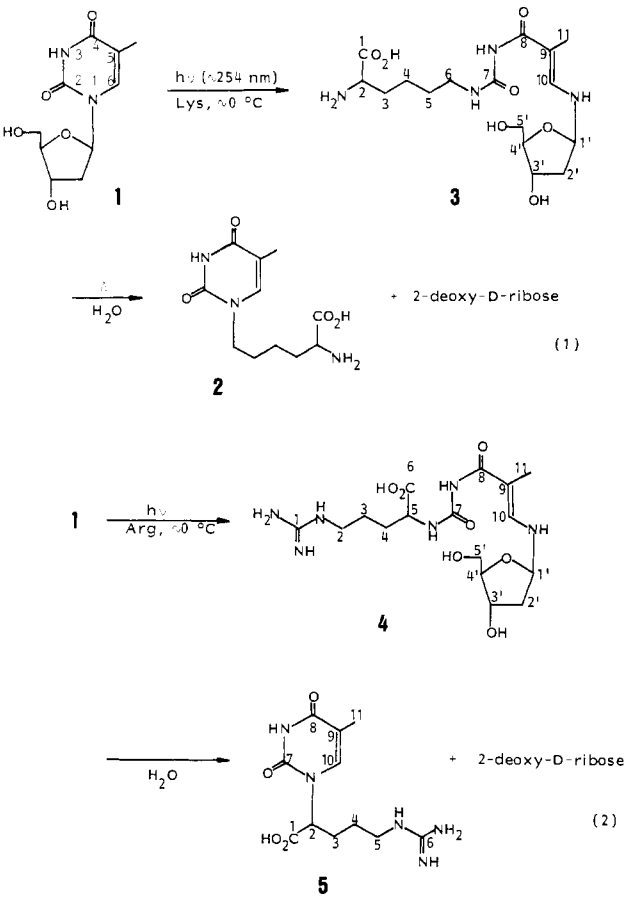
Table I. Contents of Thymine–Lysine Adduct **2** Determined by HPLC Analysis of UV-Irradiation Nucleohistone under Various Conditions[a]

pH	irradiation time, min	concn of **2**, μM	yield of **2**,[b] %
10.5	20	10	0.33
	40	18	0.60
	60	18	0.60
	210	20	0.67
8.6	210	5	0.17
7.5	210	4	0.13

[a] Solutions each containing calf thymus nucleohistone (50 mg/200 mL) in 5 mM $NaHCO_3$–Na_2CO_3 buffer were irradiated with a 400-W medium-pressure mercury lamp through a Vycor filter at 10 °C under identical conditions. Irradiated solutions were adjusted to pH 10.5, heated at 70 °C for 2 h, and then lyophilized. After hydrolysis (6N HCl), each sample was dissolved in 5 mL of water and analyzed by HPLC. [b] Calculated yields based on thymine in DNA (thymine content 27.6%) contained in calf thymus nucleohistone (DNA content 40 wt%).

actions involved in covalent linkage between DNA and histones have not be unequivocal.[3c,5] The regions of histones strongly involved in the binding of DNA to the nucleosome core in chromatin are believed to be those that are rich in lysine and arginine.[6] We report herein the results demonstrating that one major cross-linkage induced by UV irradiation of nuclei at 254-nm is the addition of the lysine ε-amino group of nucleoproteins to the C-2 carbon of thymine in DNA.

We previously reported that irradiation of thymidine (**1**) and L-lysine in aqueous solution with 254-nm light at ambient temperature led to the formation of 2-amino-6-(1-thyminyl)hexanoic acid (**2**).[7,8] In order to characterize the precursor of **2**, we have attempted low-temperature irradiation under carefully controlled conditions. When a solution (pH 10.6) of **1** (1 mM) and free L-lysine (3 mM) in distilled water was irradiated with a 400-W medium-pressure mercury lamp through a Vycor filter at 0 °C for 10 h, a new UV absorption due to the formation of a ring-opened adduct appeared at around 300 nm.[7b] The solution was lyophilized to dryness, and the residue was subjected to preparative high-performance LC (HPLC)[9] at a temperature below 10 °C to give **3**[10] in 70% yield. Heating of the aqueous solution (pH 10.5) of **3** at 90 °C instantaneously produced **2** in 90% yield together with 2-deoxy-D-ribose.[7,11] Under similar conditions irradiation of **1** (0.25 mM) and free L-arginine (0.57 mM) in distilled water at pH 11.2 (0 °C, 6 h) gave a different mode of adduct, **4**,[10] in 30% yield. The structure of **4** was again confirmed by converting it to **5**.[10] The result clearly demonstrates that the guanidino group of arginine cannot participate in the photoreaction with thymine.[12] These model experiments also suggest that the lysine ε-amino groups of histones at the sites of intimate association with thymine in DNA may undergo photo-cross-linking reaction as represented by eq 1 in DNA–histone complexes and that the



cross-linking with arginine is of minor importance. The facile conversion[11] of **3** to **2** suggests that the DNA–histone photoadducts once formed may release free histones that contain modified lysine residues by a similar thermal reaction.[7]

We next examined the UV irradiation of nucleohistone. External irradiation of calf thymus nucleohistone[13] (50 mg/200 mL) in 5 mM $NaHCO_3$–Na_2CO_3 buffer (pH 10.5) was performed with a 400-W medium-pressure mercury lamp through a Vycor filter (>250 nm) at 10 °C under nitrogen atmosphere. Following irradiation, the solution was heated at 70 °C for 2 h in order to complete the conversion of **3** to **2** and then lyophilized to dryness. The sample was subjected to extensive acid hydrolysis (6 N HCl, 110 °C). Analysis of the hydrolysate by HPLC[14] or by amino acid analyzer revealed the presence of thymine–lysine adduct **2**. The modified lysine **2** appeared between methionine and isoleucine in the output of the amino acid analyzer. Collection of the HPLC

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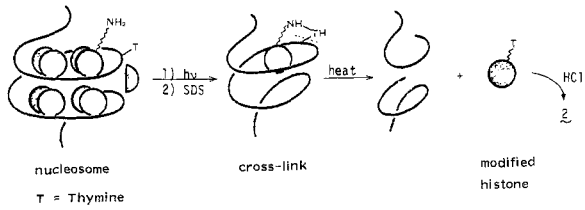
(9) Reverse-phase Nucleosil $7C_{18}$ column; acetonitrile–water (5:95); retention time 26 min at 4 mL/min.
(10) All new compounds exhibited consistent spectral data and elemental analyses. **3**: viscous oil; ^1H NMR (D_2O) δ 1.70 (s, 3 H), 1.20–2.30 (m, 8 H), 3.28 (t, 2 H, J = 6 Hz), 3.60–4.20 (m, 5 H), 4.70 (m, 1 H), 7.43 (s, 1 H); ^{13}C NMR (D_2O) δ 9.3 (C-11), 22.4 (C-4), 29.0 (C-3), 30.8 (C-5), 33.5 (C-2′), 39.6 (C-6), 55.3 (C-2), 66.9 (C-1′), 67.7 (C-5′), 68.2 (C-3′), 85.3 (C-4′), 98.2 (C-9), 146.2 (C-10), 156.4 (C-7), 171.7 (C-8), 175.4 (C-1); UV (MeOH) λ_{max} 286 nm (log ε 4.86). **4**: oil; ^1H NMR (D_2O) δ 1.72 (s, 3 H), 1.40–2.40 (m, 6 H), 3.20 (br t, 2 H, J = 7 Hz), 3.50–4.30 (m, 6 H), 7.45 (br s, 1 H); ^{13}C NMR (D_2O) δ 9.8 (C-11), 25.2 (C-3), 30.2 (C-4), 33.9 (C-2′), 41.7 (C-2), 55.8 (C-5), 67.4 (C-1′), 68.2 (C-5′), 68.7 (C-3′), 85.8 (C-4′), 98.8 (C-9), 146.8 (C-10), 156.1 (C-7), 157.6 (C-1), 172.3 (C-8), 179.3 (C-6); UV (H_2O) 290 nm (log ε 4.25). **5**: negative ninhydrin test; ^1H NMR (D_2O) δ 1.27–1.55 (m, 2 H), 1.71 (d, 3 H, J = 1 Hz), 1.73–2.13 (m, 2 H), 3.02 (t, 2 H, J = 6 Hz), 4.68 (dd, 1 H, J = 8, 4 Hz), 7.25 (q, 1 H, J = 1 Hz); ^{13}C NMR (D_2O) δ 12.3 (C-11), 25.7 (C-4), 28.2 (C-3), 41.2 (C-5), 61.1 (C-2), 111.5 (C-9), 141.6 (C-10), 153.3 (C-7), 157.5 (C-6), 167.2 (C-8), 176.5 (C-1); UV (H_2O) λ_{max} 273 nm (log ε 3.91). The *E* geometry of the carbon–carbon double bonds of **3** and **4** was assigned by their ^1H and ^{13}C NMR data as described previously.[7b]

(11) Compound **3** was slowly converted to **2** in neutral or alkaline aqueous solution even at room temperature. Acidic solution of **3** rapidly produced **2** in almost quantitative yield.
(12) Irradiation of **1** and 1-methylguanidine in aqueous alkaline solution did not produce any photoadduct.
(13) Lyophilized powder obtained from Sigma (DNA 40%, proteins 54%) was used without purification. Its spectral ratios were as follows: A_{260}/A_{230} = 1.53; A_{260}/A_{280} = 1.83; max/min = 1.69; A_{260}/A_{320} = 116. The proteins contain lysine (22.6%) and arginine (10.7%) as determined by amino acid analyzer.
(14) Reverse-phase Nucleosil $7C_{18}$ column (1 × 25 cm); 0.5% aqueous acetic acid.

Scheme I. Schematic Representation of the Proposed Mechanism for Photo-Cross-Linking in Nucleosome



peak corresponding to **2** gave a pure product whose chromatographic behaviors,[7a,15] UV spectrum,[7a] and 400-MHz ^{1}H NMR[8] are identical with those of the authentic sample obtained from the model experiment. The contents of thymine–lysine adduct **2** were directly determined by HPLC analysis of the acid hydrolysates of UV-irradiated nucleohistone under various conditions. The amount of **2** in UV-irradiated nucleohistone at pH 10.5 increased proportionally with increasing irradiation time within 40 min, and further irradiation no more increased the yield of **2** significantly (Table I). This may reflect that the lysine ϵ-amino group of intimate association with thymine in DNA is primarily responsible for the formation of **2**.[16] Irradiation at pH 7.5 proceeded less efficiently, and the yield of **2** increased with increasing pH.

In order to confirm whether a similar photo-cross-linking is feasible in irradiation of nuclei, we examined the irradiation of chicken erythrocyte nuceli[17] (0.1 mg/mL) at 0 °C in the same buffer at pH 9.5. After being heated at 70 °C, the photolysate was hydrolyzed (6 N HCl, 110 °C) and then analyzed by amino acid analyzer to reveal again the presence of **2** in the hydrolysate. Control experiments without irradiation or irradiation with light through a Pyrex filter (>280 nm) never produced **2** under the conditions. Thus, we were able to confirm that irradiation of DNA–protein systems in calf thymus nucleohistone or chicken erythrocyte nuclei with 254-nm light followed by acid hydrolysis leads ultimately to the formation of **2**. However, we are not confident whether the adduct formation is resulted solely from the photoreaction of specific DNA–histone systems because of the presence of other proteins in nucleohistone used.[18]

It has previously been reported that UV irradiation of chromatin produces DNA–histone adducts.[4,19] Kunkel and Martinson[20] have observed that a considerable amount of reversal of DNA–histone cross-links occurred in irradiation of calf thymus nuclei with light between 230 and 290 nm, i.e., the histones cross-linked to DNA are released by chemical hydrolysis or upon rechromatography on Sepharose column, while the chemical nature of the reversibility and the sites of binding have been totally unknown.[21] By knowing the chemical reactivity of **3** and by the confirmation of **2** in UV-irradiated DNA–protein systems, it seems probable that the apparent reversibility is due to the cross-links of the lysine ϵ-amino groups to thymine in DNA as outlined in eq 1. Thus, the photoexcited state of thymine N(3) monoanion[7b] in DNA would react with neighboring lysine ϵ-amino groups of histones to result in the formation of DNA–histone adducts which on subsequent heating release free histones containing partially modified lysine residues as illustrated in Scheme I. We have already demonstrated that irradiation of DNA with alkylamines and subsequent heating of the photolysate at an alkaline pH induce DNA strand scission at the sites of reacting thymine.[7b,23] It is therefore highly probable that DNA in UV-irradiated chromatin may suffer strand scission at the sites of reacting thymine upon heating under the slightly alkaline conditions, although the mechanistic details should await further studies using each of the purified histone–DNA adducts.

In conclusion, the present work has demonstrated an important role of thymine–lysine photoreaction in the photo-cross-linking of proteins to DNA. Since the thymine–lysine photoadduct **2** is readily detectable by HPLC and amino acid analyzer, irradiation with 254-nm light may provide a useful means for probing specific interaction sites between thymine and lysine residues in DNA–protein complexes. Furthermore, such type of photo-cross-linking may be relevant to the UV-induced damage on DNA in cells.

Registry No. 1, 50-89-5; **2**, 76945-38-5; **3**, 87616-31-7; **4**, 87616-32-8; **5**, 87616-33-9; 2-deoxy-D-ribose, 533-67-5; L-lysine, 56-87-1; L-arginine, 74-79-3.

(15) R_f 0.6 on silica gel TLC (iso-PrOH–30% ammonia–water, 7:1:2).
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Cobalt-Catalyzed Cocyclizations of Isocyanatoalkynes: A Regiocontrolled Entry into 5-Indolizinones. Application to the Total Synthesis of Camptothecin

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Pyridones, particularly those containing bridgehead nitrogen, constitute useful moieties in heterocycle construction of value in alkaloid[1] and drug[2] synthesis. We report the cobalt-catalyzed [2 + 2 + 2] cocyloaddition of the novel 5-isocyanatoalkynes **1** to alkynes, providing a chemo- and regioselective entry into the class of functionalized 2,3-dihydro-5(1*H*)-indolizinones. The method is readily applied to a total synthesis of the antitumor alkaloid camptothecin.[3]

The requisite starting material 5-isocyanato-1-pentyne (**1a**) [bp 85 °C (70 torr)], is available[4] from 5-hexynoic acid via acid chloride formation [$(COCl)_2$, C_6H_6, DMF (catalyst), 25–35 °C, 82%][5] and Curtius rearrangement (NaN_3, CH_3CN, Δ, 1 h, 74%), whereas **1b** [bp 80–85°C (0.2 torr)] is prepared from 3-carbomethoxypropanoyl chloride[6] according to Scheme I.

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(4) All new compounds gave satisfactory spectral and analytical data. For example, **3a**: *m/e* (relative ion current) 249.1362 (M^+, 3.3, calcd for $C_{14}H_{19}NO_3$: 249.1365), 87 (base, 7.7); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.15 (t, 3 H, *J* = 7.2), 1.62 (s, 3 H), 2.17 (tt, 2 H, *J* = 7.3, 7.7), 2.76 (q, 2 H, *J* = 7.2), 3.04 (t, 2 H, *J* = 7.7), 3.78 (m, 2 H), 4.03 (m, 2 H), 4.15 (t, 2 H, *J* = 7.3), 6.36 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 13.4, 21.2, 21.6, 26.4, 31.7, 48.8, 64.4, 77.4, 99.1, 108.6, 129.0, 146.2, 150.3, 162.6 ppm; IR (neat) 1647, 1600, 1587 cm^{-1}. **4a**: ^{1}H NMR ($CDCl_3$) δ 1.74 (t, 3 H, *J* = 7.4), 1.79 (s, 3 H), 2.13 (tt, 2 H, *J* = 7.2, 7.7), 2.79 (q, 2 H, *J* = 7.4), 3.01 (t, 2 H, *J* = 7.7), 3.82 (m, 2 H), 4.01 (m, 2 H), 4.10 (t, 2 H, *J* = 7.2), 5.90 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 15.5, 22.0, 25.0, 27.5, 32.0, 49.0, 64.5, 77.5, 104.0, 110.5, 126.0, 148.0, 153.5, 160.5.
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