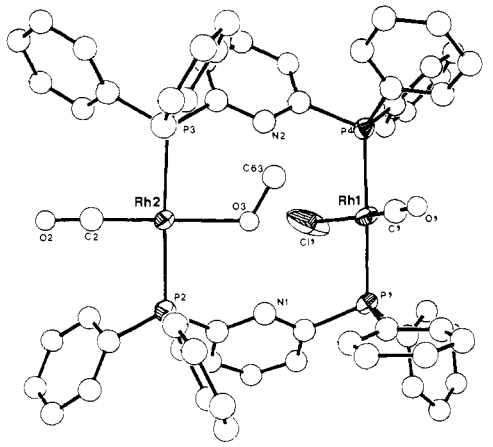
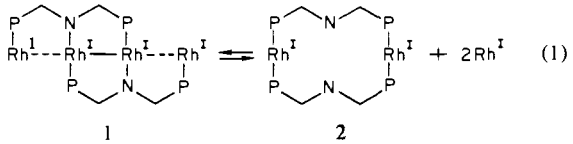


Figure 1. A perspective view of $[Rh_2[\mu\text{-}(Ph_2P)_2py]_2(CO)(CH_3OH)Cl]^+$. Some selected interatomic distances (Å) and angles (deg): Rh(1)–P(1), 2.313 (4); Rh(1)–P(4), 2.314 (4); Rh(1)–Cl(1), 2.321 (6); Rh(1)–C(1), 1.78 (1); Rh(2)–P(2), 2.333 (4); Rh(2)–P(3), 2.329 (4); Rh(2)–C(2), 1.809 (16); Rh(2)–O(3), 2.144 (6); N(1)···O(3), 2.679 (18); Rh(1)···Rh(2), 5.425 (2); Cl(1)–Rh(1)–C(1), 160.6 (2); P(1)–Rh(1)–P(4), 176.8 (1); C(2)–Rh(2)–O(3), 173.3 (9); P(2)–Rh(2)–P(3), 177.8 (2).

phosphate in methanol. The infrared spectrum of **2** shows the presence of two terminal carbonyl groups (ν(CO): 2075, 1991 cm^{-1}) and the methanol (ν(H): 3052 cm^{-1}). The $^{31}P\{^1H\}$ NMR spectrum indicates that two equally populated, rhodium-bound phosphorus environments are present (δ 31.3, ^1J(Rh,P) = 128.2 Hz; δ_2 20.4, ^1J(Rh,P) = 128.9). The crystal structure of the compound has been determined by X-ray diffraction at 140 K.[6] Figure 1 shows a view of the cation. The two rhodium atoms, the four phosphorus atoms, and the two nitrogen atoms form a nearly planar framework. Each of the two nonequivalent rhodium atoms is four coordinate and planar with trans phosphorus atoms. To complete its coordination, Rh(1) has trans carbonyl and chloride ligands, which are inclined at an angle of 67° with respect to the $Rh_2P_4N_2$ framework. Rh(2) has trans carbonyl and methanol ligands, which lie on a line that is directed 17° away from the $Rh_2P_4N_2$ plane. The orientation of ligands on Rh(2) is largely determined by the constraints imposed by hydrogen bonding N(1)···HO(3). The methanol ligand and one carbonyl group, C(1)–O(1), lie within a wall-like structure of four phenyl rings (those closest to the viewer in Figure 1). Within this cavity both the methyl group, which pivots about the N(1)···HO(3) hydrogen bond, and the carbonyl group show evidence of high thermal motion or disorder.

In the conversion of **1** to **2**, the phosphine ligands have undergone realignment and two rhodium atoms have been eliminated as shown schematically in eq 1. The rhodium atoms that are



1 2 (1)

removed are converted into well-known species, $Rh(CO)_2Cl_2^-$ and $Rh_2(\mu\text{-}Cl)_2(CO)_4$, which have been detected by infrared spectroscopy. The overall stoichiometry of the reaction is given by eq 2. This reaction is reversible. Treatment of **2** with $Rh_2(\mu\text{-}Cl)_2(CO)_4$ and $[n\text{-}Bu_4N]Cl$ in chloroform solution reforms **1**, which has been reisolated in 64% yield.

$$Rh_4[\mu\text{-}(Ph_2P)_2py]_2(\mu\text{-}CO)(CO)_2Cl_4 + CH_3OH + 3CO \rightleftarrows [Rh_2[\mu\text{-}(Ph_2P)_2py]_2(CO)_2(CH_3OH)Cl]^+ + Rh(CO)_2Cl_2^- + {}^1/_2Rh_2(\mu\text{-}Cl)_2(CO)_4 \quad (2)$$

Not all reactions of **1** result in such drastic rearrangement. Addition of sulfur dioxide to **1** yields green $Rh_4[\mu\text{-}(Ph_2P)_2py]_2(\mu\text{-}SO_2)(CO)_2Cl_4$ (**3**) (IR ν(CO) 2079, 2003, $\nu(SO_2)$ 1210, 1062; $^{31}P\{^1H\}$ NMR δ 35.7, ^1J(Rh,P) = 131 Hz; δ_2 30.7, ^1J(Rh,P) = 138), and **3** may be reconverted to **1** by the careful addition of a limited amount of carbon monoxide. The conversion of **1** to **3** appears to simply involve substitution of the bridging carbonyl by a bridging sulfur dioxide.[7]

The occurrence of the reversible realignment shown in eq 1 establishes a new aspect of the coordination chemistry of this type of polynuclear complex. In dealing with phosphine bridged complexes, it is certainly presumptive to expect that the phosphine/metal framework will remain inviolate during chemical reactions.

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Registry No. 1, 87555-67-7; **2**·CH_2Cl_2, 87555-70-2; **3**, 87566-97-0.

Supplementary Material Available: A list of atomic fractional coordinates and thermal parameters for $[Rh_2[\mu\text{-}(Ph_2P)_2py]_2(CO)_2(CH_3OH)Cl][PF_6]\cdot CH_2Cl_2$ and a stereoscopic drawing of the cation (3 pages). Ordering information is given on any current masthead page.

(6) Orange crystals (dec 250 °C) of $[Rh_2[\mu\text{-}(Ph_2P)_2py]_2(CO)_2(CH_3OH)Cl][PF_6]\cdot CH_2Cl_2$ were grown by diffusion of ethyl ether into a dichloromethane solution of the complex. At 140 K, a = 10.181 (5) Å, b = 37.708 (24) Å, c = 16.193 (11) Å, β = 105.14 (5)°. The space group is $P2_1/c$ (No. 14) with Z = 4. Of 8473 observed unique reflections, 4008 > 6$\sigma(F)$ were used in the refinements. The ω scan speed was 60° min^{-1}, using Mo (λ = 0.71069 Å) radiation. The structure was solved by Patterson methods, refinements were by least squares to R = 0.062. All atoms were included in the refinements, except the hydrogen atoms of methanol.

(7) A similar reaction sequence interconverts $Rh_2(\mu\text{-}Ph_2Ppy)_2(\mu\text{-}CO)Cl_2$ and $Rh_2(\mu\text{-}Ph_2Ppy)_2(\mu\text{-}SO_2)Cl_2$: Farr, J. P.; Olmstead, M. M.; Hunt, C. H.; Balch, A. L. *Inorg. Chem.* **1981**, *20*, 1182–1187.

Biosynthesis of Estrogens: The Steric Mode of the Initial C-19 Hydroxylation of Androgens by Human Placental Aromatase

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In this communication we report the results of our studies on the steric mode of the initial ("first") C-19 hydroxylation[1] in the biosynthetic conversion of androgens to estrogens by human placental enzymes.[2-5]

Previously we have proven that the steric mode of enzymatic hydroxylation at a primary carbon atom can be determined with the use of a substrate having a chiral methyl (labeled with ^3H, ^2H, ^1H) provided that the oxygenation involves a kinetic (normal) hydrogen isotope effect[6-9] ($k_H > k_D > k_T$) (D = ^2H; T = ^3H).

Our approach to the investigation of the elaboration of estrogens was as follows. For the sake of argument we will assume that the "first" *C-19* hydroxylation[1] of, eg., (19*R*)-[19-^{3}H,19-^{2}H,19-^{1}H]-3β-hydroxyandrost-5-en-17-one (**1**) proceeds in a retention mode and with a kinetic isotope effect $k_H > k_D > k_T$. Thus the main product of the reaction will be the (19*S*)-alcohol **2**, which will be accompanied by a minor amount of (19*R*)-alcohol **3**. Since

(1) Meyer, A. S. *Biochim. Biophys. Acta* **1955**, *17*, 441.
(2) Akhtar, M.; Skinner, S. J. M. *Biochem. J.* **1968**, *109*, 318.
(3) Akhtar, M.; Calder, M. R.; Corina, D. L.; Wright, J. N. *Biochem. J.* **1982**, *201*, 569.
(4) Fishman, J.; Raju, M. S. *J. Biol. Chem.* **1981**, *256*, 4472.
(5) Townsley, J. D.; Brodie, H. J. *Biochemistry* **1968**, *7*, 33.
(6) Caspi, E.; Shapiro, S.; Piper, J. U. *Chem. Commun.* **1981**, 76; *Tetrahedron* **1981**, *37*, 3535.
(7) Caspi, E.; Shapiro, S.; Piper, J. U. *Chem. Commun.* **1981**, 1196.
(8) Shapiro, S.; Piper, J. U.; Caspi, E. *J. Am. Chem. Soc.* **1982**, *104*, 2301.
(9) Shapiro, S.; Arunachalam, T.; Caspi, E. *J. Am. Chem. Soc.* **1983**, *105*, 1642.

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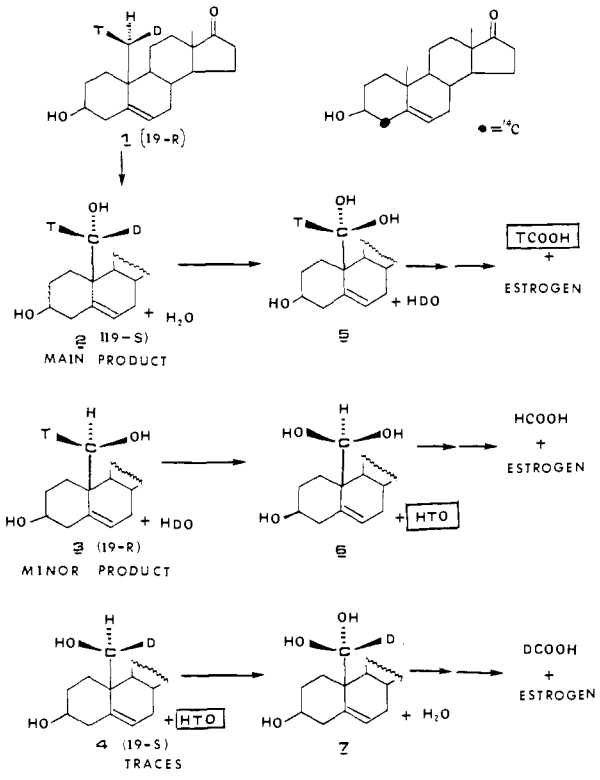
Scheme I



the tritium isotope effect is relatively large[6-10] insignificant amounts of alcohol (**4**) resulting from the abstraction of the C-19 tritium atom will be formed. It should be noted that the abstracted C-19 hydrogen, deuterium, and tritium atoms will be ultimately transferred to the water of the medium.[2,4] However considering that only minute amounts of alcohol **4** will be formed, the amounts of tritiated water produced by abstraction of tritium in the "first" hydroxylation reaction can be *disregarded.*

It was firmly established that the subsequent transformation of the 19-alcohol(s), presumably to 19-diol(s),[2] involves the loss of the 19-*pro-R* hydrogen atom Scheme I.[2,3] Most likely the "second" hydroxylation proceeds in the retention mode,[3,11] and the abstracted hydrogen is ultimately transferred to the water of the medium.[2,3] Hence the diol **5** derived from the (19*S*)-alcohol **2** will *retain a tritium* atom at C-19. In contrast the diol **6** derived from (19*R*)-alcohol **2** will be *devoid of tritium*, and the *tritium will be transferred to the water of the medium.* Obviously since **2** was the major product and **3** was a minor product, more tritium will be retained at C-19 of diol **5** while a smaller amount of tritium will be found in the water. Subsequently diols **5** and **6** will be transformed into estrogens and their C-19 atoms will be extruded as formic acid. It was proven that the *formic acid retains* the hydrogen atoms that initially had the 19-*pro-S* configuration[2,12] in alcohols **2** and **3**.

It follows therefore that if the first hydroxylation proceeds with retention and $k_H > k_D > k_T$ the aromatization of a (19*R*)-[^{3}H,^{2}H,^{1}H]-androgen **1** will yield formic acid, which will *retain a larger amount of tritium* than the amount that will be found in *the water* of the medium.[2,12]

The opposite results are expected if the "first" hydroxylation of the (19*R*)-androgen will proceed with inversion, namely *more* tritium will be released into the *water* than will be retained by the *formic* acid.

The rationalization is equally applicable to (19*S*)-3β-hydroxyandrost-5-en-17-one. In this instance the "first" hydroxylation in the retention mode will ultimately yield *formic acid* that will contain *less* tritium than the *water*, while hydroxylation in the inversion mode will *give formic acid* that will contain *more* tritium than the *water.*

In any event if the first C-19 hydroxylation is stereospecific and proceeds with an isotope effect, tritiated formic acid and tritiated water will be produced in *unequal* amounts. From the relative amounts of tritiated formic acid and tritiated water derived from the aromatization of an androgen of known C-19 chirality, the mode of the first C-19 hydroxylation can be deduced. It should be stressed that for the deductions to be valid the experiments with 19*R* and 19*S* substrates must be carried out, and the results should be complementary.

If the reaction involves *an isotope effect* but is nonstereospecific, proceeding with racemization, then *equal* (*or nearly equal*) *amounts* of tritiated formic acid and tritiated water will be produced from both 19*R* and 19*S* substrates.

However, in the *absence of an isotope effect* irrespective of the C-19 chirality of the substrate and irrespective of whether the first C-19 hydroxylation proceeds with retention, inversion, or racemization, the tritiated formic acid and tritiated water will be produced at a *constant* (*1*:*2*) *ratio.*

The (19*R*)- and (19*S*)-[19-^{3}H,19-^{2}H,19-^{1}H]-3β-hydroxyandrost-5-en-17-one and (19*RS*)-[19-^{3}H,19-^{2}H,19-^{1}H]-3β-hydroxyandrost-5-en-17-one required for these studies were synthesized in our laboratory.[13,14] For C-19-chirality determination the compounds were converted to the androst-4-ene-3,17-diones, (Jones' reagent), which were treated with selenium dioxide–hydrogen peroxide and the resulting seco acid lactones[15] were then submitted to Kuhn–Roth oxidation. The derived samples of acetic acids were assayed by the malate synthetase fumarase procedure[16-18] and showed, for 19*R*, 19*S*, and 19*RS*, F = 63, 33, and 50, respectively.[18]

The incubations of 19*R* and 19*S*, and 19*RS* substrates were carried out and processed as described for the (19*R*)-androgen. To the (19*R*)-[19-^{3}H,19-^{2}H,19-^{1}H]-3β-hydroxyandrost-5-en-17-one (2.1 μCi, 100 μg) [4-^{14}C]-3β-hydroxyandrost-5-en-17-one (1 μCi, 35 μg) was added and the mixture was incubated (1 h) (in triplicate) with human placental aromatase.[19,20] The incubations were terminated with 0.74 M phosphoric acid (1 mL) frozen (liquid N_2) and lyophilized.[21] The recovered aqueous solution of formic acid was counted, made alkaline (1.25 M NaOH) (phenolphthaleine), and frozen, and the water was removed by lyophilization. The sodium formate and the water were counted.

The residue in the incubation flask was diluted with water and extracted with ethyl acetate, and the phenolic fraction was re-

(10) Yakushin, F. S. *Russ. Chem. Rev.* (*Engl. Transl.*) **1962**, *31*, 123.
(11) Duaz, W. L.; Weeks, C.; Rohrer, D. C. *Recent Prog. Horm. Res.* **1976**, *32*, 81.
(12) Arigoni, D.; Battaglia, R.; Akhtar, M.; Smith T. *Chem. Commun.* **1975**, 185.

(13) The syntheses involved the preparation of 19-chiral alcohols which were converted to 19-chiral iodides without loss of optical purity and these were reduced to chiral 19*R*- and 19*S*-methyl groups (submitted for publication).
(14) All compounds were characterized by NMR and MS. The homogeneity of the products was determined by HPLC using a variable UV detector. Where applicable the radiochemical purity was determined with a HPLC-Radiomatic Flow-1 system.
(15) Caspi, E.; Balasubrahmanyam, S. N. *J. Org. Chem.* **1963**, *28*, 3383. This preliminary oxidation was required to circumvent scrambling of 19-methyl protons in Kuhn–Roth oxidation. The K. R. oxidation of the resulting seco acid lactones were carried out in sealed vials at room temperature (72 h).
(16) Cornforth, J. W.; Redmond, J. W.; Eggerer, H.; Buckel, W.; Gutschow, C. *Nature* (*London*) **1969**, *221*, 1212; Eur. J. Biochem. **1970**, *14*, 1.
(17) Luthy, J.; Retey, J.; Arigoni, D. *Nature* (*London*) **1969**, *221*, 1213.
(18) Floss, H. G.; Tsai, M. D. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1979**, *50*, 243.
(19) Placental aromatase was prepared from human full-term placentae.[20] The tissue was homogenized and the microsomal fraction separated out by ultracentrifugation. A microsomal aliquot equivalent to 21 g of wet tissue and containing 45 mg of protein was used for each incubation. The incubation mixture[3] contained 15 mM glucose-6-phosphate, 9 mM $NADP^+$, 5 μkat glucose-6-phosphate dehydrogenase, 100 μg of C-19 chiral substrate, and 35 μg of 4-^{14}C substrate in a total volume of 5 mL of 5.0 mM Tris buffer, pH = 7.3. Incubations were carried out for 1 h at 37–38 °C in the air and were terminated with 1 mL of 0.74 M phosphoric acid.
(20) Ryan, K. J. *J. Biol. Chem.* **1959**, *234*, 368.
(21) Following the initial removal of the volatile components, water (0.5 mL) was added and the mixture was lyophilized again. This operation was repeated twice. The distilate of the "third wash" did not contain tritium.

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		
19R[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
19RS[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	
19S[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 (19RS)-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 (19R)-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 (19S)-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 19RS 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 19R and 19S 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 19R, 19S, and 19RS substrates.

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

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.

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

(23) The average tritium ratios of products derived from (19R)- and (19S)-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 19R and 19S substrates are exactly reciprocal, it follows that the second hydroxylation proceeds without a detectable isotope effect.

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-

(1) Photoinduced Reactions. 151.

(2) (a) Smith, K. C., Ed. "Aging, Carcinogenesis and Radiation Biology: The Role of Nucleic Acid Addition Reactions"; Plenum Press: New York, 1976. (b) Kornhauser, A. *Photochem. Photobiol.* **1976**, *10*, 411. (c) Smith, K. C. *Photochem. Photobiol. Nucleic Acids* **1976**, *2*, 187.

(3) (a) Sperling, J.; Havron, A. *Biochemistry* **1976**, *15*, 1489. (b) Schimmel, P. R. *Acc. Chem. Rees.* **1977**, *15*, 1489. (c) Shetlar, M. D. *Photochem. Photobiol. Rev.* **1980**, *5*, 105.

0002-7863/83/1505-6989$01.50/0