Structures of 3β- and 17β-Oxirane Inhibitors of 3-Oxo-Δ⁵-steroid Isomerase

Setsuo Kashino,[†,⊥] Henry Katz,[†] Jenny P. Glusker,*[†] Ralph M. Pollack,*[‡] and Patricia L. Bounds[‡]

Contribution from The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, and The Laboratory for Chemical Dynamics, Department of Chemistry, University of Maryland Baltimore County, Catonsville, Maryland 21228.
Received November 14, 1986

Abstract: The crystal structures of (3*S*)-spiro[5α-androstane-3,2′-oxiran]-17β-ol (**1**β) and (17*S*)-spiro[5α-androstane-17,2′-oxiran]-3β-ol (**2**β) have been determined. Detailed three-dimensional parameters are given. These compounds act as active-site-directed irreversible inhibitors of the 3-oxo-Δ⁵-steroid isomerase of *Pseudomonas testosteroni* (EC 5.3.3.1). (3*S*)-Spiro[5α-androstane-3,2′-oxiran]-17β-ol monohydrate ($C_{20}H_{32}O_2 \cdot H_2O$, **1**β) is orthorhombic with space group $P2_12_12$, a = 10.060 (1) Å, b = 22.496 (4) Å, c = 8.036 (1) Å, V = 1818.5 (4) Å³, and Z = 4. (17*S*)-Spiro[5α-androstane-17,2′-oxiran]-3β-ol ($C_{20}H_{32}O_2$, **2**β) is also orthorhombic with space group $P2_12_12_1$, a = 10.096 (1) Å, b = 46.517 (5) Å, c = 7.467 (1) Å, V = 3506.8 (8) Å³, and Z = 8. The structures were solved by using a multiple-solution method and refined by a full-matrix least-squares method. The final residuals were 0.039 for 1821 observed reflections for **1**β and 0.070 for 2742 observed reflections for **2**β, respectively. A comparison of the structure of **1**β with that of the substrate 5-androstene-3,17-dione suggests that both the oxirane oxygen of **1**β and the carbonyl oxygen of the substrate are protonated by an acidic group of the enzyme during reaction. When the structure of **2**β is rotated 180° about an axis perpendicular to the plane of the steroid nucleus and compared with that of **1**β, it is found that the overall shapes are similar. In addition, the 17β-oxirane oxygen atom of **2**β is in almost the same relative location as the 3β-oxirane oxygen atom of **1**β. This result is consistent with the finding that **2**β can bind in two orientations to the enzyme and that inactivation by 17β-oxiranes occurs by modification of the same carboxyl group on the enzyme as that by 3β-oxiranes.

The 3-oxo-Δ⁵-steroid isomerase (EC 5.3.3.1) from *Pseudomonas testosteroni* catalyzes the isomerization of a wide variety of 3-oxo-Δ⁵-steroids to their corresponding conjugated Δ⁴ isomers (Scheme I).[1] This enzyme is of particular interest because its remarkably high turnover number (4.4×10^6 min⁻¹) puts it among the most active enzymes known. Although the isomerase has been purified to homogeneity[2] and its physical and molecular properties are well described,[1,3] there are still many unanswered questions concerning the mechanism. Several investigators, utilizing active-site-directed inhibitors and suicide inactivators, have shown both Asp-38 and Asn-57 to be present at the active site,[4-6] and Asp-38 has been implicated in the mechanism by virtue of its "hyperreactivity".[4a,b] Histidine and tyrosine residues have also been proposed to be important for catalysis,[1] although there is some doubt about the role of histidine in the mechanism.[7] Malhotra and Ringold[8] suggested a mechanism over two decades ago that involves the action of an acidic and a basic group on the enzyme to give the intermediate formation of a dienol. This mechanism is supported by the recent finding that the isomerase can catalyze the ketonization of the trienol 3-hydroxy-3,5,7-estratrien-17-one to 4,7-estradiene-3,17-dione.[9] Proton transfer has been shown to occur predominantly from the 4β position to the 6β position,[8,10] indicating that the base responsible for proton transfer must be above the β-face of the steroid. Studies by Carrell et al.[11] are in accord with this interpretation.

The nature of binding of steroids to the active site of the isomerase has also been extensively investigated. Weintraub et al.[12] have examined over 90 steroid derivatives and related compounds as inhibitors and have proposed a model for the active site. Recently, Westbrook et al.[13] reported the crystal structure of the isomerase at 6-Å resolution and proposed that the substrate binding site is a deep hydrophobic pit with one end open to solvent. Hearne and Benisek[4c] have concluded that Asp-38 is located near the base of this pit.

Work from one of our laboratories has been concerned with the irreversible inhibition of the isomerase by 3β- and 17β-oxiranes and the relationship of these results to the enzyme mechanism and to the nature of steroid binding at the active site.[5,14] For both the 3β- and 17β-oxiranes, inactivation occurs via stoichio-

Scheme I

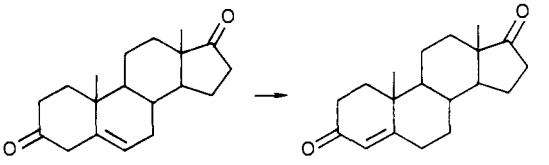


Table I. Crystal Data

	1β	**2**β
mol formula	$C_{20}H_{32}O_2 \cdot H_2O$	$C_{20}H_{32}O_2$
formula wt	322.49	304.47
F(000)	712	1344
space group	$P2_12_12$	$P2_12_12_1$
a, Å	10.060 (1)	10.096 (1)
b, Å	22.496 (4)	46.517 (5)
c, Å	8.036 (1)	7.467 (1)
V, Å³	1818.5 (4)	3506.8 (8)
Z	4	8
D(calcd), g cm⁻³	1.178	1.153
λ(Cu Kα), Å	1.5418	1.5418
μ(Cu K), cm⁻¹	5.26	4.82
cryst size, mm	0.20 × 0.03 × 0.25	0.30 × 0.13 × 0.09

[†] Institute for Cancer Research.
[⊥] Visiting Scientist, on leave from the Department of Chemistry, Okayama University, Okayama 700, Japan.
[‡] University of Maryland Baltimore County.

(1) (a) Talalay, P.; Benson, A. M. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic: New York, 1972; Vol. 6, pp 591–618. (b) Batzold, F. H.; Benson, A. M.; Covey, D. F.; Robinson, C. H.; Talalay, P.; *Adv. Enzymol. Regul.* **1976**, *14*, 243–267.
(2) Kawahara, F. S.; Talalay, P. *J. Biol. Chem.* **1960**, *235*, PC1–PC2.
(3) (a) Wang, S. F.; Kawahara, F. S.; Talalay, P. *J. Biol. Chem.* **1963**, *238*, 576–585. (b) Benson, A. M.; Jarabak, R.; Talalay, P. *J. Biol. Chem.* **1971**, *246*, 7514–7525. (c) Benson, A. M.; Suruda, A. J.; Talalay, P. *J. Biol. Chem.* **1975**, *250*, 276–280. (d) Tivol, W. F.; Beckman, E. D.; Benisek, W. F. *J. Biol. Chem.* **1975**, *250*, 271–275. (e) Weintraub, H.; Vincent, F.; Baulieu, E.-E. *FEBS Lett.* **1973**, *37*, 82–88.
(4) (a) Martyr, R. J.; Benisek, W. F. *J. Biol. Chem.* **1975**, *250*, 1218–1222. (b) Ogez, J. R.; Tivol, W. F.; Benisek, W. F. *J. Biol. Chem.* **1977**, *252*, 6151–6155. (c) Hearne, M.; Benisek, W. F. *Biochemistry* **1985**, *24*, 7511–7516.
(5) (a) Kayser, R. H.; Bounds, P. L.; Bevins, C. L.; Pollack, R. M. *J. Biol. Chem.* **1983**, *258*, 909–915. (b) Bounds, P. L.; Pollack, R. M. *Biochemistry* **1987**, *26*, 2263–2269.

0002-7863/87/1509-6765$01.50/0

metric covalent bond formation between the steroid and a carboxylate side chain within the peptide corresponding to residues 14–45 of the enzyme.[5a,14c] This carboxylate residue has been shown to be Asp-38 for both series of oxiranes.[5a,b] To accommodate these results, we have proposed that there are two modes of binding of steroids to the isomerase.[14b,f] We report here the X-ray crystal structures of the isomeric oxiranyl steroids (3*S*)-spiro[5α-androstane-3,2′-oxiran]-17β-ol (**1**β) and (17*S*)-spiro-[5α-androstane-17,2′oxiran]-3β-ol (**2**β) and the implication of these results for both the mechanism of action of the isomerase and the nature of steroid binding to the active site.

Experimental Section

The oxiranes, **1**β and **2**β, respectively, were available from previous investigations.[14a,b] Crystals were grown from hexane–ethyl acetate solutions (ca. 4:1 in volume). Cell dimensions (Table I) were obtained from a least-squares fit of angular data from 15 reflections measured on an automated 4-circle diffractometer.

Three-dimensional diffraction data were collected with graphite-monochromated Cu Kα radiation with use of the variable θ–2θ scan technique in the 2θ range 0–138° (sin θ/λ = 0.606 Å^{-1}) with the minimum scan rate of 1 deg min^{-1}. In this way, and with a scan:background time ratio of 2.5, intensities were recorded respectively for 1967 and 3752 unique data of which 1821 and 2742 had intensity $I \geq 1.0\sigma(I)$ and $2.33\sigma(I)$, respectively (where $\sigma(I)$ was determined from counting statistics) and were used in further calculations. Values of $\sigma(F)$ were obtained from the expression $\sigma(F) = (F/2[\sigma^2(I)/(I)^2 + \delta^2]^{1/2}$, where δ (=0.023 and 0.043, respectively) is an instrumental uncertainty determined from the variation in intensity of three check reflections periodically monitored during the data collection. There was no indication of deterioration of the crystal of **1**β, but a small decay correction was applied to the **2**β data. Structure amplitudes were obtained by correcting the intensities for geometric factors and were placed on an absolute scale with a Wilson plot.

The structures were solved with use of a multiple-solution method (MULTAN 78). The positions of the non-hydrogen atoms so obtained were refined by a full-matrix least-squares procedure, first with isotropic and then anisotropic temperature factors. The quantity minimized was $\sum w||F_o| - |F_c||^2$ where the weights, *w*, were $1/\sigma^2(F)$. All hydrogen atoms attached to the carbon atoms were found in a Fourier difference map and were included in the subsequent refinements with isotropic temperature factors. A difference map revealed the positions of hydrogen atoms attached to the oxygen atoms. All hydrogen atoms were included in the final least-squares refinement. An occupancy factor of 0.5 was assumed for the disordered hydrogen atom attached to O(2) in **1**β. The atomic scattering factors used were from *International Tables for X-ray Crystallography*.[15] The computer programs used were from the Crystallographic Program Library of the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA.[16]

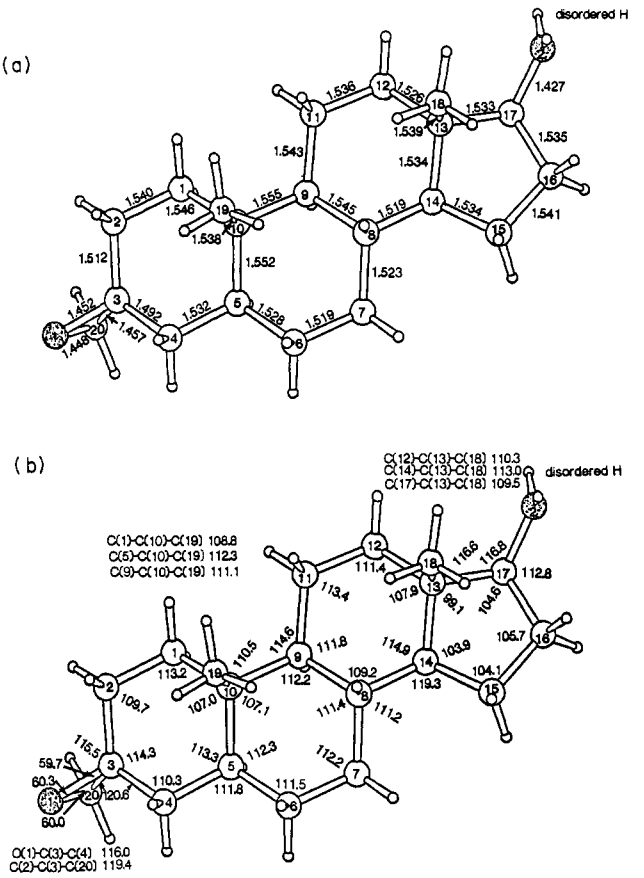
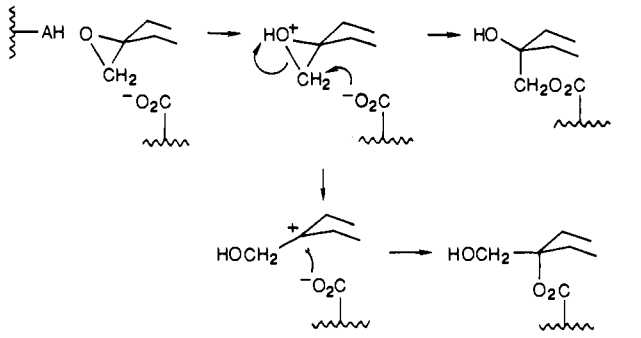


Figure 1. (a) Bond lengths and (b) interbond angles for **1**β. View from above the plane through the C and D rings. Large open circles are carbon atoms, stippled circles are oxygen atoms, and small open circles are hydrogen atoms.

Scheme II



The final residuals for **1**β were $R = 0.039$ and $wR = 0.041$, and the final difference Fourier map had no peak higher than 0.12 e Å^{-3}. For **2**β the equivalent values were $R = 0.070$ and $wR = 0.065$; the highest peak in the difference map was 0.20 e Å^{-3}.

Results

Final atomic parameters for structures **1**β and **2**β are given in Tables II and III, respectively. Bond lengths and interbond angles are shown in Figures 1 and 2, together with the atomic numbering. Some selected torsion angles of interest are listed in Table IV, and the geometry of hydrogen bonds is given in Table V. There are two molecules in the asymmetric unit of **2**β, and therefore two measurements were made of each feature of the molecular geometry of this structure.

The geometry of the molecules **1**β and **2**β resembles that commonly found in steroids with rings A, B, and C in the chair conformation and the five-membered ring D in a 13β-envelope conformation for **1**β and a 13β,14α half-chair conformation for each molecule of **2**β.

(6) (a) Penning, T. M.; Talalay, P. *J. Biol. Chem.* **1981**, *256*, 6151–6155. (b) Penning, T. M.; Covey, D. F.; Talalay, P. *J. Biol. Chem.* **1981**, *256*, 6842–6850. (c) Penning, T. M.; Heller, D. N.; Balasubramanian, T. M.; Fenselau, C. C.; Talalay, P. *J. Biol. Chem.* **1982**, *257*, 12589–12593.
(7) Benisek, W. F.; Ogez, J. R. *Biochemistry* **1982**, *21*, 5816–5825.
(8) Malhotra, S. K.; Ringold, H. J. *J. Am. Chem. Soc.* **1965**, *87*, 3228–3236.
(9) Bantia, S.; Pollack, R. M. *J. Am. Chem. Soc.* **1986**, *108*, 3145–3146.
(10) (a) Viger, A.; Marquet, A. *Biochim. Biophys. Acta* **1977**, *485*, 482–487. (b) Viger, A.; Coustal, S.; Marquet, A. *J. Am. Chem. Soc.* **1981**, *103*, 451–458.
(11) Carrell, H. L.; Glusker, J. P.; Covey, D. F.; Batzold, F. H.; Robinson, C. H. *J. Am. Chem. Soc.* **1978**, *100*, 4282–4289.
(12) Weintraub, H.; Vincent, F.; Baulieu, E.-E.; Alfsen, A. *Biochemistry* **1977**, *16*, 5045–5053.
(13) Westbrook, E. M.; Piro, O. E.; Sigler, P. B. *J. Biol. Chem.* **1984**, *259*, 9096–9103.
(14) (a) Pollack, R. M.; Kayser, R. H.; Bevins, C. L. *Biochem. Biophys. Res. Commun.* **1979**, *91*, 783–790. (b) Bevins, C. L.; Kayser, R. H.; Pollack, R. M.; Ekiko, D. B.; Sadoff, S. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1131–1137. (c) Bevins, C. L.; Bantia, S.; Pollack, R. M.; Bounds, P. L.; Kayser, R. H. *J. Am. Chem. Soc.* **1984**, *106*, 4957–4962. (d) Bantia, S.; Bevins, C. L.; Pollack, R. M. *Biochemistry* **1985**, *24*, 2606–2609. (e) Pollack, R. M.; Bantia, S.; Bounds, P. L.; Koffman, B. M. *Biochemistry* **1986**, *25*, 1905–1911. (f) Bevins, C. L.; Pollack, R. M.; Kayser, R. H.; Bounds, P. L. *Biochemistry* **1986**, *25*, 5159–5164.
(15) *International Tables for X-ray Crystallography*; Kynoch: Birmingham, England, 1962; Vol. IV, pp 71–147.
(16) Carrell, H. L. *ICRFMLS. Modification of UCLALS4 (1975)*; Gantzel, P. K., Sparks, R. A., Long, R. E., Trueblood, K. N., UCLALS4 Program in Fortran IV (1969).

Table II. Atomic Parameters with Estimated Standard Deviations in Parentheses for **1β** [(3*S*)-Spiro[5α-androstane-3,2′-oxiran]-17β-ol][a]

atom	*x*	*y*	*z*	*B*
O(1)	0.3290 (2)	0.00705 (6)	0.9922 (2)	4.69 (7)
O(2)	0.3726 (1)	0.47434 (5)	0.4973 (2)	4.11 (6)
C(1)	0.3276 (2)	0.17661 (8)	0.9701 (2)	3.48 (8)
C(2)	0.3531 (2)	0.11583 (9)	1.0524 (3)	3.82 (9)
C(3)	0.3358 (2)	0.06694 (8)	0.9252 (3)	3.64 (9)
C(4)	0.4179 (2)	0.07448 (8)	0.7721 (3)	3.72 (9)
C(5)	0.3921 (2)	0.13556 (8)	0.6940 (2)	3.01 (8)
C(6)	0.4676 (2)	0.14344 (9)	0.5305 (3)	4.01 (9)
C(7)	0.4305 (2)	0.20130 (8)	0.4448 (3)	3.58 (8)
C(8)	0.4466 (2)	0.25482 (8)	0.5592 (2)	2.84 (7)
C(9)	0.3720 (2)	0.24569 (8)	0.7253 (2)	2.73 (7)
C(10)	0.4159 (2)	0.18792 (8)	0.8161 (2)	2.82 (7)
C(11)	0.3748 (2)	0.30217 (8)	0.8342 (3)	3.41 (8)
C(12)	0.3297 (2)	0.35834 (8)	0.7414 (3)	3.64 (8)
C(13)	0.4096 (2)	0.36770 (8)	0.5822 (2)	2.88 (7)
C(14)	0.3968 (2)	0.31127 (8)	0.4763 (2)	2.85 (7)
C(15)	0.4538 (2)	0.32943 (9)	0.3068 (3)	4.01 (9)
C(16)	0.4173 (3)	0.39572 (9)	0.2907 (3)	4.37 (10)
C(17)	0.3543 (2)	0.41296 (8)	0.4577 (3)	3.48 (8)
C(18)	0.5547 (2)	0.38340 (9)	0.6242 (3)	3.95 (9)
C(19)	0.5617 (2)	0.19206 (10)	0.8727 (3)	4.17 (9)
C(20)	0.2114 (3)	0.03383 (10)	0.9201 (3)	4.88 (11)
O(W)	0.1386 (2)	0.50036 (8)	0.6564 (2)	6.61 (9)
H(1A)	0.234 (2)	0.1779 (8)	0.934 (2)	4.0 (5)
H(1B)	0.349 (2)	0.2072 (8)	1.054 (3)	4.2 (5)
H(2A)	0.441 (2)	0.1135 (9)	1.103 (3)	5.5 (6)
H(2B)	0.299 (2)	0.1092 (9)	1.146 (3)	4.3 (5)
H(4A)	0.401 (2)	0.0440 (8)	0.693 (2)	3.0 (4)
H(4B)	0.514 (2)	0.0691 (10)	0.797 (3)	5.6 (6)
H(5)	0.296 (2)	0.1387 (8)	0.667 (2)	2.7 (4)
H(6A)	0.567 (2)	0.1414 (9)	0.550 (3)	5.2 (5)
H(6B)	0.448 (2)	0.1100 (9)	0.454 (3)	4.1 (5)
H(7A)	0.482 (2)	0.2061 (9)	0.344 (3)	4.9 (5)
H(7B)	0.332 (2)	0.1984 (9)	0.420 (3)	4.2 (5)
H(8)	0.543 (2)	0.2606 (8)	0.579 (3)	3.9 (5)
H(9)	0.276 (2)	0.2388 (7)	0.693 (2)	2.4 (4)
H(11A)	0.322 (2)	0.2961 (9)	0.935 (3)	4.0 (5)
H(11B)	0.459 (2)	0.3085 (9)	0.885 (3)	4.3 (5)
H(12A)	0.230 (2)	0.3523 (9)	0.716 (3)	5.2 (6)
H(12B)	0.342 (2)	0.3926 (8)	0.812 (3)	3.6 (4)
H(14)	0.301 (2)	0.3062 (8)	0.461 (2)	2.4 (4)
H(15A)	0.552 (2)	0.3273 (10)	0.304 (3)	5.2 (6)
H(15B)	0.420 (2)	0.3067 (9)	0.212 (3)	4.0 (5)
H(16A)	0.503 (2)	0.4211 (11)	0.273 (3)	5.9 (6)
H(16B)	0.362 (2)	0.4049 (10)	0.200 (3)	4.9 (6)
H(17)	0.258 (2)	0.4076 (7)	0.451 (2)	2.2 (4)
H(18A)	0.557 (2)	0.4215 (10)	0.689 (3)	5.3 (6)
H(18B)	0.601 (2)	0.3516 (9)	0.682 (3)	4.4 (5)
H(18C)	0.608 (3)	0.3900 (11)	0.530 (3)	6.1 (6)
H(19A)	0.599 (3)	0.1568 (10)	0.918 (3)	6.2 (6)
H(19B)	0.620 (2)	0.2039 (10)	0.786 (3)	5.4 (6)
H(19C)	0.574 (3)	0.2209 (12)	0.968 (4)	7.3 (8)
H(20A)	0.191 (3)	0.0139 (10)	0.811 (3)	6.5 (7)
H(20B)	0.141 (2)	0.0430 (10)	1.005 (3)	5.2 (6)
H(0WA)	0.124 (3)	0.4792 (10)	0.671 (3)	5.9 (6)
H(0WB)	0.158 (4)	0.5014 (14)	0.765 (4)	9.8 (9)
H(02)	0.299 (5)	0.4915 (23)	0.555 (7)	6.8 (13)
H(02D)	0.445 (3)	0.4847 (15)	0.509 (5)	2.4 (8)

[a] *x*, *y*, and *z* are given in fractions of unit cell edges. *B* are averaged isotropic displacement factors ($^1/_3$ trace of the orthogonalized B matrix).

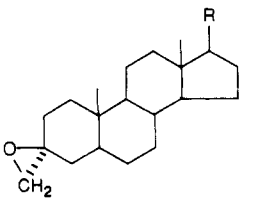
In **1β**, the C(3)–O(1) bond is equatorial and the epoxy group lies almost perpendicular to the ring A on the β-side of the molecule, with C(20) out of the molecular plane. The hydrogen atom of the hydroxyl group on C(17) is disordered, but neither of the O–H bonds is trans to the C(13)–C(17) bond (as is found in many other steroids).[17]

Similarly, in **2β** the C(17)–O(1) bond is equatorial with C(20) out of the molecular plane. The hydrogen atoms on C(20) were located with difficulty. The hydroxyl group on C(3) does not appear to be disordered although, again, the hydrogen atom position was hard to locate.

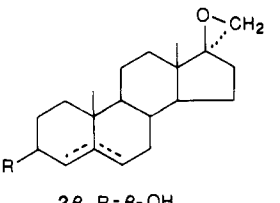
In **2β**, the two molecules in the asymmetric unit pack with epoxide O(1) near C(20′) of another molecule (at 3.406 Å) and H(02) of the hydroxyl group on C(3) forming a hydrogen bond to the epoxide O(1′). The molecules extend along the *b* axis, roughly perpendicular to the *c* axis. Molecules, related by a twofold screw axis along the *a*-direction, pack along that direction.

Discussion

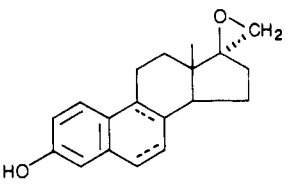
Affinity Alkylation of the Isomerase by 3β- and 17β-Oxiranyl Steroids. Both 3β-oxiranes (**1β**, **7β**, **10β**) and 17β-oxiranes (**2β**–**6β**, **8β**) have been shown to function as active-site-directed irreversible inhibitors of the 3-oxo-Δ^5-steroid isomerase from *Pseudomonas testosteroni*, although neither 3α- nor 17α-oxiranes show any signs of irreversible inhibition of the enzyme.[14a,b] For both 3β- and



1β, R = β-OH
7β, R = =O
10β, R = CHOHCH3



2β, R = β-OH
3β, R = β-OH, Δ^5
4β, R = =O, Δ^4
8β, R = =O



5β, $\Delta^{1,3,5(10)6,8}$
6β, $\Delta^{1,3,5(10)}$

17β-oxiranes, inactivation occurs by attack of an enzymic carboxylate on the methylene and spiro carbons of the oxirane (Scheme II).[14c,d] This group has been identified as Asp-38 for inactivation by the 3β-oxirane **1β**[5b] and the 17β-oxiranes **4β** and **5β**.[14]

Several lines of evidence point to a remarkable similiarity of the reaction of the 17β-oxiranes to that of the 3β-oxiranes. (1) Both types of oxiranes react with the same amino acid residue.[5,14c] (2) Two different products are formed from alkylation of the isomerase in all cases.[14c,d] (3) Cleavage of each of these products in base yields analogous steroid products for both the 3β- and 17β-isomers.[14c,d] (4) The second-order rate constants of inactivation (k_3/K_I) are similar for both series.[14a,b,f] (5) The corresponding 3α- and 17α-isomers do not irreversibly inactivate the enzyme.[14a,b]

These results were rationalized by postulating that the 17β-oxiranes can bind in two orientations.[14b,f] The binding modes are related by a 180° rotation of the steroid around an axis perpendicular to the plane of the steroid ring. Further spectral studies during the inactivation of the isomerase by (17*S*)-spiro[estra-1,3,5-pentaene-17,2′-oxiran]-3-ol (**5β**) support this interpretation.[14f]

The apparent ability of the steroid isomerase to bind steroids in two modes leads to the question of how the enzyme can interchangeably recognize both ends of the steroid molecule. The similarity of the second-order rate constants for inactivation by the 3β- and 17β-oxiranes (e.g., k_3/K_I = 66 M^{-1} s^{-1} for **1β** and 300 M^{-1} s^{-1} for **2β**) suggests that the binding of the transition state to the enzyme has similar characteristics in the two cases. A comparison of the X-ray structures of **1β** and **2β** supports this hypothesis. If **2β** is rotated 180° about an axis perpendicular to the ring, it becomes apparent that the overall size and shape of "backwards" **2β** is similar to that of **1β**. The sideways view (Figure 3) shows that the oxirane oxygens are in almost identical positions when the steroid rings are aligned. It is important to note that the methylene and ring carbons of the oxirane moiety are also

(17) Glusker, J. P. *Biochem. Actions Horm.* **1979**, *6*, 139–143.

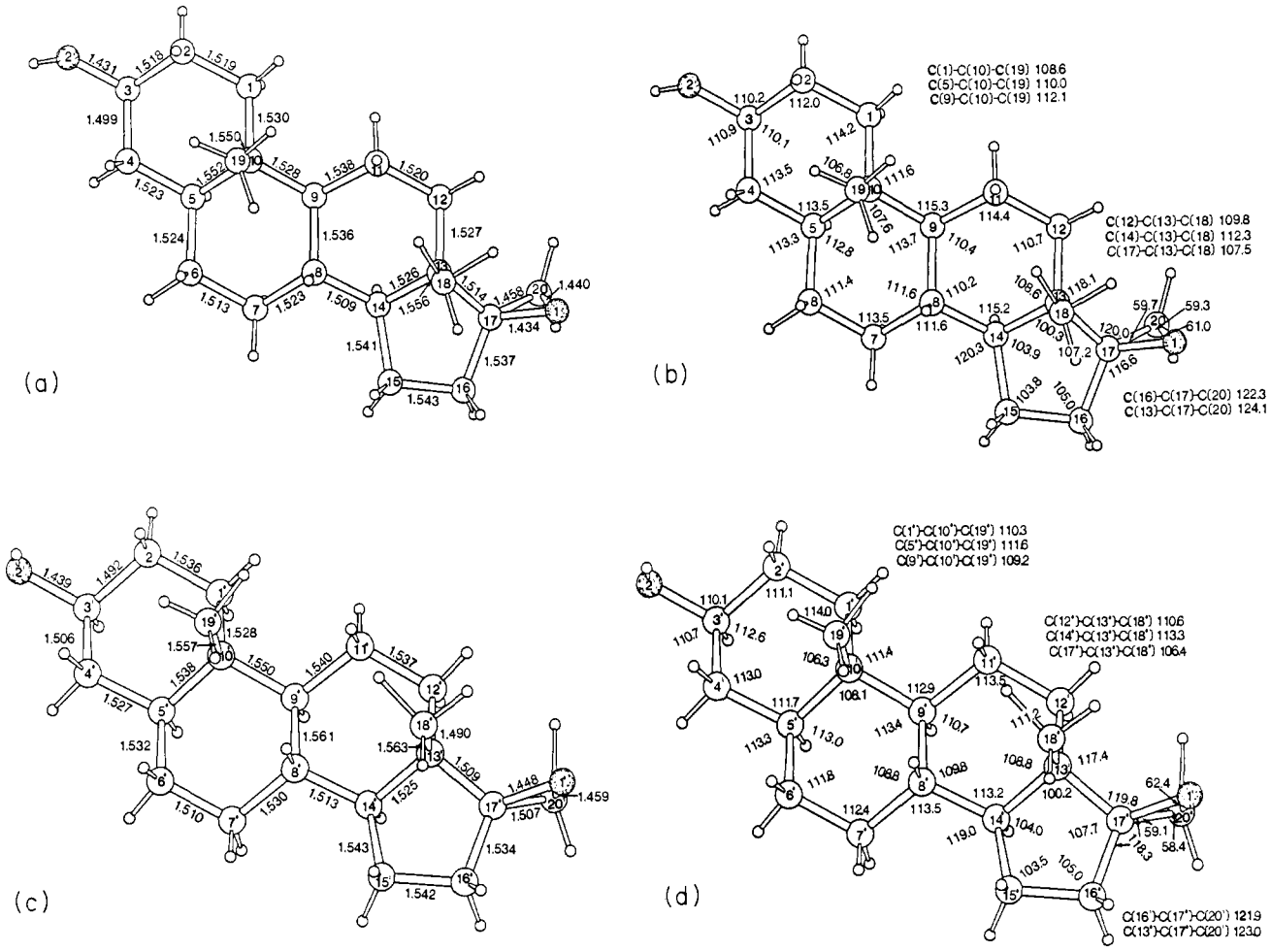


Figure 2. (a) Bond lengths and (b) interbond angles for **2**β and (c) bond lengths and (d) bond angles for the second molecule. View from above the plane through the C and D rings. Large open circles are carbon atoms, stippled circles are oxygen atoms, and small open circles are hydrogen atoms.

in comparable positions. Thus, both protonation by AH and attack by carboxylate of the enzyme could occur with almost equal facility for the two compounds.

A view from the top, looking down onto the β face, shows that complete alignment of these two structures cannot be achieved (Figure 4). In particular, overlap of the two steroid ring systems requires that the 17-oxygen of **1**β and the 3-oxygen of **2**β be unaligned. Furthermore, the relative positions of the angular methyl groups are also different. However, a slight sideways displacement of the steroid nucleus of **2**β allows the oxygens to overlap and in addition enables the angular methyl groups to be along the same axis relative to the long direction of the steroid nucleus. It is not unreasonable that the enzyme can accommodate this sideways displacement since 4,4-dimethyl steroids are able to bind to the active site.[18]

Backwards binding has been proposed for a variety of other steroid transforming enzymes.[19] Sweet and Samant[19a] and Strickler et al.[19b] have suggested that the active site of 3α,20β-hydroxysteroid dehydrogenase can bind steroids in two modes. They suggest that the two modes for this enzyme are related by a 180° rotation about the long axis of the steroid nucleus, a "flipflopping" of the α and β faces.

Implications for the Enzyme Mechanism. Since epoxide linkages are generally unreactive toward nucleophiles, the ability of these oxiranes to rapidly react with an enzyme carboxylate (Asp-38) suggests that protonation of the oxirane oxygen may be occurring.[20] It is thus likely that the oxiranes are activated toward the attack of the enzyme carboxylate via protonation of the ring oxygen by an appropriately situated acidic side chain of the enzyme. An active-site proton donor capable of protonating the 3-oxo group of the substrate has long been a feature of the proposed catalytic mechanisms.[8]

A comparison of the structures of **1**β and the specific substrate 5-androstene-3,17-dione[11] suggests that the putative enzyme acid group used to protonate the carbonyl oxygen during substrate catalysis (AH) should also be in the correct position to protonate the oxirane oxygen of **1**β. Side views of these two steroids (Figure 3) show that both the oxirane oxygen of **1**β and the carbonyl oxygen of the substrate are almost in the plane of the steroid ring. This observation, besides supporting a mechanistic basis for the inactivation reaction, supplies indirect evidence for the presence of an acidic side chain in the active site. If the structure of **2**β is rotated so that its epoxide group is juxtaposed to that of **1**β, it becomes apparent that the equatorial oxygen of the 17β-oxirane also occupies a position similar to that of the 3-oxo group of the substrate. Murray-Rust and Glusker[21] have shown that the orientation of electron pairs, and thus potential hydrogen bonds, is almost identical for oxygen atoms in both ketones and epoxides. Thus, the oxirane oxygen atom of **1**β should be a good probe for the environment of the 3-ketone oxygen atom of the substrate.

(18) (a) Neville, A. M.; Engel, L. L. *J. Clin. Endocrinol.* **1968**, *28*, 49–60. (b) Goldman, A. S. *J. Clin. Endocrinol.* **1968**, *28*, 1539–1546.

(19) (a) Sweet, F.; Samant, B. R. *Biochemistry* **1980**, *19*, 978–986. (b) Strickler, R. C.; Covey, D. F.; Tobias, B. *Biochemistry* **1980**, *19*, 4950–4954. (c) Adams, J. B.; McDonald, D. *Biochim. Biophys. Acta* **1981**, *664*, 460–468. (d) Waxman, D. J.; Ko, A.; Walsh, C. *J. Biol. Chem.* **1983**, *258*, 11937–11947.

(20) See, for example: (a) Long, F. A.; Pritchard, J. G. *J. Am. Chem. Soc.* **1956**, *78*, 2663–2667, 2667–2670. (b) Cook, C. E.; Corley, R. C.; Wall, M. W. *J. Org. Chem.* **68**, *33*, 2789–2793. (c) Ross, A. M.; Pohl, T. M.; Piazza, K.; Thomas, M.; Whalen, D. L. *J. Am. Chem. Soc.* **1982**, *104*, 1658–1665.

(21) Murray-Rust, P.; Glusker, J. P. *J. Am. Chem. Soc.* **1984**, *106*, 1018–1025.

Table III. Atomic Parameters with Estimated Standard Deviations in Parentheses for **2**β [(17*S*)-Spiro[5α-androstane-17,2′-oxiran]-3β-ol][a]

atom	*x*	*y*	*z*	*B*	atom	*x*	*y*	*z*	*B*
				Molecule 1					
O1	0.0928 (3)	0.36300 (5)	0.6648 (4)	7.8 (2)	H3	−0.039 (4)	0.1525 (9)	0.756 (6)	8.7 (13)
O2	0.0958 (3)	0.12350 (5)	0.8366 (4)	8.3 (2)	H4	0.135 (5)	0.1525 (9)	0.551 (6)	7.9 (16)
C1	0.0658 (4)	0.20299 (7)	0.9276 (6)	5.7 (2)	H4A	0.245 (4)	0.1594 (8)	0.664 (6)	6.7 (11)
C2	0.0882 (4)	0.17124 (9)	0.9645 (6)	6.7 (2)	H5	0.036 (4)	0.1993 (7)	0.584 (5)	4.3 (9)
C3	0.0651 (5)	0.15292 (9)	0.7993 (7)	6.5 (3)	H6	0.187 (5)	0.1923 (11)	0.324 (7)	11.2 (17)
C4	0.1465 (5)	0.16396 (9)	0.6462 (6)	6.5 (2)	H6A	0.298 (4)	0.2052 (7)	0.472 (5)	4.1 (9)
C5	0.1265 (4)	0.19587 (8)	0.6095 (5)	5.3 (2)	H7	0.065 (4)	0.2378 (8)	0.381 (5)	6.2 (11)
C6	0.1996 (4)	0.20643 (8)	0.4434 (6)	5.8 (2)	H7A	0.224 (4)	0.2440 (8)	0.302 (6)	6.6 (13)
C7	0.1648 (4)	0.23730 (8)	0.4005 (6)	5.7 (2)	H8	0.276 (4)	0.2585 (7)	0.579 (5)	4.2 (9)
C8	0.1794 (4)	0.25755 (8)	0.5597 (6)	4.9 (2)	H9	0.017 (3)	0.2457 (6)	0.687 (5)	3.1 (8)
C9	0.1094 (4)	0.24573 (8)	0.7268 (6)	4.9 (2)	H11	0.207 (4)	0.2692 (8)	0.932 (6)	6.6 (12)
C10	0.1509 (4)	0.21515 (8)	0.7759 (5)	4.7 (2)	H11A	0.069 (4)	0.2598 (8)	0.977 (6)	5.5 (12)
C11	0.1163 (4)	0.26768 (9)	0.8805 (5)	5.6 (2)	H12	0.080 (4)	0.3118 (7)	0.931 (5)	4.7 (9)
C12	0.0667 (4)	0.29753 (8)	0.8316 (6)	5.9 (2)	H12A	−0.023 (4)	0.2975 (8)	0.793 (6)	6.0 (12)
C13	0.1407 (4)	0.30911 (8)	0.6685 (6)	5.3 (2)	H14	0.031 (3)	0.2825 (6)	0.507 (4)	1.8 (6)
C14	0.1288 (4)	0.28729 (8)	0.5168 (6)	5.1 (2)	H15	0.145 (3)	0.2975 (7)	0.233 (5)	3.2 (8)
C15	0.1814 (5)	0.30357 (10)	0.3517 (6)	6.7 (3)	H15A	0.291 (5)	0.3013 (10)	0.356 (7)	9.5 (15)
C16	0.1333 (5)	0.33470 (10)	0.3812 (7)	7.7 (3)	H16	0.055 (5)	0.3406 (11)	0.290 (8)	9.7 (18)
C17	0.0864 (5)	0.33564 (9)	0.5769 (7)	6.4 (3)	H16A	0.249 (7)	0.3480 (13)	0.389 (10)	16.4 (23)
C18	0.2874 (5)	0.31537 (10)	0.7190 (7)	7.5 (3)	H18	0.321 (7)	0.3001 (14)	0.814 (10)	16.9 (27)
C19	0.2989 (4)	0.21344 (9)	0.8326 (6)	6.0 (2)	H18A	0.324 (5)	0.3285 (9)	0.626 (6)	6.8 (14)
C20	−0.0349 (5)	0.35029 (9)	0.6309 (8)	7.5 (3)	H18B	0.278 (5)	0.3322 (10)	0.841 (7)	9.2 (14)
HO2	0.068 (5)	0.1090 (10)	0.773 (7)	8.9 (19)	H19	0.325 (4)	0.1937 (8)	0.851 (6)	6.5 (12)
H1	0.094 (4)	0.2124 (7)	1.039 (5)	5.5 (10)	H19A	0.356 (4)	0.2276 (7)	0.753 (5)	4.7 (10)
H1A	−0.027 (4)	0.2053 (8)	0.899 (6)	7.0 (13)	H19B	0.308 (4)	0.2237 (7)	0.948 (5)	4.8 (10)
H2	0.038 (4)	0.1646 (9)	1.047 (6)	5.8 (13)	H20	−0.084 (4)	0.3605 (8)	0.537 (6)	7.2 (13)
H2A	0.180 (4)	0.1706 (8)	1.004 (6)	6.5 (12)	H20A	−0.105 (5)	0.3474 (10)	0.746 (7)	9.7 (17)
				Molecule 2					
O1′	0.4310 (3)	0.39160 (5)	0.4713 (5)	9.4 (2)	H3′	0.438 (4)	0.6046 (8)	0.539 (6)	6.8 (12)
O2′	0.6044 (4)	0.62873 (6)	0.5961 (5)	10.0 (2)	H4′	0.503 (4)	0.5937 (10)	0.843 (6)	7.4 (14)
C1′	0.5350 (4)	0.55527 (8)	0.4056 (5)	6.1 (2)	H4′A	0.649 (5)	0.5855 (11)	0.794 (8)	10.9 (18)
C2′	0.5936 (5)	0.58568 (8)	0.4186 (6)	7.2 (3)	H5′	0.389 (4)	0.5548 (8)	0.696 (5)	5.7 (11)
C3′	0.5384 (5)	0.60151 (9)	0.5756 (7)	7.3 (3)	H6′	0.441 (5)	0.5451 (9)	0.998 (5)	6.8 (13)
C4′	0.5457 (5)	0.58434 (10)	0.7463 (7)	7.5 (3)	H6′A	0.576 (4)	0.5361 (8)	0.955 (6)	5.7 (11)
C5′	0.4871 (4)	0.55422 (9)	0.7276 (6)	5.6 (2)	H7′	0.304 (6)	0.5115 (11)	0.869 (8)	14.2 (21)
C6′	0.4831 (5)	0.53758 (10)	0.9045 (6)	7.2 (3)	H7′A	0.428 (4)	0.4991 (8)	0.985 (5)	5.0 (11)
C7′	0.4165 (5)	0.50872 (10)	0.8835 (5)	7.2 (3)	H8′	0.575 (3)	0.4871 (7)	0.765 (5)	4.5 (9)
C8′	0.4808 (4)	0.49036 (9)	0.7375 (5)	5.4 (2)	H9′	0.387 (4)	0.5112 (7)	0.529 (5)	4.6 (9)
C9′	0.4823 (4)	0.50781 (8)	0.5589 (5)	5.2 (2)	H11′	0.637 (5)	0.4846 (9)	0.443 (7)	8.6 (14)
C10′	0.5531 (4)	0.53726 (9)	0.5750 (6)	5.2 (2)	H11′A	0.524 (3)	0.5000 (7)	0.302 (5)	3.2 (9)
C11′	0.5360 (5)	0.48929 (9)	0.4041 (6)	6.1 (2)	H12′	0.513 (4)	0.4503 (9)	0.300 (6)	6.0 (12)
C12′	0.4689 (4)	0.45973 (9)	0.3895 (6)	6.1 (2)	H12′A	0.370 (4)	0.4657 (8)	0.346 (5)	5.4 (10)
C13′	0.4767 (4)	0.44372 (8)	0.5619 (6)	5.4 (2)	H14′	0.314 (4)	0.4659 (7)	0.657 (5)	5.1 (9)
C14′	0.4125 (4)	0.46180 (9)	0.7085 (6)	5.4 (2)	H15′	0.328 (4)	0.4457 (8)	0.949 (6)	5.8 (11)
C15′	0.3989 (5)	0.44083 (11)	0.8675 (7)	8.0 (3)	H15′A	0.490 (5)	0.4341 (10)	0.928 (7)	8.2 (14)
C16′	0.3649 (5)	0.41196 (11)	0.7770 (8)	8.9 (3)	H16′	0.272 (5)	0.4066 (12)	0.805 (9)	11.0 (20)
C17′	0.3964 (5)	0.41648 (10)	0.5781 (8)	7.3 (3)	H16′A	0.423 (5)	0.3968 (9)	0.825 (7)	7.0 (14)
C18′	0.6233 (5)	0.43529 (10)	0.6044 (7)	7.8 (3)	H18′	0.699 (5)	0.4550 (11)	0.581 (7)	9.8 (15)
C19′	0.7031 (4)	0.53217 (9)	0.6121 (6)	6.5 (2)	H18′A	0.628 (6)	0.4252 (13)	0.711 (8)	12.1 (22)
C20′	0.3056 (6)	0.40584 (11)	0.4323 (9)	9.1 (4)	H18′B	0.649 (8)	0.4242 (16)	0.496 (10)	16.5 (33)
H02′	0.682[b]	0.6243[b]	0.615[b]	9.8[b]	H19′	0.748 (4)	0.5485 (8)	0.637 (6)	6.5 (13)
H1′	0.574 (4)	0.5457 (7)	0.315 (5)	4.4 (10)	H19′A	0.727 (5)	0.5178 (9)	0.713 (7)	10.2 (18)
H1′A	0.433 (4)	0.5590 (8)	0.378 (6)	6.8 (12)	H19′B	0.726 (7)	0.5277 (13)	0.475 (9)	14.4 (24)
H2′	0.569 (6)	0.5968 (12)	0.293 (9)	15.3 (23)	H20′	0.250 (10)	0.3932 (16)	0.509 (14)	23.3 (42)
H2′A	0.686 (4)	0.5821 (9)	0.444 (7)	8.1 (15)	H20′A	0.312 (6)	0.4233 (13)	0.247 (9)	14.2 (21)

[a] *x*, *y*, and *z* are given in fractions of unit cell edges. *B* are averaged isotropic displacement factors ($^1/_3$ trace of orthogonalized B matrix). [b] Atom not defined.

The ability of the 3β- and 17β-oxiranes to rapidly inactivate the isomerase in an active-site-directed process may be due to a large extent to the existence of an acidic group capable of protonating the oxygen. By extension then, this group should be capable of protonating the 3-oxo-Δ^5-substrates.

In both series of oxiranes, the attacking nucleophile is Asp-38.[5b,14] There is substantial evidence that this group is a participant in the enzyme mechanism. Benisek et al. have shown that Asp-38 is hyperreactive[4a,b] and is located near the A ring of a bound steroid molecule.[4c] In addition, the pH–rate profile for the enzyme-catalyzed isomerization of the substrate 5(10)-estrene-3,17-dione is virtually identical with that for the inactivation of the isomerase by (3*S*)-spiro[5α-androstane-3,2′-oxiran]-17-one, the 17-oxo analogue of **1**β, indicating that the same functional groups are important in both reactions.[14e]

We have previously shown that Asp-38 is located on the α-side of the bound 3β- and 17β-oxiranes in the productive binding mode for inactivation.[14c,d] Since proton transfer in the catalytic reaction (isomerization of 5-androsten-3,17-dione) is 4β to 6β,[8,10] we suggested that Asp-38 may not be the enzyme base that is involved in the catalytic 4β to 6β proton transfer but rather that it is the α-side base previously postulated by Viger et al.[10] to account for labilization of the 4α-proton during the catalytic reaction. In addition, we suggested that Asp-38 might function as an electrostatic catalyst.[14c]

Recent investigations by Westbrook[22] into the crystal structure of the isomerase, however, have revealed that Asp-38 may have

(22) Westbrook, E. M., personal communication.

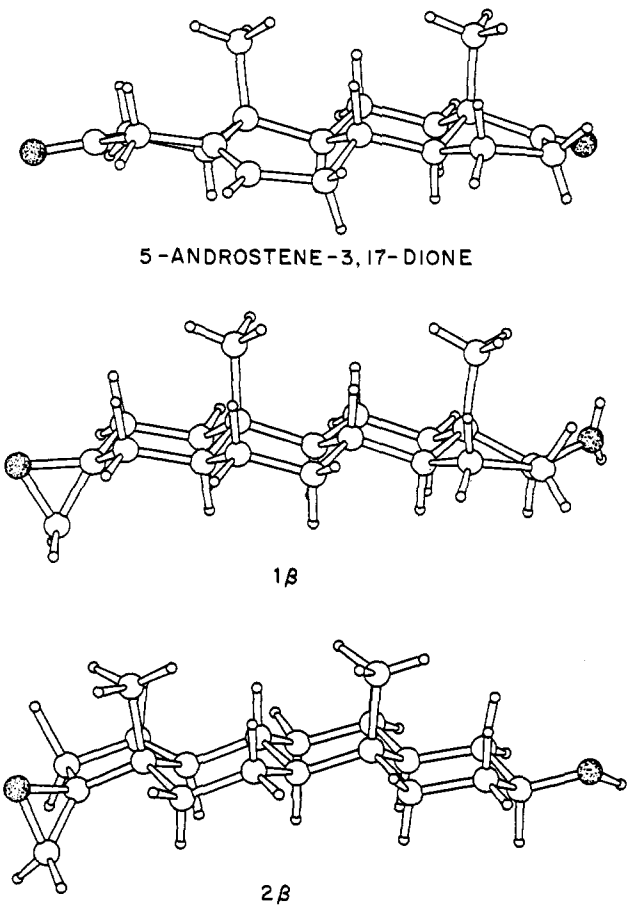


Figure 3. Comparison of 5-androstene-3,17-dione with **2β** and **1β** viewed along the C and D ring plane. Large open circles are carbon atoms, stippled circles are oxygen atoms, and small open circles are hydrogen atoms.

Table IV. Torsion Angles (deg) in the Regions of Oxygen Atoms

Structure **1β**	
C(3) epoxy group	
C(1)–C(2)–C(3)–O(1)	−167.4 (2)
C(1)–C(2)–C(3)–C(20)	−99.3 (3)
C(17) hydroxyl group	
C(15)–C(16)–C(17)–O(2)	151.6 (2)
C(13)–C(17)–O(2)–H(02)	−94 (5)
C(13)–C(17)–O(2)–H(02D)	59 (4)
Structure **2β**	
C(17) epoxy group	
C(15)–C(16)–C(17)–O(1)	152.8 (5), 153.4 (5)
C(15)–C(16)–C(17)–C(20)	−137.7 (6), −137.2 (6)
C(3) hydroxyl group	
C(1)–C(2)–C(3)–O(2)	176.1 (4), 174.1 (4)
C(4)–C(3)–O(2)–H(02)	−77 (5), 63[a]

[a] Not refined.

access to the β-face of bound steroids and is, thus, a candidate for the proton transfer group at the active site. In light of these results, it is necessary to reevaluate the evidence for two basic groups at the active site. The salient points that are consistent with the hypothesis of two basic groups at the active site are the following: (1) during the isomerization of 5-androstene-3,17-dione there is some loss of the 4α-proton, but this proton is not transferred to C-6;[10] (2) there is significant intramolecular proton transfer from C-4β to C-6β;[10] (3) Asp-38 is located at the α-face of the steroid during inactivation of the enzyme by 3β- and 17β-oxiranes;[14c,d] and (4) 3α- and 17α-oxiranes do not irreversibly inactivate the isomerase.[14a,b] Westbrook's results suggest that the isomerase may bind steroids "upside down" as well as "backwards". If the enzyme does indeed bind steroids in two modes that differ

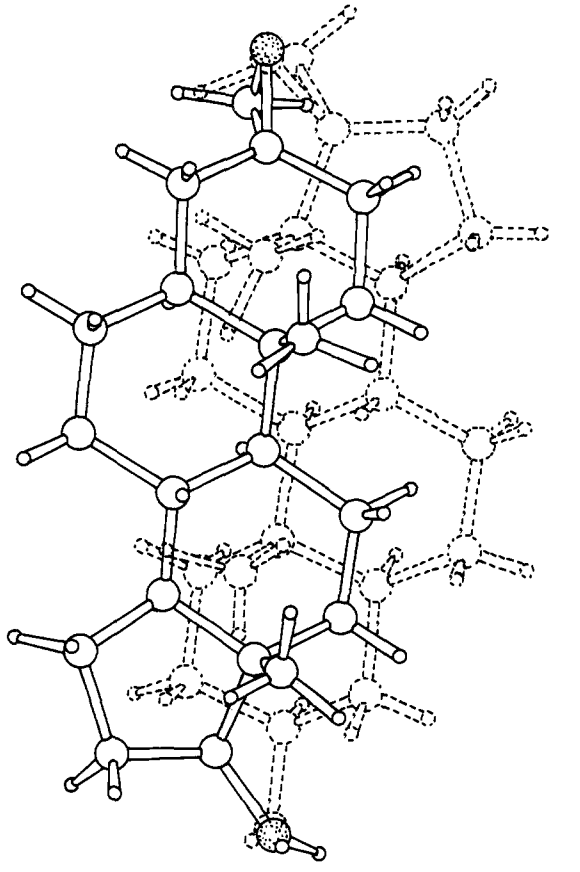


Figure 4. Top view of **1β** superimposed on "backwards" **2β**. Large open circles are carbon atoms, stippled circles are oxygen atoms, and small open circles are hydrogen atoms.

Table V. Geometry of Hydrogen Bonds

	O···O (Å)	H···O (Å)	O—H···O (deg)
(a) Compound **1β**			
$O(W^{i})$–$H(0WB^{i})$···$O(1^{iii})$	2.848 (2)	1.97 (3)	171 (3)
$O(2^{i})$—$H(02^{i})$···$O(W^{i})$	2.742 (3)	1.83 (6)	159 (5)
$O(2^{i})$—$H(02D^{i})$···$O(2^{ii})$	2.813 (2)	2.06 (4)	167 (4)
symmetry code: (i) x, y, z; (ii) $1-x, 1-y, z$; (iii) $^1/_2-x, ^1/_2+y, 2-z$			
(b) Compound **2β**			
$O(2^{i})$—$H(02^{i})$···$O(1'^{ii})$	2.923 (5)	2.29 (6)	130 (4)
$O(2'^{i})$—$H(02'^{i})$···$O(2^{iii})$	3.077 (5)	2.27[a]	166[a]
symmetry code: (i) x, y, z; (ii) $-^1/_2+x, ^1/_2-y, 1-z$; (iii) $1-x, ^1/_2+y, 1-^1/_2-z$			

[a] Hydrogen atom not refined.

by rotation about the long axis of the steroid, then a second base need not be involved in the catalytic mechanism. Abstraction of the 4α-proton by Asp-38 could occur when the substrate is bound "upside down". Similarly, alkylation of Asp-38 might occur when **1β** is bound α-side up. Although this hypothesis is attractive in view of the X-ray studies, it must be pointed out that the only clear-cut chemical evidence for the location of Asp-38, reaction with the 3β- and 17β-oxiranyl steroids, places this residue at the α-face of bound steroid.[14c,d]

In summary, the structure of the 3β-oxirane (**1β**) mimics that of the natural substrate and provides evidence for the existence of an active site acid group. The 17β-oxirane (**2β**), when rotated 180° about an axis perpendicular to the plane of the steroid ring system, gives a structure which is similar to that for **1β**, especially in the vicinity of the oxirane rings. This result is in accord with the postulated ability of steroids to bind in two orientations.

Acknowledgment. This work was supported by Grants No. GM 33059, CA-10925, CA-22780, and CA-06927 awarded by the

National Institutes of Health, by Grant No. BC-242 from the American Cancer Society, and by an appropriation from the Commonwealth of Pennsylvania. The authors thank Dr. Edwin M. Westbrook for communicating his results in advance of publication and Dr. William F. Benisek for helpful comments.

Supplementary Material Available: Tables of crystallographic details, including thermal parameters and torsion angles for **1**β and **2**β (4 pages); listing of observed and calculated structure factors for **1**β and **2**β (31 pages). Ordering information is given on any current masthead page.

Molecular Bridge Effects on Distant Charge Tunneling†

José Nelson Onuchic‡,§ and David N. Beratan*,‖

Contribution from the Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, and Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California 91109. *Received February 13, 1987*

Abstract: The question arises as to whether different hydrocarbon bridges should give different electron-transfer rates. We answer this question on the basis of energetic and topological (interference) effects that can be gleaned from one-electron models. A discussion of model compound electron-transfer rates based on this interpretation is given. The approximations implicit in the periodic models used here (and in previous work) are carefully justified.

I. Introduction

In previous studies, we presented predictions for the tunneling matrix element dependence on donor, acceptor, and bridge energetics and topology for several linkers.[1] We also distinguished the nature and importance of through-bond vs. through-space pathways for some model potentials.[2] Here, we simplify and unify the results for tunneling through organic linkers. We begin by discussing considerable simplifications that arise when charge mediation by either the bonding (valence) or the antibonding (conduction) states dominates the donor–acceptor interaction. We discuss the validity of the periodic, weak coupling, and small backscattering approximations. Next, we compare tunneling matrix elements for several linkers and show, analytically, how topological effects in cyclic bridges can enhance or decrease the matrix element. Both constructive- and destructive-interference effects are found to be important. An understanding of how these effects influence the distance decay of the matrix element for different organic donor–acceptor bridges of current experimental interest is the main goal of this paper. (Recall that the rate is proportional to the square of the matrix element in the nonadiabatic limit.[3]) We also discuss the predictions of Hush[4] and Schipper[5] that electron-transfer matrix elements decay in a polynomial rather than in an exponential fashion with distance.

The goal of this work is to show why different hydrocarbon bridges are expected to give different electron-transfer rates even for the same donors, acceptors, and transfer distance. A consistent method is given to predict the efficiency of different bridges for mediating the donor–acceptor interaction. This method is given in a simple enough form so that it can be directly applied by experimentalists when considering target bridging molecules, and it is also of use for understanding electron-transfer rates in existing model compounds. It may also be useful for designing new molecules with novel applications to microelectronics.[6]

II. One-Band Model for Bond-Mediated Electron Tunneling

In the first part of this section we show how the electron-transfer rate dependence on distance for a linear alkane bridge can be described with a one orbital per bond model. This description permits a clearer understanding of the terms electron and hole transfer. It also identifies the contributions of different "tunneling pathways" to the tunneling matrix element.

Let us represent an alkane chain by a set of sp^3 orbitals. Orbitals on the same carbon atom have an interaction γ, and orbitals in the same bond have an interaction β. For simplicity we neglect the C–H bonds in this section. For realistic parameters, $|\beta| \gg |\gamma|$. Thus, if the donor is coupled to an n-alkane with N carbon atoms ($2N$ sp^3 carbon orbitals participating in the C–C bonds), the Hamiltonian is given in (2.1). The zero of the energy scale is chosen so that $\alpha_{sp^3} = 0$. Also, β and γ are defined as negative quantities in the usual Hückel convention, which follows from the assumption that the basis functions all have the same phase.[7]

$$H_D = \Delta_D a_D^\dagger a_D + \beta_D(a_D^\dagger a_1 + a_1^\dagger a_D) + \sum_{i=1}^{N}\gamma(a_{2i-1}^\dagger a_{2i} + a_{2i}^\dagger a_{2i-1}) + \sum_{i=1}^{N-1}\beta(a_{2i}^\dagger a_{2i+1} + a_{2i+1}^\dagger a_{2i}) \quad (2.1)$$

This Hamiltonian describes N carbon atoms, each with two sp^3 orbitals participating in the backbone bonds. The hybrid orbitals participating in the C–H bonds, considered in section III, are not included at this stage for reasons of simplicity. The parameter γ couples hybrid orbitals on the same carbon atom, and β couples hybrid orbitals participating in a bond. Only nearest-neighbor interactions are included. Notice that the Hamiltonian, written here in operator notation, is just the common one-electron extended Hückel representation of the problem using a basis set of hybrid orbitals.

† Contribution No. 7546.
‡ On leave of absence from the Instituto de Física e Química de São Carlos, Universidade de São Paulo, 13560 São Carlos, SP, Brazil.
§ Division of Chemistry and Chemical Engineering.
‖ Jet Propulsion Laboratory.

(1) (a) Beratan, D. N.; Hopfield, J. J. *J. Am. Chem. Soc.* **1984**, *106*, 1584. (b) Beratan, D. N. *J. Am. Chem. Soc.* **1986**, *108*, 4321. (c) Beratan, D. N.; Onuchic, J. N.; Hopfield, J. J. *J. Chem. Phys.* **1987**, *86*, 4488.
(2) Beratan, D. N.; Onuchic, J. N.; Hopfield, J. J. *J. Chem. Phys.* **1985**, *83*, 5325.
(3) (a) Chance, B., DeVault, D. C., Frauenfelder, H., Marcus, R. A., Schrieffer, J. R., Sutin, N., Eds. *Tunneling in Biological Systems*; Academic: New York, 1979. (b) DeVault, D. *Quantum Mechanical Tunneling in Biological Systems*, 2nd ed.; Cambridge University: New York, 1984.
(4) Hush, N. S. *Coord. Chem. Rev.* **1985**, *64*, 135.
(5) Schipper, P. S. *Int. Rev. Phys. Chem.* **1986**, *5*, 283.
(6) Carter, F. L., Ed. *Molecular Electronic Devices*; Dekker: New York, 1982.
(7) (a) Yates, K. *Hückel Molecular Orbital Theory*; Academic: New York, 1971. (b) Ballhausen, C. J.; Gray, H. B. *Molecular Orbital Theory*; Academic: New York, 1978.

0002-7863/87/1509-6771$01.50/0