1:1 pentane-ether at −20 °C gave microcrystals, mp 126–129 and 135–138 °C.[33] Recrystallization from isopropyl ether–pentane gave colorless crystals: mp 128–130 °C; IR λ_{max} ($CHCl_3$) 3.40, 5.75 (C=O), 5.95 (α,β-unsaturated C=O), and 6.17 μ; NMR 0.83 (s, 3 H, C-18 CH_3), 1.20 (s, 3 H, C-19 CH_3), 1.0–2.5 (methylene envelope), and 5.76 ppm (br s, 1 H, C-4 vinyl proton); mass spectrum (70 eV) *m/e* 286 (M^+), and 244 (M − 42, from ring A).

The IR spectrum of **9** was identical with that of an authentic specimen[23] and a mixture melting point was undepressed (128.0–130.5 °C).

Acknowledgment. We are indebted to the National Institutes of Health and the National Science Foundation for support of this research.

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(4) NIH Postdoctoral Fellow, 1970–1971.
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(9) Prepared from 2-butyn-1-ol by the method of K. E. Schulte and K. P. Reiss, *Ber.*, **87**, 964 (1954).
(10) S. F. Brady, M. A. Ilton, and W. S. Johnson, *J. Am. Chem. Soc.*, **90**, 2882 (1968).
(11) An unidentified peak in the VPC was presumed to correspond to the homoallylic alcohol because it is the expectant product from reaction of methyllithium with the β,γ-unsaturated ester **17** which was known to be present in the equilibrium mixture. In our hands the methyllithium reaction was capricious, sometimes affording, in addition to some starting material, a product mixture containing a significant amount of substance tentatively identified as the methyl ketone **18** (CH_3 instead of OCH_3); hence the retreatments with methyllithium were generally necessary.
(12) For examples of cyclizations conducted with formic acid, see ref 2 and references cited therein.
(13) H. Nakata, *Tetrahedron*, **19**, 1959 (1963).
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(33) These two polymorphs have been reported previously. See ref 23.

Molecular Structures of Substrates and Inhibitors of Δ^5-3-Keto Steroid Isomerase and Their Relevance to the Enzymatic Mechanism

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Abstract: The crystal structures of a substrate, an acetylenic suicide substrate, and an allenic inhibitor of the enzyme Δ^5-3-keto steroid isomerase of *Pseudomonas testosteroni* (EC 5.3.3.1) have been determined. The enzyme catalyzes intramolecular proton transfer from C(4) to C(6), converting Δ^5- to Δ^4-3-keto steroids. The overall conformations of the acetylenic and allenic seco steroids are very much like those of substrate and product. Detailed three-dimensional parameters are given. These studies, together with the known structure of the Δ^4-3-keto steroid product have led to some suggestions on the mechanisms of this enzyme. It is proposed that binding of the C-3 carbonyl group of substrate to the enzyme ensures the correct conformation of the A and B rings for 4β hydrogen abstraction, leading to a $\Delta^{3,5}$-dienol. The conformations of the A and B rings will then dictate whether or not a proton is added at C(6) rather than at C(4). The acetylenic seco steroid is thought to bind in a similar manner and is converted enzymatically to the allenic seco steroid, which then alkylates and so inactivates the enzyme.

The Δ^5-3-keto steroid isomerase of *Pseudomonas testosteroni* (EC 5.3.3.1)[2a,b] catalyzes the conversion of Δ^5-3-keto steroids to Δ^4-3-keto steroids via β-face intramolecular proton transfer from C(4) to C(6) (Scheme I). Both the catalytic mechanism and the specificity of this enzyme have been studied in some detail.[3] The rate-limiting step in the reaction has been

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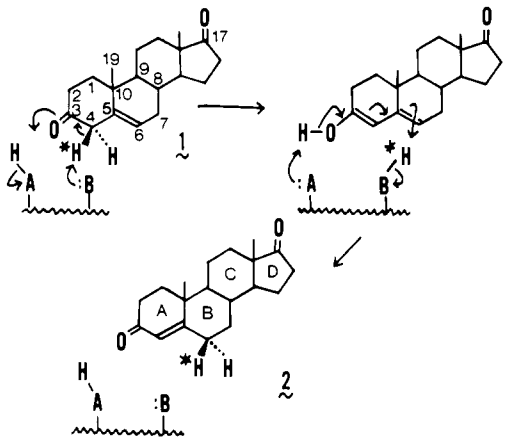
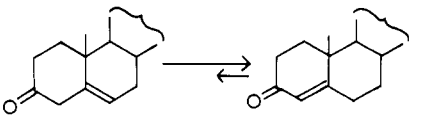
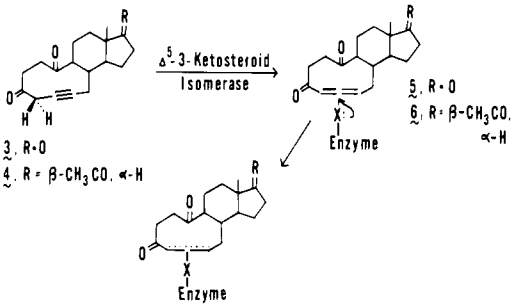
Scheme I



Scheme II

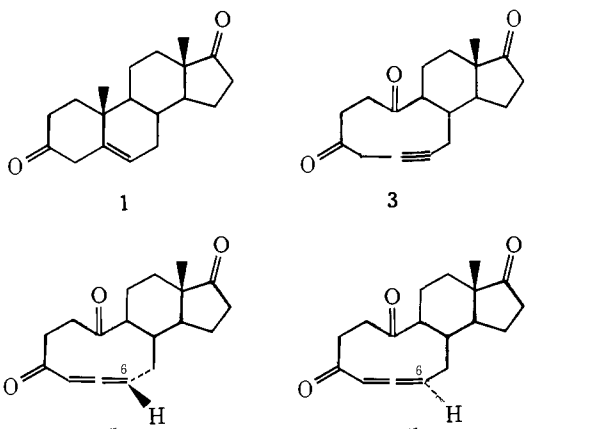


Scheme III



shown to be cleavage of the 4βH–C bond. No significant reversibility of the reaction can be shown spectrophotometrically, but reversibility has been demonstrated by coupling the isomerase reaction to that of a hydroxy steroid dehydrogenase which catalyzes the reduction of the 3-carbonyl group[4] (Scheme II).

The suicide substrates **3** and **4** have been synthesized[5] recently, and have been shown[6,7] to lead to rapid and irreversible inhibition of Δ^5-3-keto steroid isomerase. Thus, the β,γ-acetylenic 5,10-seco steroid, **3**, is a substrate for the enzyme, and is converted enzymatically to the corresponding pair of isomeric conjugated allenic ketones, represented by structures **5a** and **5b**, respectively. An analogous situation prevails for the acetylenic compound **4**. Compounds **5a** and **5b** differ in configuration at C(6). They have also been prepared nonenzymatically and have been isolated and characterized.[6] The major isomer, whether formed enzymatically or nonenzymatically from the acetylenic precursor, is stable and can be crystallized, and is shown in this study to be isomer **5a**. The minor isomer **5b** is unstable. The allenic ketones **5a** and **5b** are both powerful irreversible inhibitors of Δ^5-3-keto steroid isomerase, but **5a** is the more potent inhibitor.[5] Further studies on the mode of enzymatic formation of **5a** and **5b** are in progress. Inactivation is thought to occur through covalent bond formation at the active site of the enzyme via Michael addition of a nucleophilic amino acid residue to the conjugated allenic ketone grouping (Scheme III).

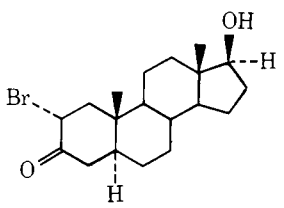


1

3

5a
(4*R*) compound

5b
(4*S*) compound



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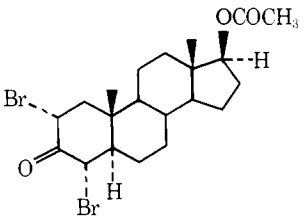

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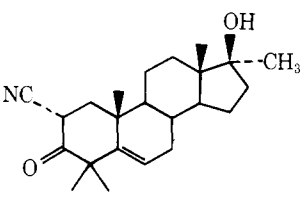

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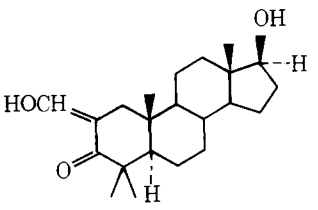

10

The efficiency of 5,10-seco compounds **3** and **4** as suicide substrates and of **5** and **6** as irreversible inhibitors of isomerase prompted detailed x-ray crystallographic studies in order to study the stereochemistry of the enzymatic reaction. An unusual opportunity is thus provided to examine in detail the conformational similarities and differences between the acetylenic ketone **3** and a normal isomerase substrate **1** on the one hand, and between an enzymatically derived allenic ketone **5a** and a normal isomerase product **2** on the other hand. In this paper, x-ray crystallographic structural data are described for the 5,10-seco compounds **3** and **5a**, and compared with the corresponding data for compounds **1** and **2**.

Experimental Section

The crystal structures of three compounds, **1**, **3**, and **5a**, were determined. Crystal data are given in Table I. Other details of the x-ray studies follow.

A. Compound 1 (Δ^5-Androstene-3,17-dione). Three-dimensional x-ray intensity data were collected with a crystal 0.2 × 0.2 × 0.2 mm in size, using a Syntex $P\bar{1}$ automated four-circle diffractometer with the ω scan technique and Mo Kα radiation (using a highly oriented graphite crystal monochromator). Scans of 1709 independent reflections in the range $\sin\theta/\lambda = 0$ to 0.65 Å^{-1} ($2\theta = 55°$) gave 1647 having intensity I greater than the threshold of $1.00\sigma(I)$. There was no falloff in intensity as a function of time. The data were converted to structure amplitudes by application of Lorentz and polarization factors and no absorption correction was applied.

The structure was solved by direct methods using the program MULTAN.[8] Isotropic, then anisotropic full-matrix least-squares refinements reduced the R value to 0.107 for the "observed" data. All hydrogen atoms were located from a difference map and their inclusion reduced the R value to 0.086. The structure was refined further by full-matrix least-squares methods (hydrogen isotropic, carbon and oxygen anisotropic) to the final residuals $R = 0.053$, weighted $R = 0.062$.

B. Compound 3 (5,10-Secoestr-5-yne-3,10,17-trione). Three-dimensional x-ray intensity data were collected on a Syntex $P\bar{1}$ auto-

Table I. Crystal Data

	5-Androstene-3,17-dione (**1**)	5,10-Secoestra-4,5-diene-3,10,17-trione (**5a**)	5(10)-Secoestra-5,6-yne-3,10,17-trione (**3**)
Molecular formula	$C_{19}H_{26}O_2$	$C_{18}H_{22}O_3$	$C_{18}H_{22}O_3$
Molecular weight	286.4	286.4	286.4
Crystal system	Monoclinic	Orthorhombic	Orthorhombic
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
Calculated density, g cm^{-3}	1.21	1.18	1.27
Cell volume, Å^3	789.5	1608.2	1501.3 (3)
Molecules per cell	2	4	4
Cell dimensions			
a, Å	12.191 (2)	11.628 (2)	8.896 (1)
b, Å	6.199 (1)	17.977 (5)	20.242 (3)
c, Å	11.024 (2)	7.693 (1)	8.337 (1)
α, deg	90.00	90.00	90.00
β, deg	108.16 (2)	90.00	90.00
γ, deg	90.00	90.00	90.00
Observed data	$1647 > \sigma(I)$	$1611 > 0$	$1165 > 2\sigma(I)$
Reliability factor	0.053	0.054	0.051
Radiation	Mo Kα	Cu Kα	Mo Kα

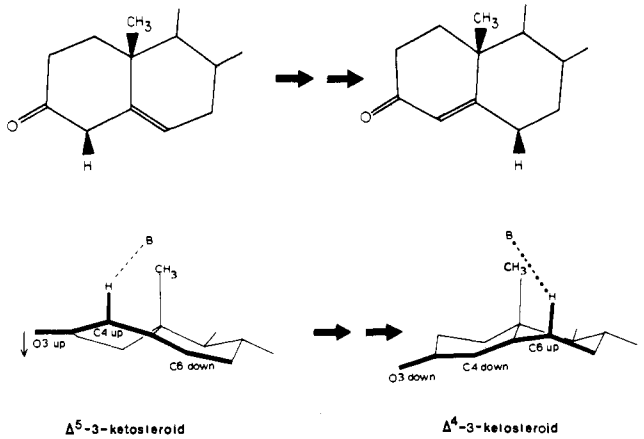

Figure 1. Suggested location of hydrogen-abstracting group (B) with respect to the hydrogen atoms on C(4) in a Δ^5-3-keto steroid and C(6) in a Δ^4-3-keto steroid.

mated four-circle diffractometer with the θ–2θ scan technique and Mo Kα radiation (using a highly oriented graphite crystal monochromator). Of the 1629 independent reflections scanned in the range $\sin\theta/\lambda = 0$ to 0.65 Å^{-1} ($2\theta = 55°$), 1165 had intensity I greater than the threshold of $2.0\sigma(I)$ (1039 with $I > 3\sigma(I)$). The crystal size was 0.06 × 0.15 × 0.20 mm. There was no falloff in intensity as a function of time and no absorption correction was applied. The data were converted to structure amplitudes by application of Lorentz and polarization factors, and placed on an absolute scale with a Wilson plot.

The structure was solved by the program MULTAN.[8] Isotropic, then anisotropic full-matrix least-squares refinements reduced the R value to 0.10 for the "observed" data. All hydrogen atoms were located from a difference map and their inclusion reduced the R value to 0.08. The structure was refined further by full-matrix least-squares methods (hydrogen isotropic, carbon and oxygen anisotropic) to the final residuals $R = 0.051$, weighted $R = 0.048$.

C. Compound 5a [(4*R*)-5,10-Secoestra-4,5-diene-3,10,17-trione]. Three-dimensional x-ray intensity data were collected on a Syntex $P\bar{1}$ automated four-circle diffractometer with the θ–2θ scan technique varying the scan from 2°/min to 24°/min and Cu Kα radiation, using a highly oriented graphite crystal monochromator; 1687 independent reflections were scanned in the range $\sin\theta/\lambda = 0$ to 0.600 Å^{-1} of which 1611 had intensity I greater than zero. The crystal size was 0.1 × 0.1 × 0.20 mm. There was no falloff in intensity as a function of time. The data were converted to structure amplitudes by application of Lorentz and polarization factors; no absorption was applied.

The structure was solved by the program MULTAN;[8] 18 of the 21 nonhydrogen atoms were located in the E map, and all the remaining atoms were located in a map with phase angles determined by the contribution of 17 of the 18 atoms. Isotropic, then anisotropic full-matrix least-squares refinements on the 21 atoms were carried out and all hydrogen atoms were located from a difference map. For the next least-squares cycle, hydrogen atoms were included in calculated positions. The structure was refined further by two full-matrix least-squares cycles on all atoms (hydrogen isotropic, oxygen and carbon anisotropic). The weighting scheme was then adjusted and the data were corrected for secondary extinction[9] ($\alpha = 6 \times 10^{-8}$). Refinements were then continued with two cycles for nonhydrogen atoms for data with $(\sin\theta/\lambda) > 0.45$, then two cycles for hydrogen atoms for data with $(\sin\theta/\lambda) < 0.45$, and then for all atoms ($(\sin\theta/\lambda) < 0.62$), to the final residuals $R = 0.054$, weighted $R = 0.072$.

D. Computer Programs. Computer programs used were ICRFMLS,[10] the CRYSNET[11] package, and other programs written in the ICR laboratory. The quantity minimized in the least-squares calculations was $\Sigma w\{||F_o| - |F_c||\}^2$. Values of $\sigma(F)$ were determined as $\sigma(F) = (F/2)\{(\sigma^2(I)/I^2) + \delta^2\}^{1/2}$ where $\sigma(I)$ was derived from counting statistics and δ is an instrumental uncertainty determined from the variation of the measured intensities of three periodically monitored standard reflections ($\delta = 0.03$ for **1**, 0.025 for **3**, and 0.03 for **5a**). The weights of the reflections, w, used during the refinement were $1/[\sigma^2(F)]$ with zero weight for those reflections below the threshold value. The atomic scattering factors used were listed values.[12]

A list of positional parameters is given in Table II and a list of thermal parameters is given in Table I of the microfilm edition. Tables of observed and calculated structure factors are also available in the microfilm edition.

Results and Discussion

A. Structures of the Compounds Studied. Views of the compounds studied are shown in Figures 1a,c,d and 2a,c,d. The structure of the analogous Δ^4-3-keto steroid (**2**), which has been determined previously,[13] is also included in Figures 1b and 2b in order to complete an analysis of the stereochemistry of the reaction catalyzed by Δ^5-keto steroid isomerase. In Figure 1 the molecules are viewed from above the β face onto the least-squares plane through the C and D rings. The remarkable similarity of the acetylenic and the allenic seco steroids to the steroids is shown here. In Figure 2 the molecules are viewed along the plane through the C and D rings and, again, the similarities between the steroids and the seco steroids (which lack the C(5)–C(10) bond) are shown.

The structure determination of the allenic seco steroid has shown that the stable major product has the 4*R* configuration. Since the unstable minor isomer has the opposite configuration at this carbon atom, and since the 4*R* isomer studied here has a structure that is very similar to that of the Δ^4-3-keto steroid, the minor isomer, which is not crystalline, will not be discussed further.

The various dimensions of the molecules are listed in tables in the microfilm edition. Interatomic distances in **1**, **3**, and **5a**

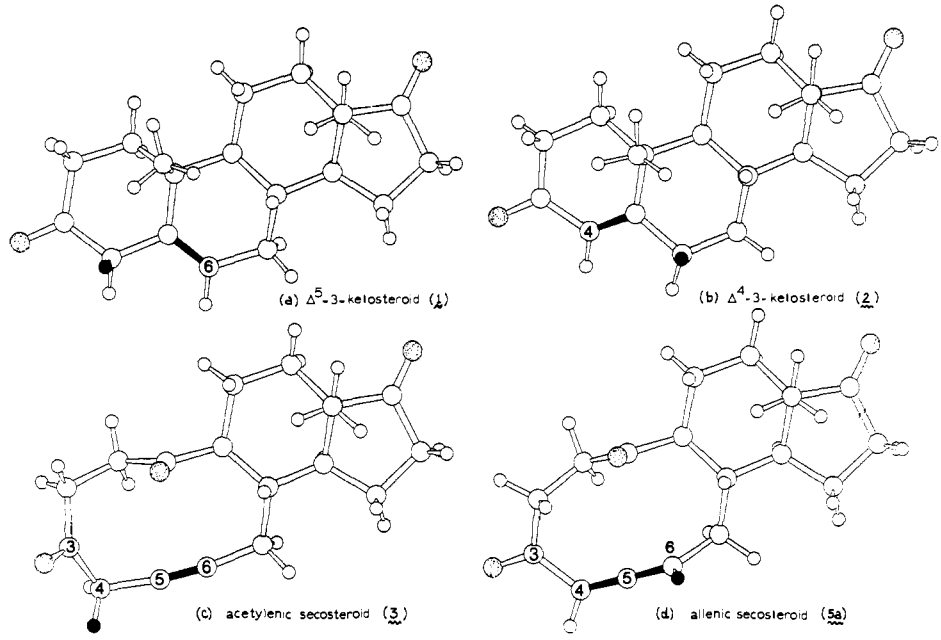

Figure 2. Views of the four steroids **1**, **2**, **3**, and **5a** from above the plane through the C and D rings. The abstracted hydrogen atoms are black. Double bonds in (a), (b), and (d) and a triple bond in (c) are indicated by black bonds. Note the similarity in overall shape of the four steroids.

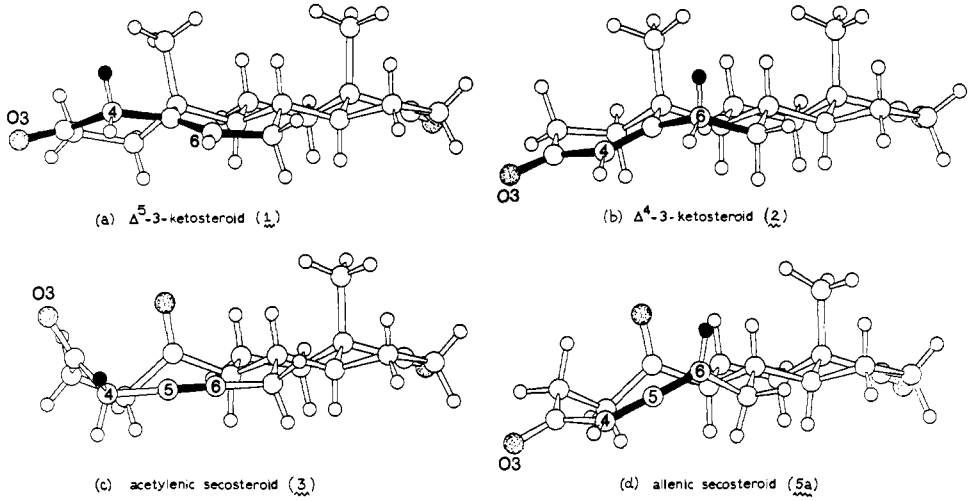

Figure 3. Side views of the four steroids shown in Figure 2. The abstracted hydrogen atoms are black. In (a) and (b) bonds are black on the side of the molecule nearer the viewer. In (c) the triple bond is black and in (d) the allenic double bonds are black.

are listed in Table II. Interbond angles are given in Table III and torsion angles in Table IV. The distances between functional groups (methyl or keto) are given in Table III of this paper.

B. Δ^5- to Δ^4-3-Keto Steroid. Detailed consideration of the crystal structures of the two steroids suggests that the areas of the steroids that are recognized and bound by the enzyme in a fixed manner during the reaction involve the C and D rings; these areas appear similar in both the Δ^5- and Δ^4-3-keto steroid. Alternate models, such as those in which binding only occurs at O(3) and O(17), do not give as many similarities between Δ^5- and Δ^4-3-keto steroids in possible interactions with an active site. However, presumably both of these oxygen atoms are involved in binding, O(17) as a D ring substituent and O(3) to anchor the A ring, as discussed later. Since the binding of the C and D rings seems to be a constant factor, views of molecules in Figures 1 and 2 have the plane through these two rings as a constant factor.

The importance of the 18- and 19-methyl groups in the binding of substrate in the active site of the enzyme has been considered further. Studies[2b] with a variety of substrates and reversible inhibitors have shown that Δ^4 and Δ^5 steroids of the 19-nor series exhibit greater affinity for the enzyme than do the corresponding compounds possessing a 19-methyl group. For example, 19-nortestosterone (K_i = 5.2 μM) binds more firmly than testosterone (K_i = 26 μM), and similarly 19-norprogesterone (K_i = 0.5 μM) binds more firmly than progesterone (K_i = 6.4 μM). Furthermore, steroids with aromatic A and B rings, such as 17β-estradiol (K_i = 10 μM) and 17β-dihydroequilenin (K_i = 6.3 μM), bind well. We are not aware of related measurements on 18-nor-Δ^4- or -Δ^5-keto steroids. However, the 18-methyl group, for example, in 19-norandrostenedione (K_i = 27 μM), can be replaced by an 18-ethyl group without loss of activity,[14] as in the analogous compound, 13β-ethylgonenedione (K_i = 18 μM).

A comparison of the Δ^4- and Δ^5-3-keto steroids is given in Figure 3, which shows, diagrammatically, part of the structure illustrated in Figure 2, i.e., the A and B rings with respect to the least-squares best plane through the C and D rings. In the enzymatic proton abstraction, the hydrogen atom, which is abstracted and transferred is on the β face of the steroid, and the hydrogen-abstracting group of the enzyme must be above

Table II. Atomic Parameters

	x	*y*	*z*
	A. 5-Androstene-3,17-dione (**1**) Parameters		
C(1)	0.4760 (3)	0.3670 (6)	0.7217 (3)
C(2)	0.5355 (3)	0.2803 (7)	0.8562 (3)
C(3)	0.4636 (3)	0.2849 (6)	0.9443 (3)
C(4)	0.3344 (3)	0.2715 (7)	0.8865 (3)
C(5)	0.2833 (2)	0.3381 (5)	0.7474 (2)
C(6)	0.1838 (3)	0.4393 (6)	0.7092 (3)
C(7)	0.1198 (2)	0.4943 (6)	0.5723 (3)
C(8)	0.1676 (2)	0.3715 (5)	0.4792 (2)
C(9)	0.3005 (2)	0.3851 (5)	0.5257 (2)
C(10)	0.3528 (2)	0.2773 (5)	0.6597 (2)
C(11)	0.3558 (3)	0.2991 (8)	0.4271 (3)
C(12)	0.3027 (3)	0.3986 (7)	0.2930 (3)
C(13)	0.1736 (3)	0.3616 (6)	0.2488 (3)
C(14)	0.1214 (2)	0.4657 (6)	0.3450 (3)
C(15)	−0.0088 (3)	0.4673 (7)	0.2758 (3)
C(16)	−0.0149 (3)	0.5228 (8)	0.1378 (3)
C(17)	0.1036 (3)	0.4774 (7)	0.1269 (3)
C(18)	0.1436 (3)	0.1188 (7)	0.2246 (3)
C(19)	0.3554 (3)	0.0291 (7)	0.6493 (3)
O(3)	0.5076 (2)	0.2872 (0)[a]	1.0595 (2)
O(17)	0.1371 (2)	0.5225 (6)	0.0379 (2)
H(1) α	0.465 (3)	0.512 (8)	0.725 (3)
H(1′)	0.525 (3)	0.337 (6)	0.667 (3)
H(2)	0.602 (3)	0.346 (7)	0.888 (3)
H(2′) β	0.559 (4)	0.128 (10)	0.856 (4)
H(4)	0.298 (3)	0.351 (8)	0.939 (4)
H(4′) β	0.322 (4)	0.114 (10)	0.895 (5)
H(6)	0.144 (3)	0.483 (7)	0.765 (3)
H(7) α	0.126 (3)	0.657 (7)	0.554 (4)
H(7′)	0.042 (3)	0.472 (7)	0.555 (4)
H(8) β	0.145 (3)	0.213 (7)	0.480 (3)
H(9) α	0.320 (2)	0.530 (6)	0.541 (3)
H(11)	0.352 (2)	0.133 (6)	0.419 (3)
H(11′) β	0.437 (3)	0.325 (6)	0.452 (3)
H(12)	0.345 (3)	0.346 (7)	0.239 (3)
H(12′) α	0.318 (3)	0.549 (9)	0.296 (4)
H(14) α	0.146 (2)	0.616 (6)	0.351 (3)
H(15)	−0.055 (3)	0.570 (8)	0.312 (3)
H(15′) β	−0.036 (3)	0.324 (8)	0.278 (4)
H(16) α	−0.029 (4)	0.671 (11)	0.126 (5)
H(16′) β	−0.069 (4)	0.456 (9)	0.073 (5)
H(18)	0.190 (4)	0.064 (11)	0.181 (5)
H(18′)	0.057 (3)	0.104 (8)	0.186 (4)
H(18″)	0.165 (3)	0.038 (9)	0.302 (4)
H(19)	0.282 (3)	−0.022 (8)	0.599 (4)
H(19′)	0.365 (4)	−0.041 (9)	0.732 (5)
H(19″)	0.416 (4)	0.003 (10)	0.617 (4)
	B. 5,10-Secoestra-4,5-diene-3,10,17-trione (**5a**) Parameters		
C(1)	0.6835 (3)	0.1939 (2)	0.3767 (5)
C(2)	0.5691 (3)	0.1661 (2)	0.4461 (5)
C(3)	0.4673 (3)	0.1832 (2)	0.3318 (5)
C(4)	0.4688 (3)	0.1599 (2)	0.1484 (5)
C(5)	0.5532 (3)	0.1236 (2)	0.0751 (4)
C(6)	0.6384 (3)	0.0869 (2)	0.0066 (4)
C(7)	0.7440 (3)	0.1189 (2)	−0.0760 (4)
C(8)	0.8538 (2)	0.1029 (1)	0.0305 (3)
C(9)	0.8602 (3)	0.1523 (2)	0.1954 (4)
C(10)	0.7668 (3)	0.1332 (2)	0.3225 (4)
C(11)	0.9772 (3)	0.1460 (2)	0.2903 (4)
C(12)	1.0804 (3)	0.1589 (2)	0.1726 (5)
C(13)	1.0740 (3)	0.1049 (2)	0.0207 (4)
C(14)	0.9606 (3)	0.1170 (2)	−0.0776 (4)
C(15)	0.9776 (3)	0.0743 (2)	−0.2474 (4)
C(16)	1.1042 (3)	0.0903 (3)	−0.2928 (5)
C(17)	1.1586 (3)	0.1169 (3)	−0.1245 (5)
C(18)	1.0909 (3)	0.0248 (2)	0.0822 (6)
O(3)	0.3858 (2)	0.2172 (2)	0.3862 (5)
O(10)	0.7605 (2)	0.0722 (1)	0.3875 (3)
O(17)	1.2542 (2)	0.1439 (3)	−0.1142 (4)

Table II (*Continued*)

	x	*y*	*z*
H(1)	0.727 (3)	0.223 (2)	0.473 (6)
(H1′) α	0.666 (3)	0.224 (2)	0.283 (5)
H(2) β	0.578 (4)	0.105 (2)	0.456 (6)
H(2′)	0.551 (3)	0.185 (2)	0.569 (5)
H(4)	0.392 (4)	0.164 (2)	0.102 (6)
H(6) β	0.636 (2)	0.030 (2)	0.011 (4)
H(7)	0.752 (3)	0.093 (2)	−0.200 (5)
H(7′) α	0.724 (3)	0.171 (2)	−0.085 (5)
H(8) β	0.853 (3)	0.051 (2)	0.066 (4)
H(9) α	0.852 (3)	0.208 (2)	0.152 (5)
H(11)	0.979 (3)	0.177 (2)	0.384 (6)
H(11′) β	0.979 (3)	0.093 (2)	0.341 (5)
H(12) α	1.072 (3)	0.207 (2)	0.112 (6)
H(12′)	1.160 (4)	0.159 (3)	0.232 (7)
H(14) α	0.955 (3)	0.173 (2)	−0.100 (5)
H(15) β	0.961 (3)	0.017 (2)	−0.237 (5)
H(15′)	0.924 (4)	0.095 (2)	−0.346 (6)
H(16) β	1.136 (4)	0.045 (2)	−0.335 (6)
H(16′) α	1.109 (6)	0.135 (4)	−0.386 (10)
H(18)	1.167 (4)	0.027 (2)	0.145 (6)
H(18′)	1.028 (4)	0.001 (2)	0.161 (6)
H(18″)	1.099 (4)	−0.002 (2)	−0.017 (6)
	C. 5(10)-Secoestra-5,6-yne-3,10,17-trione (**3**) Parameters		
C(1)	0.1132 (5)	0.3259 (2)	0.5451 (5)
C(2)	0.1897 (5)	0.3819 (2)	0.4555 (5)
C(3)	0.2798 (5)	0.4293 (2)	0.5531 (4)
C(4)	0.2233 (6)	0.4546 (2)	0.7132 (5)
C(5)	0.2240 (5)	0.4028 (2)	0.8367 (4)
C(6)	0.2218 (5)	0.3566 (2)	0.9234 (4)
C(7)	0.2089 (4)	0.2954 (2)	1.0163 (5)
C(8)	0.2328 (4)	0.2313 (2)	0.9188 (4)
C(9)	0.1517 (4)	0.2324 (2)	0.7550 (4)
C10)	0.2202 (5)	0.2811 (2)	0.6371 (4)
C(11)	0.1503 (5)	0.1639 (2)	0.6692 (5)
C(12)	0.1056 (4)	0.1063 (2)	0.7766 (5)
C(13)	0.1992 (4)	0.1063 (2)	0.9291 (5)
C(14)	0.1786 (4)	0.1724 (2)	1.0166 (5)
C(15)	0.2403 (5)	0.1600 (2)	1.1847 (5)
C(16)	0.1842 (6)	0.0894 (2)	1.2214 (5)
C(17)	0.1505 (5)	0.0586 (2)	1.0591 (5)
C(18)	0.3653 (5)	0.0895 (2)	0.8948 (6)
O(3)	0.3971 (4)	0.4507 (2)	0.5012 (4)
O(10)	0.3543 (4)	0.2810 (1)	0.6123 (4)
O(17)	0.0925 (4)	0.0054 (1)	1.0396 (4)
H(1)	0.056 (5)	0.298 (2)	0.469 (6)
H(1′) α	0.049 (6)	0.349 (2)	0.622 (6)
H(2) β	0.269 (8)	0.358 (3)	0.382 (8)
H(2′)	0.112 (5)	0.403 (2)	0.405 (6)
H(4)	0.291 (5)	0.490 (2)	0.751 (6)
H(4′) α	0.119 (6)	0.472 (2)	0.707 (7)
H(7)	0.285 (5)	0.295 (2)	1.112 (5)
H(7′) α	0.109 (5)	0.294 (2)	1.066 (5)
H(8) β	0.342 (4)	0.228 (2)	0.897 (4)
H(9) α	0.040 (4)	0.246 (1)	0.776 (4)
H(11)	0.078 (6)	0.172 (2)	0.579 (6)
H(11′) β	0.254 (5)	0.156 (2)	0.638 (5)
H(12) α	−0.004 (4)	0.109 (2)	0.811 (4)
H(12′)	0.122 (5)	0.068 (2)	0.720 (5)
H(14) α	0.074 (5)	0.178 (2)	1.027 (5)
H(15) β	0.338 (6)	0.162 (2)	1.190 (6)
H(15′)	0.194 (5)	0.189 (2)	1.268 (6)
H(16) β	0.262 (7)	0.064 (3)	1.270 (7)
H(16′) α	0.085 (6)	0.085 (3)	1.281 (7)
H(18)	0.375 (5)	0.046 (2)	0.836 (6)
H(18′)	0.412 (5)	0.123 (2)	0.829 (6)
H(18″)	0.414 (7)	0.089 (3)	0.997 (8)

[a] *y* of O(3) kept constant during refinement.

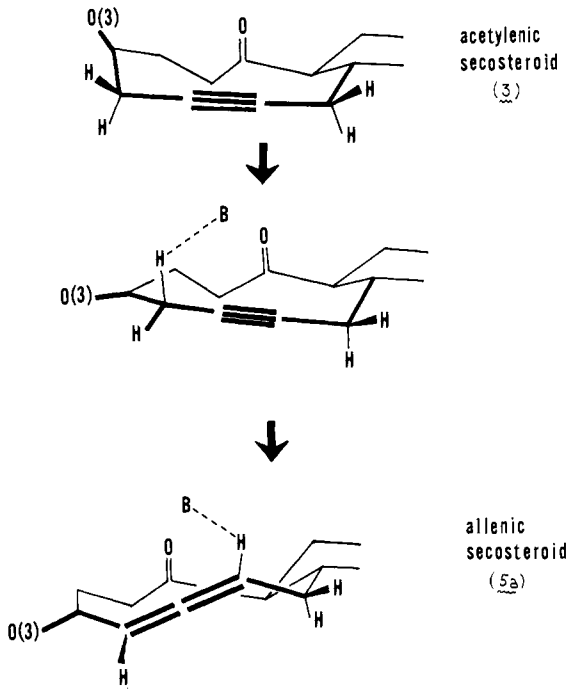

Figure 4. Diagram of the suggested conversion of acetylenic to allenic seco steroid in the enzymatic active site.

Table III. Interatomic Distances between Functional Groups

	1	5a	3
O(3)···O(17)	10.847 (3)	10.887 (4)	10.427 (5)
C(18)···O(3)	8.946 (4)	9.200 (5)	8.020 (5)
C(18)···O(17)	3.226 (5)	3.236 (5)	3.200 (6)
O(10)···O(3)		5.077 (4)	3.578 (4)
O(10)···O(17)		7.036 (4)	7.016 (4)
O(10)···C(18)		4.582 (5)	4.537 (5)
C(19)···O(3)	4.607 (4)		
C(19)···O(17)	7.119 (4)		
C(19)···C(18)	4.621 (5)		

the plane of the steroid (β face), and thus, presumably, symmetrically located with respect to the 19-methyl group (Figure 3). Since the distances from the abstracted hydrogen atoms to the hydrogen atoms of the 19-methyl groups are approximately van der Waals distances (H···H > 2.0 Å), removal of the 19-methyl group may facilitate substrate binding by eliminating a nonbonded interaction between the 19-methyl group and the hydrogen-abstracting group of the enzyme. For example, the substrate 19-nor-Δ^5-androstenedione binds more tightly (K_m = 130 μM) than the corresponding 19-methyl substrate (Δ^5-androstenedione, K_m = 290 μM). On the other hand, the V_{max} for the 19-nor steroid is less (V_{max} = 3.8 mol substrate transformed per minute per micromole of enzyme) than that for the 19-methyl substrate (V_{max} = 10).[14] The 19-methyl group may play a role in the enzymatic reaction, possibly by guiding the proton to the appropriate site for protonation (along the surface of the van der Waals sphere of the 19-methyl group), and it may also aid in the ejection of product from the active site.

Comparison of the side views of the two keto steroids in Figure 1 shows that O(3) is higher (with respect to the plane through the C and D rings) by 0.75 Å in the Δ^5-keto steroid than in the Δ^4-keto steroid. Since enolization of the 3-keto group is postulated to be an important part of the enzyme mechanism, presumably the steroid is held in the active site of the enzyme by hydrogen bonding to O(3). The proposed enol intermediate shows (molecular models) a very flattened A–B ring system because of the conjugated 3,5-diene grouping, and

Table IV. Distances between Atoms of Interest in a Study of Catalytic Activity (Å)

Molecule	C(4)–C(6)	C(19)···H	C(19)···C(4)	C(19)···C(6)
1	2.258 (4)	2.91 to H(4′)	3.095 (5)	3.485 (5)
2	2.451	2.86 to H(6)	3.389	3.185
		O(10)···H	O(10)···C(4)	O(10)···C(6)
5a	2.608 (5)	3.32 to H(6)	4.168 (4)	3.267 (4)
3	2.647 (5)	4.42 to H(4)	3.796 (5)	3.234 (5)
		4.46 to H(4′)		

O(3) is now pulled down (with respect to the plane through the C and D rings). The crystallographic results for the Δ^4-3-keto steroid show that C(6) is nearer to the postulated hydrogen-abstracting group by 0.46 Å, and C(4) is further from the hydrogen-abstracting group by 0.83 Å than it is in the Δ^5-3-keto steroid. Thus protonation of the postulated enol may be more likely to occur at C(6) rather than at C(4).

The area of the active site adjacent to O(3) and its neighboring carbon atoms C(2) and C(4) apparently permits not only movement of O(3), but also accommodation of bulky groups at C(2) and C(4). Thus, the 2α-bromo- and the 2α,4α-dibromo-3-keto compounds (K_i = 8.2 and 6.7 μM, respectively) (**7** and **8**) proved[15] to be powerful competitive inhibitors of the enzyme, as did[16] a 2α-cyano-4,4-dimethyl-3-keto (K_i = 0.4 μM) (**9**) and a 2-hydroxymethylene-3-keto compound (K_i = 1.5 μM) (**10**).

B. 5,10-Seco Steroid Inhibitors. It has been shown that the acetylenic 5,10-seco steroid (**3**) is converted very rapidly by Δ^5-3-keto steroid isomerase to the conjugated allenic ketone system (**5**) which irreversibly inhibits the enzyme.[7] The latter process is thought to involve Michael addition of an active site amino acid residue to the allenic ketone grouping in **5**, as noted earlier.

Comparison of the solid state structures of the allenic ketone (**5a**) (Figures 2d and 3d) and of the Δ^4-3-keto steroid (**2**) (Figures 2b and 3b) reveals remarkable conformational similarity. The relative orientations of the β hydrogen at C(6) and of the O(3) carbonyl group in compound **5a** closely resemble those in compound **2**. Furthermore, the carbonyl group at C(10) in the allenic ketone (**5a**) should project into the area of the active site occupied by the 19-methyl group in compound **2**.

An interesting difference, shown in Table IV, is that the 10-carbonyl group in compound **5a** is much further (O(10)···H(6), 3.32 Å) from the 6β hydrogen than is the 19-methyl group from the 6b hydrogen in compound **2** (C(19)···H(6), 2.87 Å). As suggested above for 19-nor steroids, the consequent lack of a nonbonded interaction between the 19-methyl group and the hydrogen-abstracting group of the enzyme may offset the loss of the hydrophobic contribution of the 19-methyl group in compound **5a**. An additional consideration is that carbonyl groups themselves can show some hydrophobicity in spite of their dipolar nature. For example, in an alkylated peptide[17] the peptide carbonyl groups interacted with both the hydrophilic and the hydrophobic areas in the crystal structure.

An analogous situation (except for the O(3) carbonyl) prevails on comparing the acetylenic 5,10-seco steroid (**3**) (Figures 1c and 2c) with the Δ^5 substrate (**1**) (Figures 1a and 2a). Thus, the C(10) carbonyl group in compound **3** again occupies the same region of the molecule as does the 19-methyl group in Δ^5-3-keto steroid **1**, and the distance between C(4) and C(6) is similar (2.65 and 2.26 Å) for compounds **3** and **1**. The latter is an important consideration in view of the enzymatically catalyzed transfer of the C(4) β hydrogen to C(6). Finally, the C(10) carbonyl group in seco steroid **3** is much further (4.42 Å, see Table IV) from the 4β hydrogen than is

the 19-methyl group from the 4β hydrogen in Δ^5 steroid **1** (2.91 Å).

The O(3) carbonyl group in the acetylenic compound (**3**) sticks up (1.146 Å above the planes of the C and D rings) much more than does O(3) in the Δ^5 compound (**1**) (where it lies 1.00 Å below this plane). However, it is possible that a different orientation prevails in solution, or that the enzyme induces a conformational change in compound **3**, or even that the enzyme is not entirely rigorous in its requirements for the relative orientation of the O(3) carbonyl group. Rotation of the ten-membered ring in compound **3** readily produces a conformation (Figure 4) closely similar (as judged by molecular models) to the Δ^5 ketone (**1**), suggesting that conformational change on the enzyme, or in solution, may indeed occur. An estimate of the energy barrier for such a conformational inversion of the ten-membered ring in compound **3** would be helpful, and variable temperature NMR studies are in progress.

In summary, we suggest that the substrates are positioned in the active site of the enzyme mainly by the C and D rings. Apparently substitution in the A ring has little adverse effect on the binding of substrate. An amino acid side chain in the active site, interacting with O(3), would ensure the correct conformation of the A and B rings for 4β hydrogen abstraction. Formation of the $\Delta^{3,5}$-dienol after proton abstraction is accompanied by a conformational change which now may facilitate addition of a proton at C(6) rather than at C(4). The 19-methyl group may also guide this protonation. Because the acetylenic seco steroid is converted, presumably by an analogous mechanism, to an allenic seco steroid, it is likely that it binds to enzyme in a manner similar to that of the Δ^5-keto steroid. Subsequently alkylation of the enzyme by the electrophilic allenic compound can occur.

Acknowledgment. This research was supported by Grants CA-10925, CA-06927, RR-05539, AM-15918, and CA-16418 from the National Institutes of Health, U.S. Public Health Service, and AG-370 from the National Science Foundation, and an appropriation from the Commonwealth of Pennsylvania.

Supplementary Material Available: Anisotropic temperature factors, interatomic distances and angles, conformation angles, and a table of structure factors (36 pages). Ordering information is given on any current masthead page.

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Conformation of Cyclic Peptides. 10. Conformational Averaging in Peptides with the Sequence *cyclo*-(Gly-D-Xxx-L-Yyy)$_2$

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Abstract: NMR studies, supported by circular dichroism measurements, indicate that *cyclo*-(Gly-D-Val-L-Leu)$_2$ in methanol, and *cyclo*-(Gly-D-Val-L-Leu-Gly-D-Orn-L-Orn) and *cyclo*-(Gly-D-Phe-L-Phe-Gly-D-Orn-L-Orn) in water, favor a backbone conformation with extended D-series residues connecting L-Xxx-Gly turns. Evidence for a single backbone conformation is not apparent for *cyclo*(Gly-D-Leu-L-Leu)$_2$ in methanol, nor is it for any of the peptides in dimethyl sulfoxide, and rapid exchange among two or more backbone conformation types is suggested. The temperature coefficient of N-H proton chemical shift is an unreliable guide to solvent exposure in these cases. A conformation is proposed for a related peptide, *cyclo*-(Gly-D-Val-L-Leu-L-Ala-D-His-L-His). Synthesis of the peptides is outlined, and key steps including the cyclizations are described.

Peptides containing proline residues exhibit well-studied conformational heterogeneity resulting from rotation about peptide bonds to the proline ring nitrogen.[1-3] Because of the ~20-kcal barrier to this rotation, coexisting backbone conformers give individual NMR spectra at room temperature. Backbone conformers differing by rotations about the C^{α}–C′ and N–C^{α} bonds are generally connected by lower barriers, and are in fast exchange for NMR at room temperature, although Deber has suggested one case in which this may not be so.[4] In the fast exchange cases it is difficult to come by proofs that differing backbone types, e.g., turns at differing positions in a sequence, contribute to the single spectrum observed.

Recent reports are that in crystals the peptides *cyclo*-(Gly-L-Tyr-Gly)$_2$[5] and *cyclo*-(Gly-L-Leu-Gly)$_2$[6] have nonsymmetric conformations in which the backbone is approximately centrosymmetric, so that the two L residues are not identically situated. However, NMR spectra of these two peptides and others like them[8,9] show only one kind of L residue

0002-7863/78/1500-4289$01.00/0