Cardenolide Analogues. 4. (20*R*)- and (20*S*)-Cardanolides: On the Roles of the 20(22)-Ene and 14β-Hydroxyl in Genin Activity[1,2]

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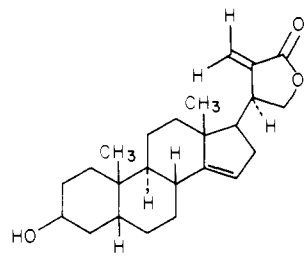
(20*R*)-20,22-Dihydrodigitoxigenin (**3a**) and (20*S*)-20,22-dihydrodigitoxigenin (**3b**) were isolated from (20*R*,*S*)-20,22-dihydrodigitoxigenin (**3**) by three fractional crystallizations each from ethyl acetate. The two diastereomers have distinct NMR spectra and similar (Na^+,K^+)ATPase inhibitory activities (I_{50} = 1.1–1.4 × 10^{-5} M)—about $^1/_{100}$ as active as digitoxigenin (**1**). Their activity compared with other cardenolide analogues suggests a passive geometric role for the 20(22) double bond in eliciting (Na^+,K^+)ATPase inhibition, keeping the lactone carbonyl in the proper orientation. (20*S*)-3β,14β-Dihydroxy-22-methylene-5β,14β-cardanolide (**7a**) was then synthesized from **3a**, and (20*R*)-3β,14β-dihydroxy-22-methylene-5β,14β-cardanolide (**7b**) from **3b**. They were found to be equivalently active in inhibiting (Na^+,K^+)ATPase, with I_{50} values of 7.0 × 10^{-5} M. Although it has been usually believed that the 14β-hydroxyl of cardenolides increases binding to the receptor, **2b** (the 14-ene derivative of **7b**) was more than twice as active (I_{50} = 3.0 × 10^{-5}) than either **7a** or **7b**.

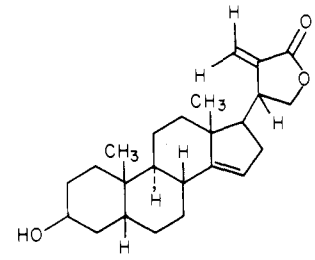
20,22-Dihydrocardanolides have been in the center of cardenolide structure–activity studies for two decades.[3] Models of digitalis–receptor binding[4,5] have focused on the approximately two orders of magnitude drop in activity[6-10] caused by hydrogenating the 20(22) double bond of cardenolides such as digitoxigenin (**1**).[11] An *active* role has generally been envisioned for the 20(22) double bond in the receptor-binding process.[4,5] Several papers have also discussed whether the 20,22-dihydro analogues might or might not be less toxic than the 20(22)-enes.[7,9] Individual (20*R*)- and (20*S*)-20,22-dihydrocardanolide diastereomers have never been characterized or evaluated for their activity toward (Na^+,K^+)ATPase.[12-14] It may be recalled that (Na^+,K^+)ATPase is believed to be the receptor, or very closely associated with it, for cardiac glycoside action in vivo. The pharmacological action of these drugs is most likely mediated by inhibition of this enzyme system.[3]

Recent studies in our laboratories have suggested that C(17) side group geometry may have a greater influence on cardenolide–receptor binding than electronic factors.[15,16] Understanding the cause of the decreased activity of the 20,22-dihydro analogues relative to the 20(22)-enes is pivotal in delineating the roles of geometric and electronic factors in the binding process. Two questions arise: (1) Is the decreased activity of the 20,22-dihydro analogues primarily due to geometric changes in the orientation of the lactone ring caused by C(20) changing from sp^2 to sp^3? (2) Is the activity of the 20,22-dihydro analogues due to the presence of a trace of unreacted 20(22)-ene?

This paper reports the preparation, characterization, and biological activities of rigorously pure (20*R*)- (**3a**) and (20*S*)-20,22-dihydrodigitoxigenin (**3b**). These diastereomers were then converted to their 22-methylene derivatives **7a** and **7b**. In 1977, we reported **2a** and **2b**—the 14-ene analogues of **7a** and **7b**.[15] Neither was as active as predicted by electronic models of cardenolide–(Na^+,K^+)-ATPase binding nor by the increased activity of α-methylene butyrolactones relative to their endo-unsaturated analogues.[15] Interestingly, **2b** was much more active than **2a**, suggesting an important role of geometry and stereochemistry in cardenolide–(Na^+,K^+)ATPase binding. Since the 14β-hydroxyl of natural cardenolides has been believed to bind to this enzyme receptor,[3,10] we wanted to make the presumably more active **7a** and **7b** for further studies.



2a



2b

Chemistry. Digitoxigenin (**1**) was hydrogenated[15,17] to (20*R*,*S*)-20,22-dihydrodigitoxigenin (**3**). No absorption at 217 nm from unreacted 20(22)-ene[18] could be detected for **3** in ethanol. A TLC system was developed with which individual spots of all diastereomers described in this paper could be separated,[1] including all four of **4**, thus permitting the progress of the purifications to be easily followed.

Initially, pure **3a** and **3b** were obtained in poor yield by four fractional crystallizations from ethyl acetate of their tetrahydropyranyl (THP) ethers **4a** and **4b**, followed by hydrolysis. From 8.9 g of **3** was thus obtained 15 mg of **3a** and 53 mg of **3b**. However, we found that **3a** and **3b** can be directly obtained by fractional crystallization of their 20*R*,*S* mixture **3** and in much better yield. With three fractional crystallizations (for each **3a** and **3b**) from ethyl acetate, 3.2 g of **3a** and 4.5 g of **3b** could be obtained from 20 g of **3**. Analytical samples of **4a** and **4b** were subsequently made from **3a** and **3b** obtained in this manner.

Both methyls are together in the NMR of **3a** but are

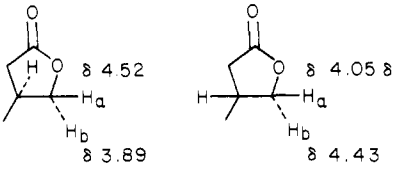
0022-2623/79/1822-0529$01.00/0

Table I. Na^+,K^+-Dependent ATPase Inhibition Studies[a]

steroid[d]	I_{50}, M, without preincub	I_{50}, M, with 10-min preincub[b]
3a	$1.4 \pm 0.5 \times 10^{-5}$	$1.1 \pm 0.5 \times 10^{-5}$
3b	$1.4 \pm 0.5 \times 10^{-5}$	$1.2 \pm 0.5 \times 10^{-5}$
7a	$7.0 \pm 0.8 \times 10^{-5}$	$6.0 \pm 0.8 \times 10^{-5}$
7b	$7.0 \pm 0.8 \times 10^{-5}$	$7.0 \pm 0.8 \times 10^{-5}$
2a[c]	$1\text{-}3 \times 10^{-4}$	$1\text{-}3 \times 10^{-4}$
2b	$3.0 \pm 1.5 \times 10^{-5}$	$2.0 \pm 1.0 \times 10^{-5}$
digitoxigenin	$3.5 \pm 0.5 \times 10^{-7}$	$3.5 \pm 0.5 \times 10^{-7}$

[a] I_{50} values are for two to four runs. Appropriate Mg^{2+} and Na^+ tubes were included to determine the basal activity of the (Na^+,K^+)ATPase. This was then subtracted from activity in the presence of Mg^{2+}, Na^+, and K^+. [b] Steroid added to (Na^+,K^+)ATPase medium lacking K^+ to allow the steroid and enzyme time to begin binding. After 10 min, KCl was added to begin (Na^+,K^+)ATPase activity. [c] Precise value was not possible to obtain due to insolubility of the steroid in the medium. [d] The steroids were added in ethanol to the (Na^+,K^+)ATPase tubes. In no case was more than 20 μL of ethanol added per tube. Independent studies showed that significant inhibition (over 3%) of the enzyme preparation by ethanol does not occur until over 25 μL of ethanol is added.

separated by 1 Hz in **3b**. The most obvious difference in the NMR spectra of **3a** and **3b** are the positions of the



3a **3b**

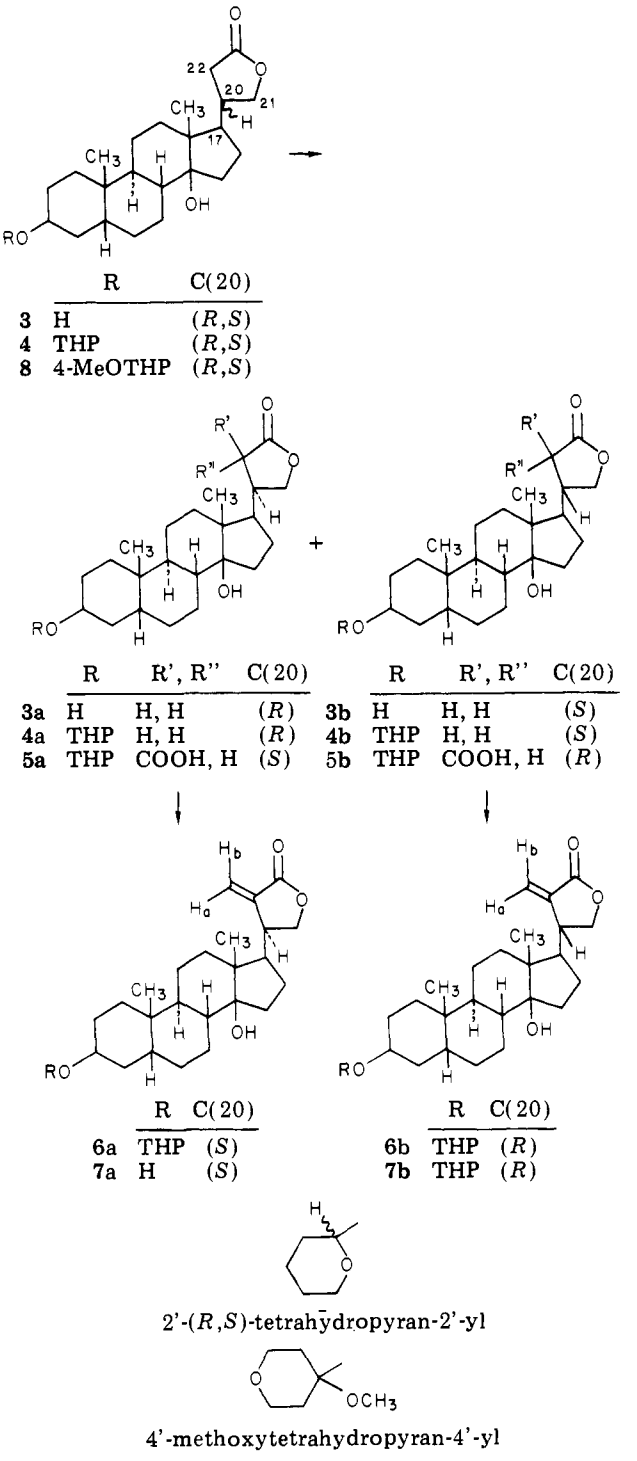
C(21) protons. Each appears as a triplet (J = 8 Hz, overlapped doublet of doublets). Models show that one C(21) proton of each **3a** and **3b** comes closer to the 14β-OH, and it has been assigned the downfield absorption as shown.

The enolates of **3a** and **3b** were individually treated with anhydrous CO_2[19] to give intermediates (presumably **5a** and **5b**), which as crude products were immediately treated with refluxing aqueous formaldehyde and diethylamine[20,21] to 22-methylene lactone products **6a** and **6b** (see Scheme I). Finally, **6a** and **6b** were hydrolyzed with dilute HCl in methanol to (20*S*)- (**7a**) and (20*R*)-3β,14β-dihydroxy-22-methylene-5β,14β-cardanolide (**7b**). The overall yield from **3a** to **7a** was 64% and **3b** to **7b** was 76%. This route is essentially the same we used to prepare **2a** and **2b**,[15] with THP ethers used to protect the 3β-hydroxyls instead of *tert*-butyldimethylsilyl (*t*-BuMe_2Si) ethers. The *t*-BuMe_2Si ethers could not be removed by acid hydrolysis without dehydration of the 14β-hydroxyl nor removed by tetra-*n*-butylammonium fluoride.[15]

In preliminary studies,[2] 22-methylene lactones **7a** and **7b** were also prepared from the (2′*R*,*S*)-4′-methoxytetrahydropyran-4′-yl (4′-MeOTHP) ether[22,23] (**8**) by the same route. However, the overall yield from **3** to a 20*R*,*S* mixture of **7a** and **7b** was only about 8% due to insufficient stability of the 4-MeOTHP group. Although pure **7a** could be obtained by fractional crystallization, **7b** could only be obtained in 85% (by LC) purity free of **7a**.

The NMR spectra of **7a** and **7b** are very similar to those of **2a** and **2b**. The C(18) methyls are in the same relative position to each other—those of the 20*R* diastereomers slightly upfield from those of the 20*S* diastereomers. Further, the C(18) absorptions of **7a** and **7b** are slightly downfield from those of the 14-enes—as expected, based on the same relationship of C(18) absorptions as digitoxigenin and 14(15)-anhydrodigitoxigenin.[24] Differences

Scheme I



in chemical shifts were also observed for the C(22) H_a protons of **7a** and **7b** (Figure 1) (assignments of H_a and H_b based on the studies of Ohga and Matsuo[25]) and for one of the C(21) protons.

Absolute assignment of structure and stereochemistry was confirmed by X-ray crystallographic analysis[2,26] of **7a** and by dehydrating the 14β-OH of **7a** and **7b** (as the crude trifluoroacetates in pyridine, with $SOCl_2$[27]) to **2a** and **2b**. The distinct NMR spectra of these 14-ene diastereomers were identical to those previously reported.[15]

Biology. The in vitro (Na^+,K^+)ATPase inhibitory activities of **2a**, **2b**, **3a**, **3b**, **7a**, **7b**, and digitoxigenin (**1**) were determined with rat brain (Na^+,K^+)ATPase (EC 3.6.1.3).[28,29] (Our rationale for using this system has been

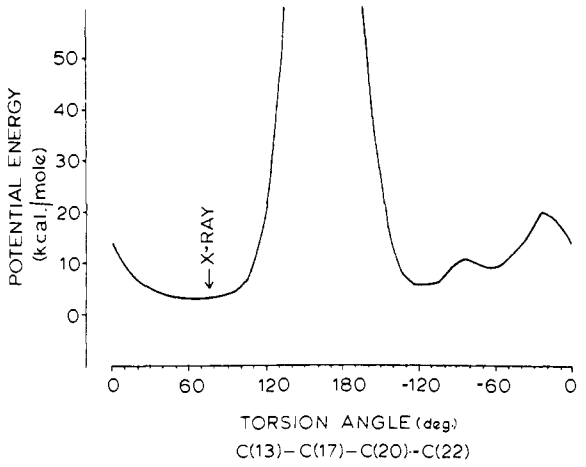


Figure 1. Potential energy diagram for rotation of the C(17) side group of digitoxigenin (1).

given previously.[16]) Comparisons of the inhibitory activities of these compounds leads to two surprising conclusions.

First, it has been generally believed that the 14β-hydroxyl greatly enhances the inhibition of (Na^+,K^+)-ATPase.[3,10] However, **2b** without the 14β-hydroxyl is more active than the corresponding 14β-hydroxy analogues **7a** and **7b**.

Second, it has been also generally believed that the C(20)–C(22) double bond in the lactone ring has an active role in inhibiting the (Na^+,K^+)ATPase.[3-5] A lactone with an exocyclic double bond would be predicted to be even more reactive than one with an endocyclic double bond[5,15] and certainly more active than one with no double bond. However, **7a** and **7b**, which should therefore be more active than digitoxigenin, are much less active. Furthermore, both 20,22-dihydro analogues **3a** and **3b**, though about 100 times less active than digitoxigenin, are more active than **7a** and **7b** despite the absence of the 20(22) double bond.

The changes in the position of the lactone carbonyl caused by variations in the geometry of the lactone ring and/or of the D ring provide a common explanation for the surprising differences in activity of these compounds. Earlier studies have also suggested that geometry is a greater determinant of cardenolide activity than electronic factors (e.g., ability to form a dipole across the enone system).[2,15,16] For example, if electronic factors predominate in cardenolide binding, the C(17) α,β-unsaturated aldehyde analogue should have been up to 123 times more active than digitoxigenin (1); however, it was less active than 1.[15] Combined with the (Na^+,K^+)ATPase-inhibiting activities presented in this paper, there is a strong indication that the C(20)–C(22) double bond in cardenolides is simply serving a passive function in (Na^+,K^+)ATPase inhibition—keeping the lactone ring in the right geometric orientation for maximal binding.

These straightforward geometric effects can be easily seen with Dreiding models and have been confirmed with crystallographic and preliminary conformational energy studies.[2] When the 20(22) double bond of digitoxigenin is hydrogenated to **2a**, **2b**, **3a**, **3b**, **7a**, and **7b**, C(20) changes from sp^2 to sp^3, moving the lactone carbonyl from its "digitoxigenin position". Thus, all six analogues become less active than digitoxigenin. However, when the 14β-hydroxyls of **7a** and **7b** are lost to form **2a** and **2b**, C(14) changes from sp^3 to sp^2, causing a perturbation in ring D which moves the lactone. The result is that the carbonyl of **2b** moves *closer* to the "digitoxigenin position", while **2a** moves even farther *away*. Thus, **2b** is more active than **2a**, **7a**, and **7b**, and **2a** is the least active of all. The greater activity of 14-ene **2b** than either 14β-hydroxy analogues **7a** or **7b** may also suggest that the 14β-OH is not contributing as much to (Na^+,K^+)ATPase binding as previously believed.

Previous studies have shown that the cardenolide lactone rings of digoxigenin,[2] digitoxigenin, and strophanthidin[30] have two preferred crystallographic orientations. To relate these observations to the present study, a conformational energy diagram for rotation of the side group of digitoxigenin (1) in 10° steps was generated using a version of the molecular mechanics program CAMSEQ,[31] which was specially modified to be used in conjunction with the NIH PROPHET computer system.[32] The energy for each point was calculated using the nonbonded and electrostatic potentials built into the CAMSEQ program, resulting in the potential energy plot shown in Figure 1. The previously described geometric changes are consistent with digitoxigenin's lactone ring being in the crystallographically observed torsion angle, C(13)–C(17)–C(20)–C(22), of 76.3°. Furthermore, the energy minima at 76° and −104° corresponds to the two preferred crystallographic orientations of cardenolide lactone rings.

More detailed studies with the PROPHET system are in progress.

Experimental Section

Elemental analyses were performed by MHW Laboratories, Phoenix, Ariz. Mass spectra were obtained at the Oregon State University Mass Spectroscopy Laboratory, Department of Agricultural Chemistry. The 100-MHz NMR spectra were taken at the Oregon State University NMR Spectroscopy Laboratory, Department of Chemistry. Melting points were determined with a Thomas-Hoover melting point apparatus and are corrected. Thin-layer chromatographies were on 0.25-mm EM silica gel 60 F-254 glass TLC plates using techniques and solvent combinations previously described for these compounds.[1] Optical rotations in chloroform were taken on a Perkin-Elmer 141 polarimeter. UV spectra were on a Cary 15 in ethanol. Optical rotations in chloroform were taken on a Perkin-Elmer 141 polarimeter.

(20*R*,*S*)-20,22-Dihydrodigitoxigenin (3). Digitoxigenin (1) was hydrogenated with 5% $Pd/CaCO_3$ catalyst as previously reported,[17] worked up, and rehydrogenated with new catalyst. The absence of any unreacted digitoxigenin in the final crystallized product was confirmed by examining a 3.75×10^{-3} M solution of **3** in ethanol by UV spectroscopy. This solution exhibited a gradually rising end absorption identical to that of γ-butyrolactone (Aldrich). On this same instrument, for example, a 3.75×10^{-5} M (i.e., 1% of **3**) solution of 1 had an absorbance of 0.6 (λ_{max} 217 nm, ε 16 000). Not the slightest 217-nm shoulder could be seen on the end absorption of the 3.75×10^{-3} M solution of **3**, showing that there was virtually no unreacted digitoxigenin remaining.

(20*S*)-3β,14β-Dihydroxy-5β,14β-cardanolide [3b, (20*S*)-20,22-Dihydrodigitoxigenin]. Twenty grams (0.52 mol) of (20*R*,*S*)-20,22-dihydrodigitoxigenin (**3**) was recrystallized from ethyl acetate, giving 8.9 g of crystals enriched in **3b**. Two additional crystallizations from ethyl acetate gave 4.5 g (45%, based on an approximately 1:1 mixture of *R* and *S* in **3**) of **3b**, free of **3a** both on TLC and 100-MHz NMR: mp 213–214 °C; IR ($CHCl_3$) 1770 cm^{-1}; NMR ($CDCl_3$) δ 4.43 (t, J = 8 Hz, 1, C_{21}-H_b), 4.14 (m, 1, C_3-H), 4.05 (t, one peak under the 4.14 m, J = 8 Hz, 1, C_{21}-H_a), 2.85 (m, 1, C_{17}-H), 2.12–2.26 (m, with major peaks at 2.66, 2.57, 2.20, 2.12, 2, C_{22}-H), 0.97 and 0.98 (each s, 6, C_{18}-H and C_{19}-H); $[\alpha]^{26}_D$ +14.8° (*c* 0.189); MS *m/e* 376 (M^+), 358 ($M - H_2O$). Anal. ($C_{23}H_{36}O_4$) C, H.

(20*R*)-3β,14β-Dihydroxy-5β,14β-cardanolide [3a, (20*R*)-20,22-Dihydrodigitoxigenin]. The mother liquor from the first crystallization of **3** in ethyl acetate was evaporated and the residue crystallized in ethyl acetate to give 11.0 g of crystals enriched in **3a**. Two additional crystallizations from ethyl acetate gave 3.2 g (32%, based on an approximately 1:1 mixture of *R* and *S* in **3**), free of **3b** both on TLC and 100-MHz NMR: mp 218–220 °C; IR ($CHCl_3$) 1770 cm^{-1}; NMR ($CDCl_3$) δ 0.52 (t, J = 8 Hz, C_{21}-H_a),

4.13 (m, 1, C_3-H), 3.89 (t, J = 8 Hz, C_{21}-H_b), 2.90 (m, 1, C_{17}-H), 2.20–2.73 (m, with major peaks at 2.20, 2.30, 2.38, 2.48, 2.55, 2.73, 2, C_{22}-H), 0.97 (s, 6, C_{18}- and C_{19}-H), $[\alpha]^{26}_D$ +5.64 (c 0.248); MS m/e 376 (M^+), 358 (M - H_2O). Anal. ($C_{23}H_{36}O_4$) C, H.

(20*S*)-3β,14β-Dihydroxy-5β,14β-cardanolide 3β-(2′*R*,*S*)-Tetrahydropyran-2′-yl Ether (4b). A solution of 2.0 g (5.31 mmol) of **3b**, 5.53 g (65.7 mmol, 6 mL) of dihydropyran (Aldrich 99+%), 20 mg of *p*-toluenesulfonic acid, and 20 mL of anhydrous dioxane was stirred for 15 min. To the solution was added 12 mL of half-saturated ammonia in methanol, and the mixture was evaporated at room temperature in vacuo. The residue was dissolved in 30 mL of methylene chloride, and the solution was washed once with water. The methylene chloride was dried with $MgSO_4$, the solvent was evaporated in vacuo, and the crude product was crystallized in ethyl acetate to give 2.15 g (88.1%) of **4b** (two distinct spots on TLC,[1] corresponding to the mixture of 2′ diastereomers): mp 175–178 °C; IR ($CHCl_3$) 1770 cm^{-1}; NMR ($CDCl_3$) δ 4.63 (m, 1, $C_{2'}$-H), 4.42 (t, J = 8 Hz, 1, C_{21}-H_b), 4.02 (t, one peak under the 3.90, J = 8 Hz, 1, C_{21}-H_a), 3.90 (m, 1, C_3-H), 3.45 (m, 2, C_6-H), 2.83 (m, 1, C_{20}-H), 2.06–2.60 (m, with major peaks at 2.63, 2.54, 2.16, 2.09, 2, C_{22}-H), 0.95 and 0.92 (each s, 6, C_{18}-H and C_{19}-H). Anal. ($C_{28}H_{44}O_5$) C, H.

(20*R*)-3β,14β-Dihydroxy-5β,14β-cardanolide 3β-(2′*R*,*S*)-Tetrahydropyran-2′-yl Ether (4a). A solution of 500 mg (1.3 mmol) of **3a**, 1.38 g (16.41 mmol, 1.5 mL) of dihydropyran, 5 mg of *p*-toluenesulfonic acid, and 5 mL of anhydrous dioxane was stirred for 15 min. Workup and crystallization as described for **4b** gave 520 mg (85.2%) of **4a** (two distinct spots on TLC,[1] corresponding to the mixture of 2′ diastereomers): mp 182–185 °C; ($CHCl_3$) 1770 cm^{-1}; NMR ($CDCl_3$) δ 4.64 (m, 1, $C_{2'}$-H), 4.52 (t, J = 8 Hz, $C_{2'}$-H_a), 3.71–3.98 (m, 2, C_{21}-H_b and C_3-H), 3.48 (m, 2, C_6-H), 2.9 (m, 1, C_{20}-H), 2.20–2.73 (m, with major peaks at 2.20, 2.31, 2.38, 2.48, 2.56, 2.73, 2, C_{22}-H), 0.97 (s, 6, C_{18}-H and C_{19}-H). Anal. ($C_{28}H_{44}O_5$) C, H.

(20*S*)-3β,14β-Dihydroxy-22-methylene-5β,14β-cardanolide 3β-(2′*R*,*S*)-Tetrahydropyran-2′-yl Ether (6a). To an ice-bath cooled solution of 1.0 mL (0.8 g, 5.67 mmol) of *N*-isopropylcyclohexylamine in 4 mL of anhydrous tetrahydrofuran (THF) under N_2 was added 2.0 mL (5.2 mmol) of 2.6 M butyllithium in hexane. The solution was stirred for 10 min and cooled to −78 °C, and 20-min later 200 mg (0.43 mmol) of **4a** in 5 mL of anhydrous THF was added. After stirring the solution for 40 min, anhydrous CO_2 (Matheson, Bone-Dry grade) was bubbled into the solution and the dry ice bath was removed. Bubbling continued for 25 min, during which time the solution warmed to room temperature. The reaction mixture was poured into 10 mL of 5% HCl and ice and extracted twice with ether; the ether solution was washed once with 5% $NaHCO_3$, once with water, and dried over $MgSO_4$. Evaporation of the ether solution gave a crude product, presumably **5a**, to which was added 6 mL of 37% aqueous CH_2O solution (with 10% MeOH, equivalent to 2.22 g, 74 mmol of CH_2O) and 1.6 mL (2.2 g, 30.2 mmol) of diethylamine. The solution was heated on a steam bath for 30 min and worked up as described for **5a**, to give 370 mg (90%) of **6a**. The analytical sample was obtained by recrystallization from ether: yield 120 mg in the first crop (30%); mp 187–191 °C; IR (KBr) 1750 cm^{-1}; NMR ($CDCl_3$) δ 6.28 (d, $J_{H_a\text{-}H_b}$ = 2 Hz, 1, C_{24}-H_b), 5.73 (d, $J_{H_a\text{-}H_b}$ = 2 Hz, 1, C_{24}-H_a), 4.65 (br s, 1, $C_{2'}$-H), 4.49–4.16 (m, 2, C_{21}-H), 3.80–3.99 (m, 1, C_3-H), 3.26–3.60 (m, 3, C_6-H and C_{20}-H), 0.95 and 1.00 (two s, 6, C_{18}- and C_{19}-H). Anal. ($C_{29}H_{44}O_5$) C, H.

(20*R*)-3β,14β-Dihydroxy-22-methylene-5β,14β-cardanolide 3β-(2′*R*,*S*)-Tetrahydropyran-2′-yl Ether (6b). To an ice-bath cooled solution of 4.0 mL (3.2 g, 22.7 mmol) of *N*-isopropylcyclohexylamine in 16 mL of anhydrous THF under N_2 was added 8.0 mL (22.4 mmol) of 2.6 M butyllithium in hexane. The solution was stirred for 10 min and cooled to −78 °C, and 20-min later 1.6 g (3.4 mmol) of **4b** in 20 mL of anhydrous THF was added. After stirring for 40 min, the solution was treated with CO_2 and worked up as described for **5a**, to give a crude product, presumably **5b**. To this product was added 24 mL of 37% aqueous CH_2O solution (with 10% MeOH, equivalent to 8.88 g, 0.296 mol of CH_2O) and 6.4 mL (8.83 g, 0.12 mol) of diethylamine. The solution was heated on a steam bath for 30 min and worked up as described for **5a**, to give 1.5 g (91.5%) of **6b**. The analytical sample was obtained by recrystallization from ether: yield 700 mg (42%) in the first crop; mp 192–196 °C; IR (KBr) 1750 cm^{-1}; NMR ($CDCl_3$) δ 6.31 (d, $J_{H_a\text{-}H_b}$ = 3 Hz, 1, C_{24}-H_b), 5.57 (d, $J_{H_a\text{-}H_b}$ = 2 Hz, 1, C_{24}-H_a), 4.66–4.81 (m, 1, C_{21}-H), 4.65 (br s, 1, $C_{2'}$-H), 4.29–4.47 (m, 1, C_{21}-H), 3.80–3.99 (m, C_3-H), 3.26–3.60 (m, 3, C_6- and C_{20}-H), 0.92 and 0.94 (each s, 6, C_{19}-H and C_{18}-H). Anal. ($C_{29}H_{44}O_5$) C, H.

(20*S*)-3β,14β-Dihydroxy-22-methylene-5β,14β-cardanolide (7a). A solution of 300 mg of **6a** in 30 mL of MeOH and 0.75 mL of 5% HCl was stirred at room temperature for 1 h. Workup as described for **5a** and crystallization from ethyl acetate gave 255 mg (84%) of **7a**. The analytical sample was obtained by recrystallization from ethyl acetate: yield 152 mg (50%) in the first crop; mp 223–224 °C; IR (KBr) 1750 cm^{-1}; NMR ($CDCl_3$) δ 6.28 (d, $J_{H_a\text{-}H_b}$ = 2 Hz, 1, C_{24}-H_b), 5.73 (d, $J_{H_a\text{-}H_b}$ = 2 Hz, 1, C_{24}-H_a), 4.19–4.50 (m, 2, C_{21}-H), 4.16 (m, 1, C_3-H), 3.29 (m, 1, C_{20}-H), 1.00 (s, 3, C_{18}-H), 0.97 (s, 3, C_{19}-H); $[\alpha]^{26}_D$ −46.7° (c 0.225); MS m/e 388 (M^+), 370 (M - H_2O). Anal. ($C_{24}H_{36}O_4$) C, H.

(20*S*)-3β,14β-Dihydroxy-22-methylene-5β,14β-cardanolide (7b). A solution of 500 mg of **6b**, 30 mL of MeOH, and 0.5 mL of 5% HCl was stirred at room temperature for 1 h. Workup as described for **5a** and recrystallization from ethyl acetate gave 390 mg (94.9%) of **7b** as an amorphous powder: mp 105–108 °C; IR (KBr) 1750 cm^{-1}; NMR ($CDCl_3$) δ 6.31 (d, $J_{H_a\text{-}H_b}$ = 3 Hz, 1, C_{24}-H_b), 5.57 (d, $J_{H_a\text{-}H_b}$ = 3 Hz, 1, C_{24}-H_a), 4.65–4.81 (m, 1, C_{21}-H), 4.28–4.47 (m, 1, C_{21}-H), 4.14 (m, 1, C_3-H), 3.27 (m, 1, C_{20}-H), 0.97 (s, 3, C_{19}-H), 0.92 (s, 3, C_{18}-H); $[\alpha]^{26}_D$ +73.0 (c 0.195); MS m/e 388 (M^+), 370 (M - H_2O). Anal. ($C_{24}H_{36}O_4$) C, H.

Acknowledgment. The financial assistance of the National Institutes of Health (Grant HL 21457), the Oregon and Minnesota Heart Associations, the Oregon State University Research Council (through NIH Biomedical Research Support Grant RR 07079), and the Veterans Administration Medical Research Fund is gratefully acknowledged. We are also appreciative of the technical assistance of Gregory Quarfoth and Ms. Renae Roelofs in the biological studies and Ms. Susan Randall in the 100-MHz NMR studies. The organization and analysis of the data base associated with this investigation were carried out in part using the PROPHET system, a unique national resource sponsored by the NIH. Information about PROPHET including how to apply for access can be obtained from the Director, Chemical/Biological Information-Handling Program, Division of Research Resources, NIH, Bethesda, Md. 20014.

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Conformational Analogy between β-Lactam Antibiotics and Tetrahedral Transition States of a Dipeptide

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The three-dimensional structures of various penicillins and cephalosporins are compared to the spatial characteristics of glycylglycine and the tetrahedral adducts formed when a nucleophile attaches to the amide carbonyl carbon of this dipeptide. The dipeptide is taken to model the D-alanyl-D-alanine terminus of the precursors of bacterial cell-wall peptidoglycan cross-links. Least-squares fitting shows that the spatial match between the dipeptide and the antibiotic depends on the thiazolidine or dihydrothiazine ring conformation, as well as the conformation of the dipeptide. In general, the tetrahedral adducts fit somewhat better than the parent dipeptide. A previously unobserved 3-cephem conformer is found by molecular mechanics calculations to be less stable than the usual crystallographically observed conformer.

The final stage in peptidoglycan biosynthesis of bacterial cell walls involves cross-linking between the peptide side chains of glycan polymers.[1] The nascent pentapeptide side chains terminate in D-alanyl-D-alanine. Cross-linking in the peptidoglycan is regulated by various transpeptidases, carboxypeptidases, and endopeptidases. The former two enzymatic activities involve rupture of the D-Ala–D-Ala bond, presumably by a nucleophilic attack mechanism involving a tetrahedral adduct. The β-lactam antibiotics are known to upset the balance of the various cell-wall enzymatic activities[1] and thereby exert their antibacterial action.

Using molecular models, it was evident to earlier workers[2,3] that a structural analogy existed between the penicillins and what seemed to be possible transition-state (TS) structures for the scission of the peptide bond in D-Ala–D-Ala. Tipper and Strominger[2] described a TS structure only vaguely in terms of a nonplanar amide nitrogen. Lee[3] described a TS in terms of a severe twisting about the amide C–N bond so that the bond is weakened and the amide nitrogen is tetrahedrally hybridized.

Recently, some preliminary but detailed structural information on the transition states of a dipeptide became available from molecular-orbital calculations.[4] These calculations, for practical reasons, used glycylglycine as a model of D-Ala–D-Ala. A simple nucleophile, OH^-, was allowed to approach the peptide carbonyl carbon on either the α or β face. Produced were two transition intermediates with tetrahedral hybridization at the reaction center and slight pyramidal hybridization at the amide nitrogen. For convenience, they will be referred to as α-face TS and β-face TS. Although these structures probably represent energy minima on the gas-phase reaction surface, it is not known whether they are intermediates or TS's on a condensed-phase reaction surface.[4,5] It was visually apparent from molecular-structure drawings that for one pertinent dipeptide conformation the α-face TS was closer in certain spatial features to a penicillin G structure than were either the β-face TS or the Gly-Gly reactant.[4]

The purpose of this disquisition is to give a more quantitative and thorough comparison of Gly-Gly structures to β-lactam antibiotic structures. These include not only penicillin G but also penicillins with other thiazolidine conformations and cephalosporins with two possible conformations of the dihydrothiazine ring. In order to make the comparisons, a least-squares fitting of dipeptide

0022-2623/79/1822-0533$01.00/0