Stereochemistry of Macrolides. I.[1a] Conformation of Aglycones of Pikromycin and Narbomycin and Their Derivatives

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Abstract: The stereochemistry of kromycin (**1**), narbomycin (**2a**), and anhydronarbonolide (**3**) has been studied. Reduction of **1** led to **3**, both of which have the same configurations. Conformation of both macrolactones (**1** and **3**) was determined from their NMR and CD spectra in the state of solution. For the determination of NMR and CD studies, anhydrodihydronarbonolide (**5a**) of **2a**, dihydrokromycin (**5b**), and reduced compounds (**6** and **7**) were prepared. A new "diamond lattice" modified conformation model for kromycin (**1**) was proposed.

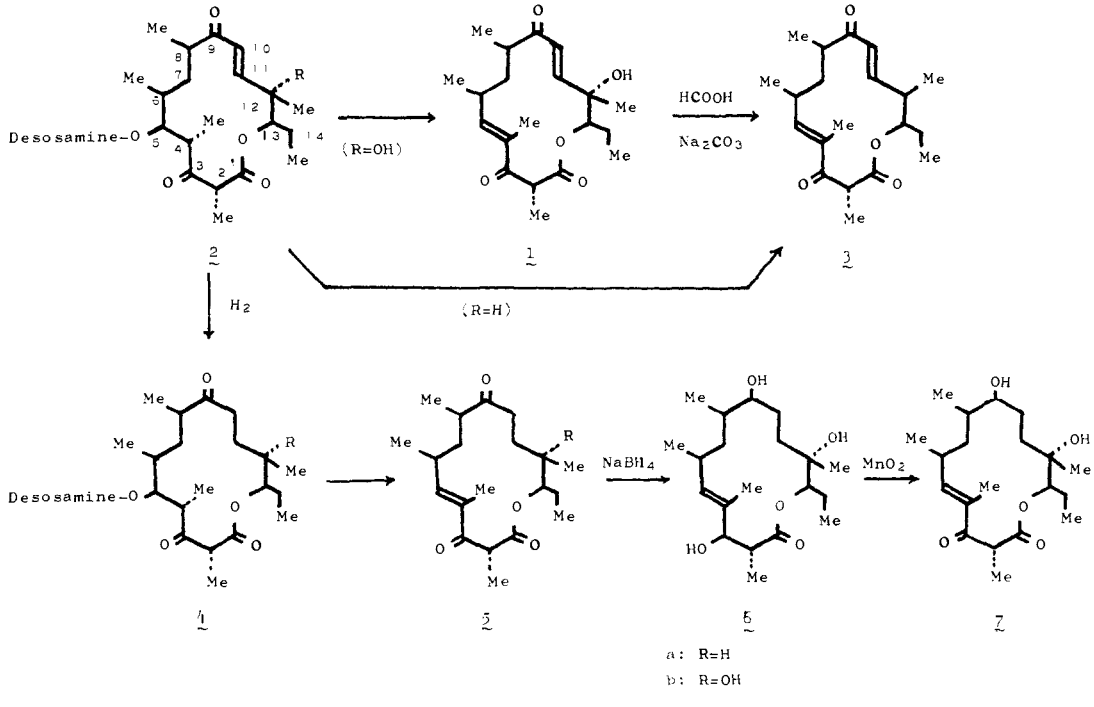
Recently, the structure of kromycin was fully confirmed by an X-ray diffraction study.[2] An earlier application of Dale's "diamond lattice"[3] conformational model A for macrolide antibiotics was proposed by Celmer.[4,5] Egan et al.[6] also reported the alternate Dale "diamond lattice" conformational model B as applied to 14-membered macrolactones of erythromycin antibiotics.

In the present paper, we describe the configurational and conformational studies of kromycin (**1**), and its relation to narbomycin (**2a**), and anhydronarbonolide (**3**) of **2a** from NMR and CD spectra.[7] During the course of this work, a new "diamond lattice" modified conformation model C for the 14-membered macrolactone of kromycin (**1**) and its derivatives was proposed.

Kromycin (**1**) was treated with formic acid and sodium carbonate[8] to obtain deoxykromycin (**3**), mp 114–115°, which had also been produced by a gentle hydrolysis of narbomycin[9] (**2a**). This result confirmed the stereochemistry of narbomycin (**2a**) which was clearly illustrated to be the same as that of kromycin (**1**).

For the preparation of anhydrodihydronarbonolide (**5a**), dihydronarbomycin (**4a**) was hydrolyzed under the same conditions as for **2b**. This reaction was already reported by Prelog et al.,[10] and they obtained five compounds (**5aA, 5aB, 5aC, 5aD,** and **5aE**). On the other hand, Suzuki and Aota[8] obtained six compounds (**5aA, 5aB, 5aC, 5aD, 5aE,** and **5aF**) from the same reaction. In our experiment, two compounds (**5aE** and **5aF**) were obtained in a pure state. From the NMR, CD, and mass spectral studies as described later in this article, both compounds, **5aE** and **5aF**, are diastereoisomers, and the former compound (**5aE**) has a *Z* conformation at the 4 position (cis isomer), while the latter compound (**5aF**) has an *E* conformation at the 4 position. Dihydropikromycin (**4b**) was hydrolyzed to a single compound (**5b**), which was found to correspond to the configuration of **5aF** by NMR and CD spectra. Sodium borohydride reduction of **5b** gave a triol (**6**) which was oxidized to **7** with manganese dioxide.

Chart I



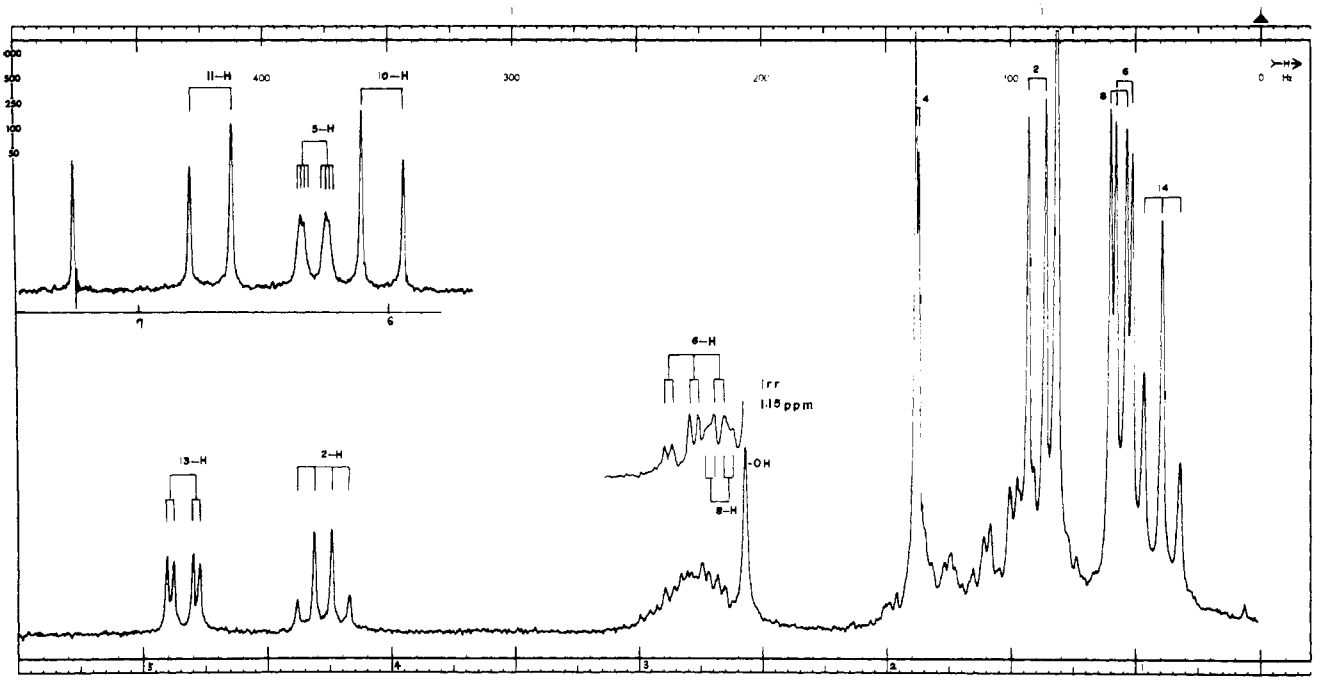


Figure 1. NMR spectrum of kromycin (**1**) in $CDCl_3$.

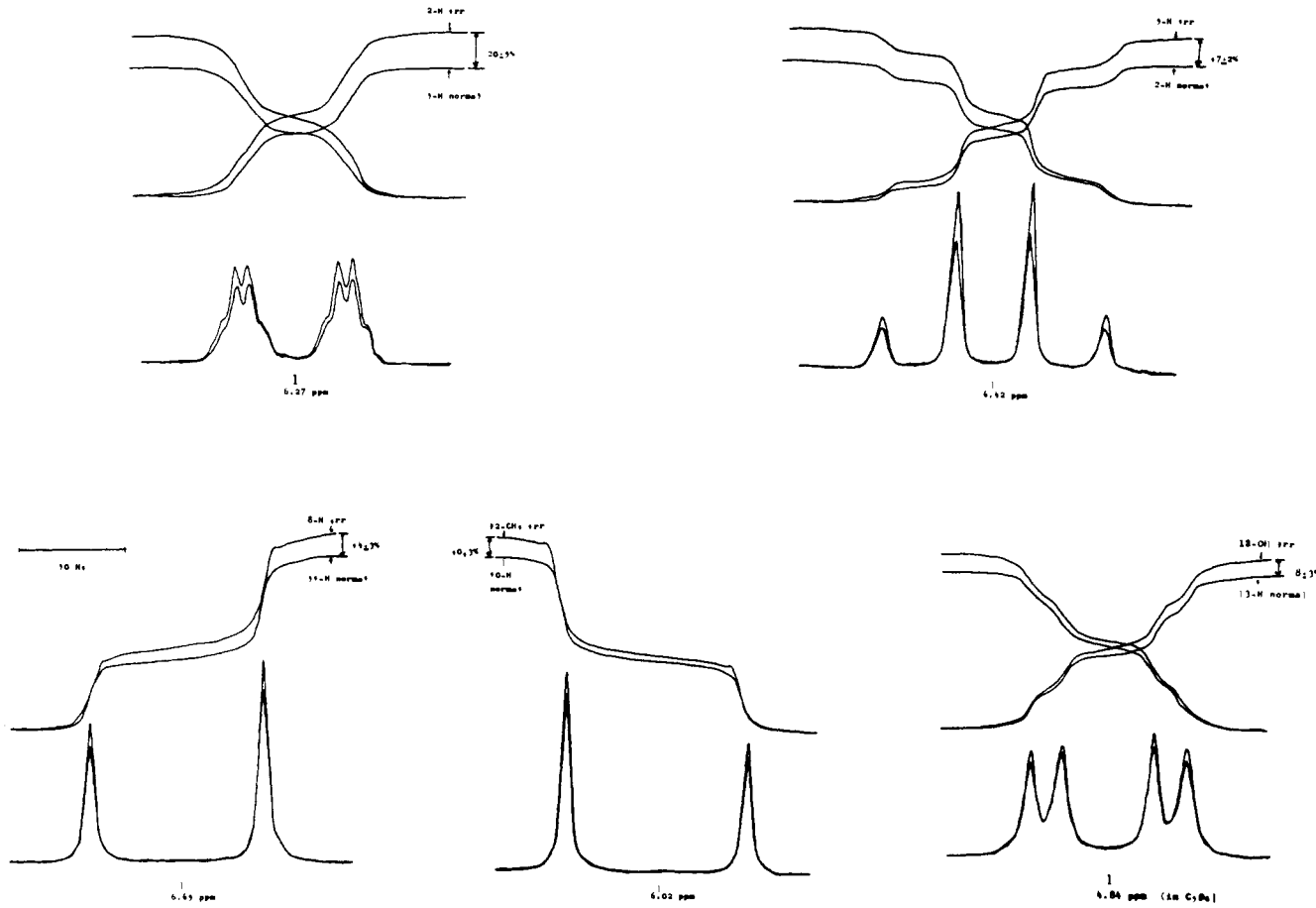


Figure 2. NOE data of kromycin (**1**) in C_6D_6.

Results and Discussion

I. Kromycin (1) and Anhydronarbonolide (3). The NMR data of kromycin (**1**), shown in Figures 1 and 2 and Table I, revealed the same conclusion as reported by Hughes et al.[2] The J values of the 5-H (4-CH_3) were 1.4 and 1.2 Hz in $CDCl_3$ and C_6D_6, respectively, and a 20% NOE effect was observed between 2-H and 5-H. This evidence indicates the 4-*E* and 2-*R* configuration. The 2-*R* configuration was confirmed by derivation of *S*-2-hydroxymethylpropanoic acid.[11] Other NOE's were observed between 8-H and 11-H, 10-H and 12-CH_3, and 13-H and 12-OH. The solvent shift at 10-H was negative [(J = 17.1 ($CDCl_3$) and 16.5 Hz (C_6D_6)].[12] These data reveal the s-trans conformation of

Table I. NMR Data of Kromycin (1)

	Chemical shifts (δ ppm; J value, Hz)			
	$CDCl_3$	C_6D_6	$\Delta^{CDCl_3}_{C_6D_6}$	CD_3OD[a]
2H	4.24, q, $J_{2,2Me}$ = 7.1 $J_{2,5}$ = 0.1–0.2	3.88	0.36	4.45
2Me	1.41, d, J = 7.1	1.37	0.04	1.46
4H				
4Me	1.89, d, J = 1.4	1.82	0.07	1.93
5H	6.27, dq, $J_{5,4Me}$ = 1.4 $J_{5,6}$ = 10.0	6.01	0.26	6.45
6H	2.83, m, $J_{6,6Me}$ = 6.7	(2.5_5)	0.2_8	
6Me	1.06, d, $J_{6,7}$ = 10 and 3.5	0.72	0.34	1.13
7H				
8H	2.73, m, $J_{8,7}$ = 7 and 4	(2.7_0)	0.0_3	
8Me	1.08, d, $J_{8Me,8}$ = 6.5	1.04	0.04	1.13
10H	6.02, d, $J_{10,8}$ = 0.1 $J_{10,11}$ = 17.1	6.20	−0.18	6.11
11H	6.69, d, $J_{11,10}$ = 17.1 $J_{11,12OH}$ = 0.3	6.74	−0.05	6.85
12OH	2.64, s	2.22	0.42	
12Me	1.32, s	1.14	0.18	1.41
13H	4.82, dd, $J_{13,14}$ = 10 and 2.7	4.84	−0.02	4.86
14H				
14Me	0.92, t, $J_{14Me,14}$ = 7.0	0.79	0.13	0.94

[a] 60 MHz.

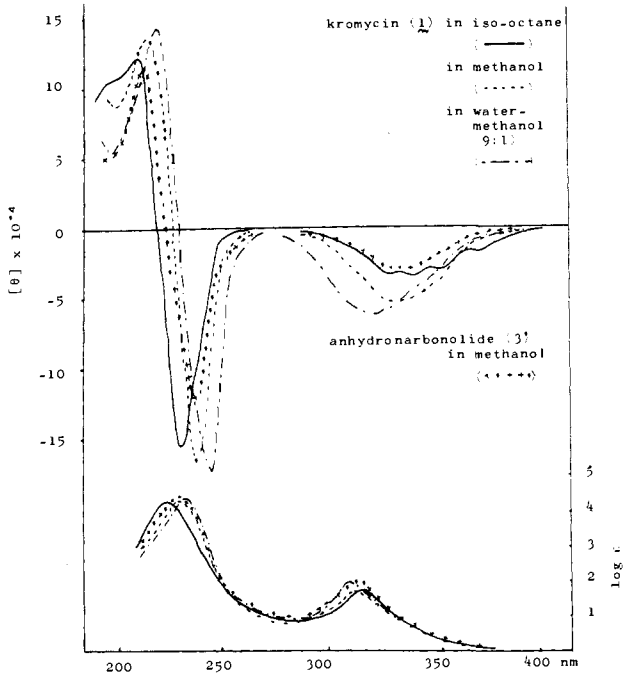


Figure 3. CD and uv spectra of **1** and **3** in various solvents.

10-E α,β-unsaturated carbonyl group at C-9 position. It was therefore concluded that kromycin (**1**) has an s-trans conformation in which the intramolecular hydrogen bond between C-9 carbonyl and 12-OH groups is absent. In CD_3OD solution, the data obtained were similar to that in $CDCl_3$ and C_6D_6 (Table I).

CD spectra of kromycin (**1**) in various solvents shown in Figure 3 reveal a strong negative Cotton effect at 230–240 nm ([θ] −165000) and a positive Cotton effect at 210–220 nm ([θ] +132000) owing to the π–π* transition of α,β-unsaturated carbonyl groups. This pair of Cotton effects of opposite signs can be interpreted as the typical intramolecular exciton coupling of two α,β-unsaturated ketones in kromycin (**1**), because these Cotton effects of opposite signs and of exceptionally large amplitudes are characteristic of

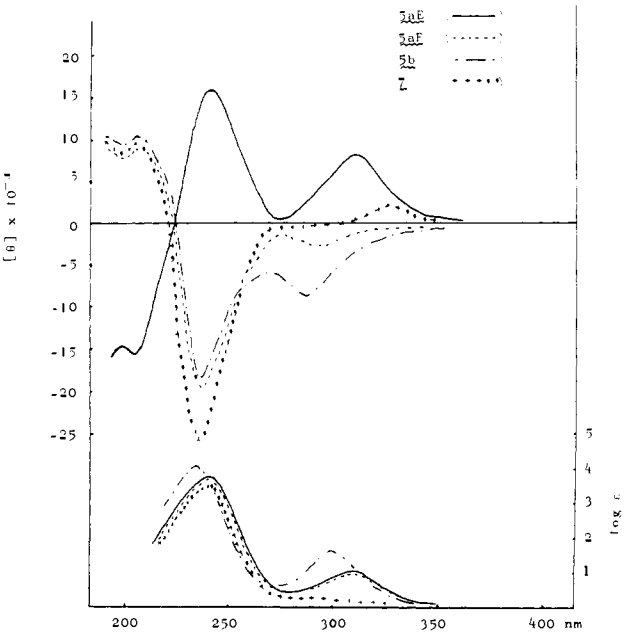


Figure 4. CD and uv spectra of **5aE**, **5aF**, **5b**, and **7** in methanol.

the dipole-coupling Cotton effects.[13] This was further supported by the fact that the centroid of the two lobes was very close to the maximal point of uv absorption (Figure 3). On the other hand, CD spectra of aglycone F (**5aF**) and dihydrokromycin (**5b**) containing one enone system showed only a weak negative Cotton effect ($[\theta]_{236}$ −18510, $[\theta]_{235}$ −18430, respectively), and the uv absorption maximal points were close to the maximal points of their Cotton effects (Figure 4). Thus, it is obvious that the CD of kromycin (**1**) is mainly attributable to the exciton chirality between two conjugated ketones.[14]

The negative sign of the first Cotton effect clearly indicates that a bis α,β-unsaturated ketone system in the molecule has a negative chirality in solution.[14] Although the relationship between the actual conformation and the negative chirality is obscure at the present stage because of the complex ring flexibility, it is quite interesting that the CD Cotton effects due to exciton chirality are observable also in macrocyclic compounds.

Anhydronarbonolide (**3**) (12-deoxykromycin) showed a CD curve similar to that of kromycin (**1**) shown in Figure 3. The coupling constant of 5-H and 6-H (10 Hz) showed the

Table II. NMR Data of 5b, 5aE, and 5aF

	Chemical shifts ($CDCl_3$, δ ppm; J value, Hz)		
	5b	5aE	5aF
2H	4.31, q, $J_{2,2Me}$ = 6.8		
2Me	1.40, d, $J_{2Me,2}$ = 6.8		
4Me	1.86, d, J = 1.5	2.02 (3 H, s)	2.00 (3 H, d, J = 1.5)
5H	6.34, dq, $J_{5,4Me}$ = 1.5 $J_{5,6}$ = 10.0	5.24 (1 H, d, J = 10)	5.24 (1 H, dq, J = 10 and 1.5)
6H	2.73, m, $J_{6,5}$ = 10.0		
6Me	1.08, d, $J_{6Me,6}$ = 6.5		
7H			
8H	2.46, m		
8Me	1.01, d, $J_{8Me,8}$ = 6.5		
10H	2.46, m		
11H			
12Me	1.18, s		
12OH	2.09, s		
13H	4.79, dd, $J_{13,14}$ = 10 and 3.1		
$14H_a$	1.37, dq		
$14H_b$	1.85, dq		
14Me	0.93, t, $J_{14Me,14}$ = 7.0		

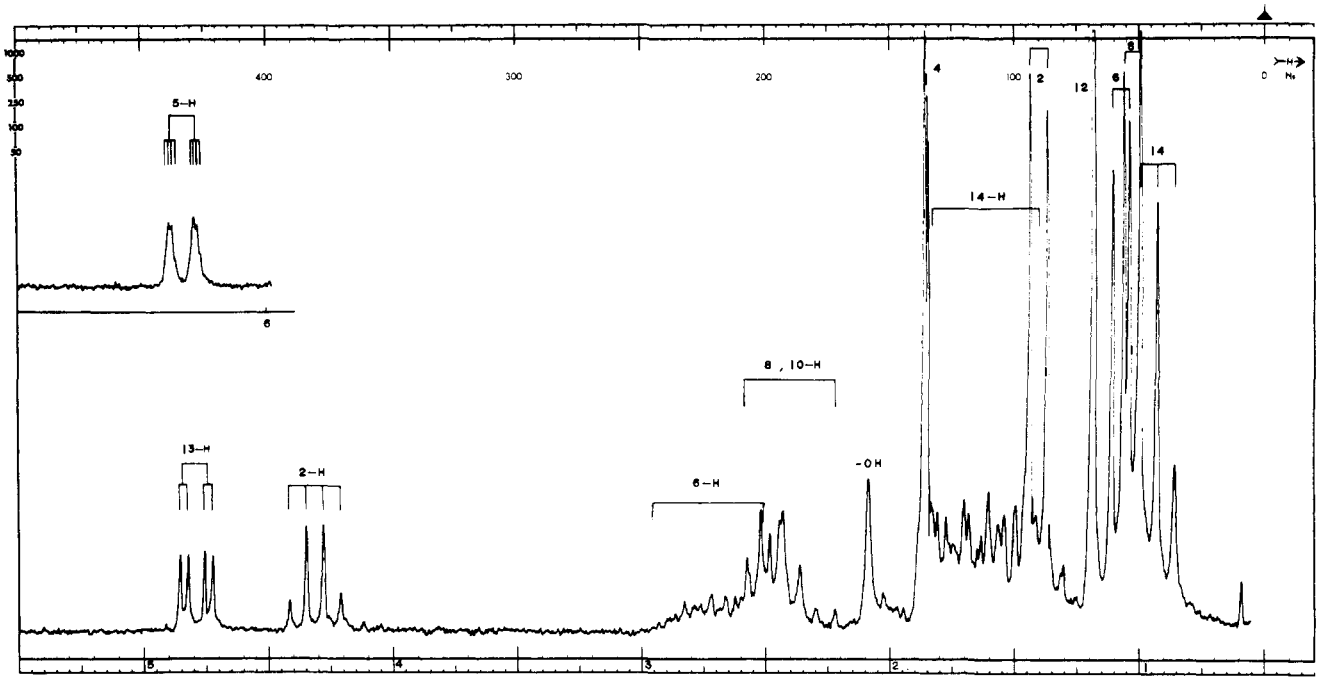


Figure 5. NMR spectrum of dihydrokromycin (**5b**) in $CDCl_3$.

same value as that of **1**. From these data, **3** has the same stereochemistry as **1**.

II. Anhydrodihydronarbonolide (5a) and Dihydrokromycin (5b). The NMR data of **5aF** and **5b** are summarized in Figure 5 and Table II, and they revealed similar conformation as that of **1** from the *J* values and NOE data [between 2-H and 5-H (29%), 5-H and 2-H (16%)]. On the other hand, anhydrodihydronarbonolide (**5aE**) showed a slight difference at 4-CH_3 (2.02 ppm, singlet) and 5-H (5.24 ppm, doublet) from **5aF** (4-CH_3, doublet; 5-H, d-quartet). This result indicates 4-CH_3 group in **5aE** to be in cis configuration to 5-H, and this was confirmed by CD data shown in Figure 4. Compounds **5aF** and **5b** have a negative Cotton effect for the α,β-unsaturated carbonyl group at 236 nm (−18510) and at 235 nm (−18430), respectively, while **5aE** showed a positive Cotton effect at 242 nm (+15810). These CD data were further confirmed from CD data of 9-hydrodihydrokromycin (**7**) which showed a positive Cotton effect at 322 nm (+980) and a negative Cotton effect at 236 nm (−24560)for assigned n-π^* and π-π^* transitions of α,β-unsaturated carbonyl group, respectively.

In conclusion, **5aF, 5b,** and **7** have the counterclockwise s-trans α,β-unsaturated carbonyl group at the 3 position,[1,15] and **5aE** has the s-cis configuration of the α,β-unsaturated carbonyl group at the 3 position.[15]

III. "Diamond Lattice" Conformation Model. The most favorable "diamond lattice" conformation model C of kromycin (**1**) from the data of the X-ray analysis,[2] NMR, and CD spectral studies is proposed in Figure 6. From this conformation, the ethyl group at the 13 position and the hydroxyl group at the 12 position of kromycin (**1**) are placed in equatorial configurations, and both double bonds at the 4 and 10 positions are assigned the E configuration. Further application of this model to deoxykromycin (**3**), **5aF,** and **5b** is probable from the NMR and CD spectra.[16]

Experimental Section[17]

Anhydronarbonolide (3). (a) From Kromycin (1). To a solution of **1** (200 mg) in formic acid (10 ml) was added sodium carbonate (100 mg), and the mixture was refluxed for 6 hr. The cooled reaction mixture was poured into water and extracted with chloroform. Evaporation of the dried chloroform solution left a crude product, which was chromatographed on silica gel with petroleum ether-

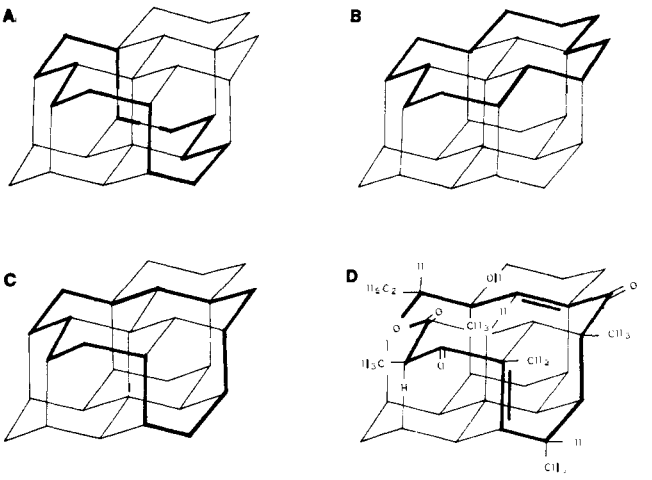


Figure 6. (A) Celmer-Dale conformation model A; (B) Perun-Dale conformation model B; (C) a new "diamond lattice" conformation model C; (D) a new "diamond lattice" conformation model C of kromycin.

ether (9:1) to yield 25 mg of **3** as white needles: mp 114–115°; uv λ_{max} (EtOH) 225 nm (log ϵ 4.42), 320 (2.10); mass *m/e* (calcd for $C_{20}H_{30}O_4$, 334.214) found, 334.214 ($M^{+\cdot}$).

(b) From Narbomycin (2a). A solution of **2a** (30 mg) in 1 *N* hydrochloric acid (10 ml) was adjusted to pH 6.8 with 0.1 *N* sodium hydroxide. After refluxing for 4 hr, the reaction mixture was extracted with chloroform. After evaporation of the dried chloroform, the residue was purified by preparative TLC to yield 4 mg of **3** as white needles, mp and mmp 114–115°.

Anhydrodihydronarbonolide (5a). A solution of **2a** (100 mg) in ethanol (20 ml) was hydrogenated over 5% Pd/C (50 mg), yielding dihydronarbomycin (**4a**), mp 92–93° (lit.[18] mp 96–99°). This was used without further purification. A solution of **4a** in 1 *N* hydrochloric acid (10 ml) was adjusted to pH 6.8 with 0.1 *N* sodium hydroxide. After refluxing for 4 hr, the cooled reaction mixture was extracted with ether. The residue from the ether extract was chromatographed over silica gel with petroleum ether–ether (9:1), and 4.2 mg of **5aE** was obtained as white needles: mp 108–109° (lit.[9] mp 108–110°); ir (KBr) 1726, 1710, 1696, and 1631 cm^{-1} (CO); uv λ_{max} (EtOH) 245 nm (log ϵ 3.76); mass *m/e* (calcd for $C_{20}H_{32}O_4$, 336.230) found, 336.234 ($M^{+\cdot}$).

From the second eluate, 3.8 mg of **5aF** was obtained as white needles: mp 106–107°; ir (KBr) 1720, 1710, 1698, 1632 cm^{-1}

(CO); uv λ_{max} (EtOH) 245 nm (log ϵ 3.74); mass *m/e* (calcd for $C_{20}H_{32}O_4$, 336.230) found, 336.229 ($M^{+\cdot}$).

Dihydrokromycin (5b). A solution of **2b** (1.0 g) in ethanol (50 ml) with hydrogenated over 5% Pd/C (0.25 g). The filtrate was evaporated under a reduced pressure to yield crude **4b** as a white powder (0.95 g), and this was used for the next reaction without further purification. **4b** was dissolved in 1 *N* hydrochloric acid (20 ml), which was adjusted to pH 6.8 with 0.1 *N* sodium hydroxide. After warming the solution at 60° for 120 hr, the separated crystals were recrystallized from petroleum ether–ether to **5b** (0.12 g) as colorless needles: mp 134–136°; ir (KBr) 1720 (lactone), 1701, 1665, 1632 cm^{-1} (CO); uv λ_{max} (EtOH) 233 nm (log ϵ 4.13), 297 (1.90).

Anal. Calcd for $C_{20}H_{32}O_5$: C, 68.15; H, 9.15. Found: C, 68.12; H, 9.20.

Tetrahydrokromycin (7). To a solution of **5b** (100 mg) in methanol (3 ml), a solution of sodium borohydride (100 mg) in methanol (10 ml) and water (1 ml) was added. After standing for 12 hr at room temperature, the reaction mixture was treated with acetone to decompose the excess reagent. The residue after evaporation of the ether extract was crystallized from petroleum ether–ether to yield 80 mg of **6** as white needles: mp 112–113°; mass *m/e* 356 ($M^{+\cdot}$). This was used for the next reaction without further purification.

A solution of **6** (70 mg) in acetone (30 ml) was stirred for 24 hr at room temperature with active manganese oxide (1 g). After evaporation of the filtrate, the residual white powder was crystallized from petroleum ether–ether to yield **7** (28 mg) as white needles: mp 137–138°; $[\alpha]^{20}D$ −15.4 (*c* 1.0, MeOH); ir (KBr) 3480 (OH), 1726 (lactone), 1670, 1635 cm^{-1} (CO); uv λ_{max} (EtOH) 245 nm (log ϵ 3.65); mass *m/e* (calcd for $C_{20}H_{34}O_5$, 354.241) found, 354.240 ($M^{+\cdot}$).

Anal. Calcd for $C_{20}H_{34}O_5$: C, 67.76; H, 9.67. Found: C, 67.55; H, 9.46.

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Bovine Serum Albumin as a Catalyst. II. Characterization of the Kinetics

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Abstract: The accelerated decomposition of the Meisenheimer complex, 1,1-dihydro-2,4,6-trinitrocyclohexadienate, by bovine serum albumin (BSA) is reported in detail. In the pH range of 7–9 the BSA-catalyzed reaction is accelerated by a factor of about 10^4 relative to the rate of decomposition of the substrate alone. This catalysis requires an unprotonated base on the protein (pK_a = 8.4) and can be stoichiometrically inhibited by binding of 1 equiv of pyridoxal phosphate (PP) to the protein. Chemical modification with acetic anhydride and fluorodinitrobenzene along with previous studies on the PP-BSA complex indicates that the active site of the molecule is rich in lysine and is probably coincident with a unique PP binding site in the protein. This unusual catalytic function has also been used as a sensitive probe to generate new information on some of the ligand-binding properties of the protein.

Numerous examples of catalytic activity of apparently nonbiological significance have been reported for a variety of proteins,[1,2] synthetic macromolecules,[3,4] and micellar complexes.[5,6] Recent examples include the bovine serum albumin (BSA) catalyzed decomposition of *p*-nitrophenyl acetate[1] and the catalyzed hydrolysis of 4-nitrocatechol sulfate by a synthetic polymer.[3] Often there are distinct differences between these activities and those associated with enzymes. Generally speaking, manifestation of specificity for both substrates and inhibitors is considerably more restricted in enzymes. In addition, but for one outstanding exception,[3] the relative rate enhancement due to the "nonbiological" catalyst is generally significantly less than the rate enhancements observed in typical enzyme systems.