two extremes can be almost fully realized within the pH range 4–8, at 30°, and with an overwhelming dependence on buffer catalysis in both directions. Since a comparable reaction could not be detected in the absence of the *complete* lock, it is impossible to make rate enhancement or equilibrium enhancement comparisons, as was done in our earlier studies. As emphasized in the preceding papers,[2] the overall effect of rate or equilibrium enhancement should be attributed not to the loss of rotational freedom alone, but also to a combination of secondary consequences, which may include improvement in orientation, reduced solvation, interorbital distortion and penetration, and perhaps other phenomena not yet recognized. The overall result is a considerable diminution of the free energy barrier between ground and transition states.

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Correlation of Reaction Rate Acceleration with Rotational Restriction. Crystal-Structure Analysis of Compounds with a Trialkyl Lock

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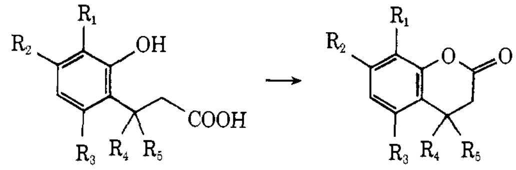
Abstract: Enzyme-promoted reactions have a rate advantage of 10^{11}–10^{15} over their nonenzymatic counterparts. The enzyme is believed to associate closely with the substrate in such a manner as to restrict the conformation of the substrate to a geometry favorable for the reaction to take place. A similar enhancement of reaction rate of lactonization in *o*-hydroxyhydrocinnamic acid has been achieved by substitution of methyl groups both on the ring and the side chain (Milstien and Cohen). Crystal-structure analyses of the "over-methylated" lactone pentamethylhydrocoumarin [space group $P2_1$ (disordered); $a = 10.148$, $b = 8.766$, and $c = 7.802$ Å; $\beta = 115.45°$] and the alcohol analog of pentamethyl-*o*-hydroxyhydrocinnamic acid [space group $C\bar{1}$; four independent molecules per asymmetric unit; $a = 11.615$, $b = 25.987$, and $c = 18.150$ Å; $\alpha = 93.66°$; $\beta = 90.38°$; $\gamma = 98.36°$] show that the conformation of the acid has been restricted by the presence of the "trialkyl lock" to be similar to that of the lactone. The four independent molecules of the alcohol analog of the acid are almost identical in bond lengths and angles and do not display rotational isomerism. Large angular distortions occur within and without the aromatic ring in order to accommodate the overmethylation.

The great enhancement of the reaction rate of a substrate promoted by an enzyme suggests that the enzyme may impose conformational restrictions on the substrate. Both Jencks and Bruice support the view that approximation of reactants in a rigid system provides *via* an entropy factor the type of rate enhancement desired for enzymatic reactions.[1] Such restriction of the rotational freedom of a substrate by an enzyme, with the accompanying decrease of rotational entropy, may be comparable to chemical alteration of an organic compound, *e.g.*, by alkylation, in order to narrow its conformational range. A study has been performed by Milstien and Cohen[2] on the rate of lactonization of *o*-hydroxyhydrocinnamic acid and its methyl derivatives. The substitution of CH_3 groups for H at the sites R_1 to R_5 enhances the rate of reaction by factors up to 10^{11} as shown in Table I. Milstien and Cohen[2] proposed that the interlocking of the CH_3 group at R_3 with the two CH_3 groups on the side chain at R_4 and R_5 restricted the acid group to the proximity of the OH group and thus was a major factor in promoting the rapid lactonization. To confirm this proposal as well as to establish the precise conformations of these highly methylated compounds, a crystal-structure analysis was undertaken. The very reactive acid IV is too unstable for an X-ray diffraction analysis, hence a crystal of the alcohol analog VI was used.[3] In addition, the crystal structure of the lactone V with R_1–$R_5 \equiv CH_3$ was established.



I–IV

V

Table I. Specific Rate Constants[a] for Lactonization (M^{-1} sec^{-1})

Alkyl substitution	$K'_{H_3O^+}$[b]	Rel rate
I, R_1–$R_5 \equiv$ H	5.9×10^{-6}	1.0
II, R_1–$R_3 \equiv CH_3$	4.0×10^{-5}	6.7
III, R_4–$R_5 \equiv CH_3$	2.6×10^{-2}	4.4×10^{3}
IV, R_1–$R_5 \equiv CH_3$	2.0×10^{6}	3.4×10^{11}

[a] Reference 2. [b] At 30°, in 20% dioxane, $\mu = 0.3$ *M*.

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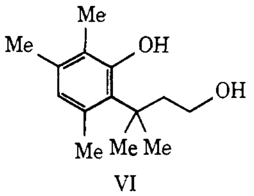
[1] See, *e.g.*, D. F. Koshland, K. W. Carraway, G. A. Dafforn, J. D. Gass, and D. R. Storm, *Cold Spring Harbor Symp. Quant. Biol.*, 36, 13 (1972); T. C. Bruice, *ibid.*, 36, 21 (1972); W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969.

[2] S. Milstien and L. A. Cohen, *Proc. Nat. Acad. Sci. U. S.*, 67, 1143 (1970); *J. Amer. Chem. Soc.*, 94, 9158 (1972).

[3] R. T. Borchardt and L. A. Cohen, *J. Amer. Chem. Soc.*, 94, 9166, 9175 (1972).

Table II. Crystallographic Data for Pentamethyl-*o*-hydroxyhydrocinnamyl Alcohol VI and Pentamethylhydrocoumarin V

Mol formula	$C_{14}H_{22}O_2$		$C_{14}H_{18}O_2$
Mol wt	222.33		218.30
Mp, °C	79–80		76–77
Color	Colorless, opaque		Colorless, opaque
Habit	Elongated prismatic on *a*		Elongated prismatic on *b*
Size, mm	1.0 × 0.13 × 0.13 mm		0.25 × 1.0 × 0.25 mm
Space group	$C\bar{1}$	$P\bar{1}$	$P2_1$ (disordered)
a, Å	11.615 ± 0.012	11.615	10.148 ± 0.007
b, Å	25.987 ± 0.025	14.983	8.766 ± 0.008
c, Å	18.150 ± 0.015	18.150	7.802 ± 0.005
α, deg	93.66 ± 0.12	93.03	90.0
β, deg	90.38 ± 0.08	90.38	115.45 ± 0.05
γ, deg	98.36 ± 0.10	120.90	90.0
V	5408.3	2704.4	626.7
Z	16	8	2
Density (calcd), g/cm³	1.092		1.157
Radiation	Cu Kα		Cu Kα
Wavelength, Å	1.54178		1.54178
Linear absorption coeff, cm⁻¹	5.74		6.19
No. of independent reflections	5020		1086



VI

Unusual features distinguish these two structure determinations. The crystal of compound VI has four independent molecules in the asymmetric unit, thus requiring that the coordinates for 64 atoms (other than hydrogen), unrelated by symmetry, be located. The structure analysis revealed that there are no rotational isomers in the crystal. Each of the four molecules has nearly the same conformation, except for the existence of right- and left-handed molecules. The crystal of lactone V is disordered. The systematic extinctions due to symmetry indicate space group $P2_1$ or $P2_1/m$ and, although the statistical distributions of the normalized structure factors are consistent with a centrosymmetric cell, there are only two molecules in the unit cell. Thus two different molecules related by a mirror plane must occupy the same space on a statistical basis. The nature of the disorder will be described in the Discussion.

Experimental Section

Crystals of both materials were provided by Dr. Louis A. Cohen of the National Institutes of Health. The cell parameters and other physical characteristics are tabulated in Table II. Compound VI crystallizes in the triclinic system and, although it is always possible to choose a primitive cell such as the $P\bar{1}$ cell shown in Table II, the end-centered cell $C\bar{1}$ with angles near 90° is easier to visualize and has been used in this investigation. X-Ray intensity data were collected on a four-circle automatic diffractometer using Cu Kα radiation and the θ–2θ scan technique. For both compounds the scan width was $2.0° + 2\theta(\alpha_2)° - 2\theta(\alpha_1)°$ with a rate of 2°/min and a background count of 10 sec at each end of the scan. Three reflections, one on each axis, were monitored after each interval of 50 measurements. The intensities of the monitored reflections remained constant during the period of data collection. Lorentz and polarization corrections were made but no absorption correction was applied since the linear absorption coefficients (Table II) are quite small. Normalized structure factor magnitudes $|E|$ were derived from the intensities.

Phases for both structures were determined directly from the observed normalized structure factor magnitudes by means of the symbolic addition procedure,[4] using formulas which are based on inequality (34) of Karle and Hauptman[5] and its probabilistic implications. E maps[6] based on these phases revealed all the atoms (excluding H atoms) in both structures.

A least-squares refinement of the coordinates and isotopic thermal factors for the 64 C and O atoms in VI was followed by a refinement with anisotropic thermal factors. Difference maps at this stage revealed 68 out of 88 hydrogen atoms for the four molecules of compound VI. One more cycle of refinement with anisotropic thermal factors and the inclusion of 68 H atoms with constant parameters resulted in an R factor of 9.5% for 3837 data with $|F_o| > 5$. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$ where $w = 30/|F_o|$ for $|F_o| > 30$, $w = 1$ for $30 > |F_o| > 5$, and $w = 0$ for $|F_o| < 5$. The restricted set of data was used in the refinement in order to conserve computer time and costs for this large problem.

Refinement of data for V was carried out in space group $P2_1/m$ with each atom weighted at one-half occupancy on a statistical basis. In this disordered structure, atoms C(7) and C(8) are close enough to atoms C(7′) and C(8′) from another molecule to cause their y coordinates to merge in the process of refinement, resulting in an average value of $y = 1/4$ accompanied by thermal ellipsoids quite elongated along the b direction. Hence the y parameters for C(7) and C(8) were determined empirically by choosing values such that the resulting values of the B_{22} terms in the expression for thermal parameters would be near those observed for neighboring atoms. In subsequent cycles of refinement, the y parameters of C(7) and C(8) were held constant. All the H atoms were readily found in a difference map. Inclusion of the 22 H atoms as constant parameters in the least-squares refinement resulted in an R factor of 8.3%. Coordinates and thermal factors for the C and O atoms for the diol VI and lactone V are listed in Tables III and IV.

Results

Lactone. The conformation of the lactone molecule is shown in Figure 1a. Bond lengths and angles are illustrated in Figure 2a. The values for the bond lengths are normal, that is, within the range usually found for phenyl groups, ester groups, and C_{ar}–CH_3 linkages. The angles, on the other hand, are quite extraordinary, deviating significantly from idealized values of 120° in order to accommodate the CH_3 groups in positions 10, 11, and 12, as well as those at 13 and 14. The phenyl group is distorted with angles ranging from 115.4 to 126.0°. In addition, the increase in angle C(5)–C(4)–C(12) to 124.0° and in angle C(4)–C(5)–C(7) to 126.7° results in a distance of 3.03 Å between C(11) and C(12) which are separated by

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(4) See, *e.g.*, J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).

(5) J. Karle and H. Hauptman, *ibid.*, **3**, 181 (1950).

(6) I. L. Karle, H. Hauptman, J. Karle, and A. B. Wing, *ibid.*, **11**, 257 (1958).

Table III. Fractional Coordinates and Thermal Parameters[a] for the Four Molecules of the Diol, Pentamethyl-*o*-hydroxyhydrocinnamyl Alcohol

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
1A	0.5949	0.0736	0.0626	5.48	4.69	6.30	−0.54	−0.05	0.21
2A	0.6156	0.0537	−0.0089	5.94	6.15	6.23	−2.16	0.75	0.51
3A	0.7020	0.0225	−0.0173	6.12	7.72	4.07	−1.83	0.56	0.01
4A	0.7641	0.0071	0.0418	5.70	5.31	6.49	−1.34	1.28	−0.36
5A	0.7442	0.0258	0.1151	3.87	4.98	6.13	−1.00	−0.14	−0.44
6A	0.6589	0.0596	0.1221	5.35	5.14	5.35	−0.30	−0.51	0.28
7A	0.7970	0.0095	0.1893	6.12	5.36	6.23	0.21	−0.46	−0.13
8A	0.8542	0.0568	0.2383	4.94	5.71	5.92	−1.51	−0.49	−0.72
9A	0.9437	0.0950	0.1987	8.61	5.22	6.34	−2.21	−0.89	−1.22
10A	0.6941	−0.0208	0.2311	8.45	6.91	8.07	−1.40	0.73	2.90
11A	0.8870	−0.0284	0.1778	9.33	5.91	10.46	1.99	−1.78	−1.41
12A	0.8540	−0.0305	0.0213	7.70	9.70	9.68	2.96	2.62	−1.16
13A	0.5491	0.0660	−0.0770	9.03	9.86	5.16	−1.82	−2.14	2.01
14A	0.5028	0.1071	0.0739	8.58	7.48	7.90	3.00	−2.02	1.08
O(1A)	0.6373	0.0798	0.1916	8.34	6.37	5.04	1.42	0.02	−0.92
O(2A)	1.0052	0.1328	0.2523	5.46	4.76	8.29	−0.67	−0.78	−0.77
1B	−0.1487	0.2039	0.4356	5.05	5.67	5.78	0.95	1.52	0.97
2B	−0.1146	0.2171	0.5100	7.54	7.41	4.07	2.54	0.74	0.61
3B	−0.0154	0.2522	0.5256	9.65	6.54	4.92	0.79	1.08	−0.84
4B	0.0488	0.2785	0.4718	7.62	5.99	6.16	0.79	0.43	−1.40
5B	0.0174	0.2689	0.3962	5.98	4.92	4.78	0.34	0.67	−0.62
6B	−0.0830	0.2304	0.3812	4.80	4.97	5.21	1.30	0.96	0.31
7B	0.0742	0.2989	0.3295	8.01	4.95	5.77	−0.21	1.33	0.19
8B	0.1258	0.2594	0.2754	6.57	4.46	5.86	−0.42	1.87	0.50
9B	0.2095	0.2276	0.3096	6.49	6.74	5.82	1.06	0.52	−0.11
10B	−0.0194	0.3240	0.2897	10.48	5.10	9.14	2.75	2.07	2.92
11B	0.1785	0.3426	0.3499	9.62	5.63	10.18	−2.87	0.45	−0.60
12B	0.1601	0.3144	0.5025	10.62	10.55	7.96	−2.07	−2.08	−2.02
13B	−0.1836	0.1893	0.5707	12.77	9.06	4.91	1.51	4.10	1.40
14B	−0.2513	0.1624	0.4144	6.84	6.51	7.04	−0.36	1.26	2.22
O(1B)	−0.1122	0.2153	0.3073	5.21	5.33	3.89	−0.14	0.61	0.36
O(2B)	0.2368	0.1870	0.2569	5.37	5.38	6.41	0.37	1.49	0.39
1C	0.1053	0.0704	0.4545	6.13	6.01	6.06	−1.84	1.80	−0.53
2C	0.1282	0.0498	0.5233	6.51	7.28	6.27	−2.78	0.76	0.20
3C	0.2106	0.0168	0.5239	7.65	8.26	6.02	−2.43	−0.40	1.90
4C	0.2715	0.0014	0.4634	6.44	6.19	8.39	−2.05	−0.71	1.61
5C	0.2532	0.0217	0.3934	5.06	5.27	6.02	−1.16	0.31	−0.07
6C	0.1655	0.0543	0.3932	4.45	5.15	6.37	−0.78	1.18	−0.10
7C	0.3084	0.0062	0.3191	5.93	6.38	8.03	−0.32	1.31	−0.63
8C	0.3536	0.0545	0.2753	5.66	6.61	6.12	−0.81	1.20	−0.85
9C	0.4299	0.0984	0.3193	6.08	6.16	6.88	−0.60	0.41	1.24
10C	0.2132	−0.0291	0.2713	9.11	6.62	10.02	−1.38	0.17	−2.36
11C	0.4112	−0.0247	0.3224	8.13	10.04	15.76	4.45	4.16	0.71
12C	0.3546	−0.0399	0.4776	9.69	8.36	15.70	2.07	−1.61	4.91
13C	0.0659	0.0624	0.5937	10.19	12.82	5.81	−3.04	3.42	−0.66
14C	0.0181	0.1068	0.4474	7.49	8.74	8.00	2.74	2.34	−0.89
O(1C)	0.1402	0.0725	0.3258	7.22	8.30	4.75	1.46	0.65	1.49
O(2C)	0.4547	0.1440	0.2749	5.63	6.78	8.96	−1.01	1.22	2.22
1D	0.3503	0.2104	0.0434	5.58	4.70	5.74	0.73	−0.15	−0.46
2D	0.3913	0.2072	−0.0292	7.32	6.35	4.42	1.68	−0.47	−0.22
3D	0.4949	0.2391	−0.0460	7.48	6.84	5.40	1.15	0.96	0.55
4D	0.5536	0.2752	0.0039	6.27	6.09	6.58	0.76	1.42	1.02
5D	0.5232	0.2778	0.0800	5.52	4.76	5.38	0.87	1.15	0.30
6D	0.4143	0.2460	0.0943	5.09	4.91	5.15	0.66	0.44	0.10
7D	0.5869	0.3137	0.1438	5.33	5.17	6.38	0.08	0.07	−0.12
8D	0.6046	0.2821	0.2123	5.05	7.46	4.74	−0.08	−0.45	−0.19
9D	0.6617	0.2343	0.1949	7.92	5.95	6.08	1.00	0.03	1.02
10D	0.5137	0.3573	0.1647	7.82	5.38	11.59	1.43	−0.63	−2.63
11D	0.7119	0.3410	0.1274	5.67	7.04	9.40	−1.41	−0.21	2.04
12D	0.6577	0.3102	−0.0294	9.30	10.25	8.25	−1.01	4.38	2.07
13D	0.3295	0.1687	−0.0871	10.21	9.08	4.84	1.52	−1.68	−1.28
14D	0.2395	0.1787	0.0662	4.96	6.55	7.59	−1.23	0.02	−0.66
O(1D)	0.3743	0.2506	0.1657	6.13	6.07	4.97	−0.75	1.71	−1.05
O(2D)	0.6664	0.2062	0.2065	5.53	10.21	8.94	0.25	−1.46	4.84

[a] The thermal parameters are expressed in the form $T = \exp[-{}^1/_4(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)]$.

three atoms as compared to 2.99 Å between C(13) and C(14) which are separated by two atoms. The C(10)···C(12) distance is 3.80 Å.

Despite the large angular distortions, the six atoms of the phenyl group are nearly coplanar with a maximum deviation of 0.023 Å, Figure 3a. The atoms C(13) and C(14) deviate by ±0.05 Å from the least-squares plane of the phenyl group and C(12) deviates by +0.125 Å. In addition, O(1) and C(7) of the lactone moiety, which may be expected to be coplanar with

Table IV. Fractional Coordinates and Thermal Parameters[a] for the Lactone, Pentamethylhydrocoumarin

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{13}	B_{23}	B_{33}
C(1)	0.3025	−0.0534	0.3616	3.45	3.09	3.25	−0.15	1.19	0.22
C(2)	0.3543	−0.1728	0.2887	3.35	2.93	4.42	−0.05	1.15	−0.01
C(3)	0.3482	−0.1637	0.1089	3.49	3.23	4.02	−0.04	1.68	−0.25
C(4)	0.2893	−0.0376	−0.0098	3.58	3.66	3.30	−0.05	1.57	−0.47
C(5)	0.2289	0.0830	0.0537	3.04	2.51	3.06	−0.30	1.28	0.12
C(6)	0.2418	0.0709	0.2377	3.52	2.58	2.83	−0.15	1.26	−0.18
C(7)	0.1534	0.2280	−0.0593	3.86	2.66	2.69	0.00	1.31	0.00
C(8)	0.0359	0.2700	0.0077	4.18	3.41	3.48	0.00	1.55	0.00
C(9)	0.1032	0.2989	0.2183	6.10	2.95	4.59	1.42	3.52	1.05
C(10)	0.2649	0.3563	−0.0167	5.59	3.22	4.88	−0.69	2.04	0.79
C(11)	0.0669	0.2040	−0.2760	5.22	5.55	2.50	1.09	1.28	0.64
C(12)	0.2986	−0.0378	−0.1985	6.80	5.44	4.77	0.98	3.89	−0.43
C(13)	0.4191	−0.3158	0.4091	5.89	3.44	6.21	1.31	2.48	0.71
C(14)	0.3112	−0.0539	0.5592	5.16	4.19	3.03	0.06	1.33	0.62
O(1)	0.2012	0.1931	0.3256	5.47	2.93	2.92	0.84	1.97	0.04
O(2)	0.0773	0.4027	0.2991	11.56	4.36	4.94	3.60	4.50	0.87

[a] The thermal parameters are expressed in the form $T = \exp[-{}^1/_4(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}\cdot klb^*c^*)]$.

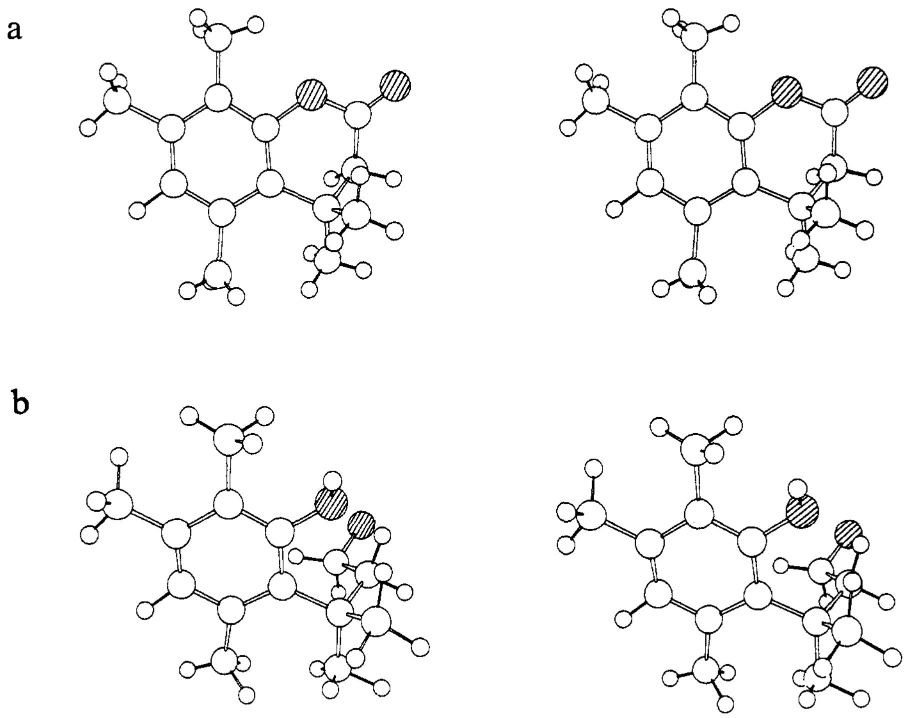


Figure 1. (a) Stereodiagram depicting the conformation of the lactone, pentamethylhydrocoumarin. (b) Stereodiagram depicting the conformation of pentamethyl-*o*-hydroxyhydrocinnamyl alcohol.

the phenyl group, also show significant deviations of +0.133 and −0.068 Å, respectively. The lactone ring which contains two saturated carbon atoms is markedly puckered, while the C(8)–C(9)–C(1)–O(2) moiety is planar to within ±0.003 Å.

A diagram of a possible mode of packing is shown in Figures 4 and 5. The packing is ordered in the *b* direction, where the molecules are related simply by a translation of one unit cell, and in the *c* direction, where the molecules alternate between site A (x, y, z) and site B (x, $^1/_2 - y$, z). This type of alternation in the *c* direction is required in order to provide spatial accommodation for the molecules; otherwise the C(14)···C(12′) distance (translation along *c*) would be only ~2.0 Å. If the structure were completely ordered, the *c* axis would be doubled. The disorder takes place in the *a* direction since it is immaterial to the packing scheme whether the molecules are related by a center of symmetry or a twofold screw, Figure 5. In the proposed packing scheme, the intermolecular approaches are all greater than 3.50 Å except for C(13)···C(1′) and C(13)···C(14′) at 3.34 and 3.36 Å, respectively, for the case when two molecules are related by x, y, z and $1 - x$, $^1/_2 + y$, $1 - z$.

Diol. All four independent molecules of the diol in the asymmetric unit are nearly the same. A representative molecule is shown in Figure 1b. The individual bond lengths and angles are shown in Table V. It is readily ascertained that the comparable bond lengths and bond angles in the individual molecules differ very little. The rms deviation for the bond lengths averages 0.011 Å while that for the angles averages 0.9°. The average values of the bond lengths and angles for the four molecules of the diol are shown in

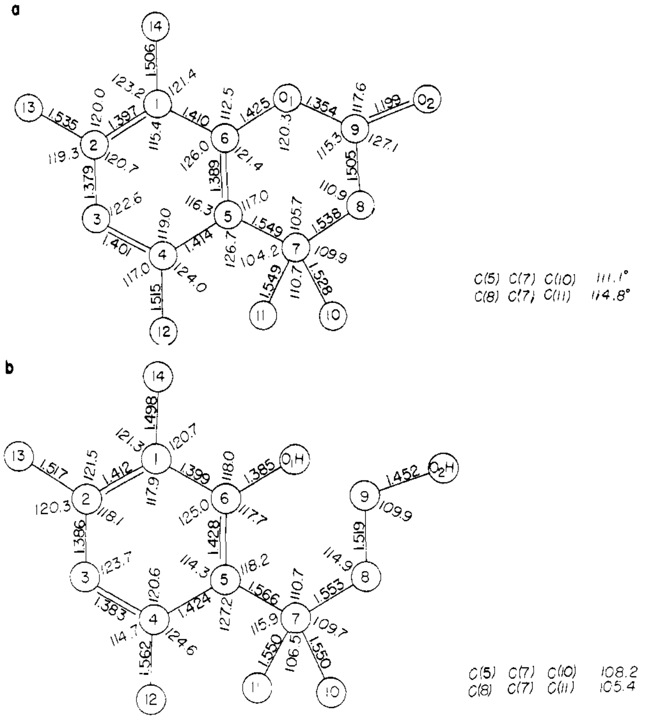


Figure 2. (a) Bond lengths and angles for the lactone. Standard deviations, based on the least-squares fit, are 0.008 Å for bond lengths and 0.5° for angles. (b) Bond lengths and angles for the diol. The figures represent the average values obtained from the four independent molecules in the asymmetric unit. Values for the individual molecules are listed in Table V.

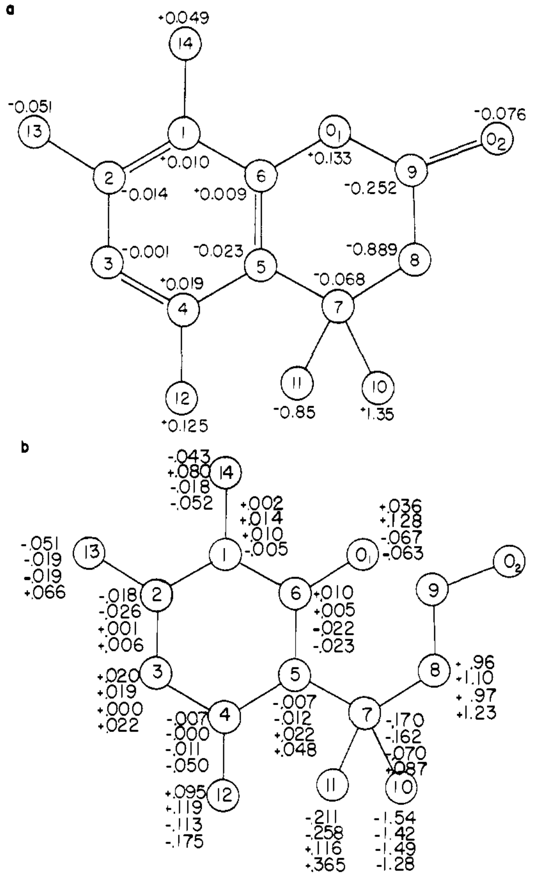


Figure 3. (a) Deviations of atoms in ångströms from a least-squares plane through atoms C(1)–C(6). (b) Deviations of atoms in ångströms from a least-squares plane through atoms C(1)–C(6). The four values by each atom represent the deviations in each of the four molecules of the diol. From top to bottom the values are for molecules A, B, C, and D.

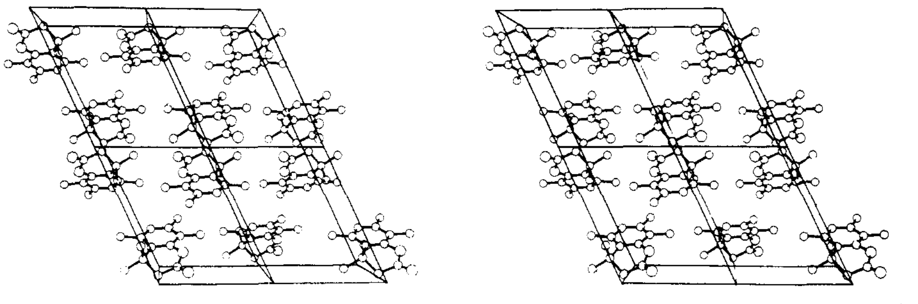


Figure 4. Stereodiagram for a possible scheme for packing in the disordered structure for the lactone. Four unit cells are shown. The directions of the axes are: *a* ↑, *c* →, and *b* up from the page.

Figure 2b for easy comparison with the lactone. Angular distortions in the six-membered aromatic ring are in the same direction in both compounds, with angles C(2)–C(1)–C(6) and C(4)–C(5)–C(6) quite small and angle C(5)–C(6)–C(1) quite large, the deviations from 120° being as great as 6.0°. Furthermore, the angles in the vicinity of the "trialkyl lock" are near 124° for C(12)–C(4)–C(5) and near 127° for C(4)–C(5)–C(7) in both the lactone and the diol. This distortion from the ideal value of 120° provides added space for the accommodation of the C(11) and C(12) methyl groups. Curiously, the C(11)···C(12) intramolecular distances which range from 2.86 to 2.94 Å in the four diol molecules are even shorter than the 3.03-Å C(1)···C(12) distance in the lactone despite the expectation that there could be more rotational freedom about the C(5)–C(7) bond in the diol than in the lactone.

Planarity is preserved in the phenyl ring in the diol just as in the lactone, Figure 3b, despite the crowding caused by substitution at five of the six carbon positions. Atoms O(1), C(7), and C(12) deviate by 0.06–0.17 Å from the plane of the ring. Atom C(11) in the diol is much closer to the plane of the phenyl ring, with a deviation of 0.11–0.36 Å in the four molecules, than in the lactone where C(11) is displaced by 0.85 Å from the plane of the phenyl ring.

The diol molecules have the side chain extended approximately perpendicularly to the plane of the phenyl group. In molecules A and B the chain is on one side of the plane of the ring, while in molecules C and D the chain is on the other side (see Figure 6). The configuration of A and B is related to that of C and D very nearly by a mirror.

There is no intramolecular hydrogen bonding in the

Table V. Bond Lengths for the Four Independent Molecules of Pentamethyl-*o*-hydroxyhydrocinnamyl Alcohol

Bond	A	B	C	D	Av	Rms deviation
			Bond Lengths, Å			
C(1)-C(2)	1.401	1.411	1.429	1.407	1.412	0.010
C(2)-C(3)	1.383	1.378	1.376	1.406	1.386	0.012
C(3)-C(4)	1.400	1.388	1.379	1.364	1.383	0.013
C(4)-C(5)	1.419	1.414	1.433	1.428	1.424	0.007
C(5)-C(6)	1.417	1.434	1.416	1.443	1.428	0.012
C(6)-C(1)	1.404	1.405	1.391	1.394	1.399	0.006
C(1)-C(14)	1.481	1.516	1.494	1.500	1.498	0.013
C(2)-C(13)	1.528	1.524	1.513	1.505	1.517	0.009
C(4)-C(12)	1.561	1.558	1.576	1.554	1.562	0.008
C(5)-C(7)	1.583	1.579	1.552	1.552	1.566	0.015
C(6)-O(1)	1.374	1.398	1.385	1.384	1.385	0.009
C(7)-C(8)	1.534	1.561	1.556	1.562	1.553	0.011
C(7)-C(10)	1.562	1.543	1.551	1.543	1.550	0.011
C(7)-C(11)	1.545	1.560	1.535	1.560	1.550	0.011
C(8)-C(9)	1.543	1.517	1.519	1.507	1.519	0.013
C(9)-O(2)	1.445	1.451	1.470	1.442	1.452	0.011
			Bond Angles, deg			
C(6)-C(1)-C(2)	119.0	117.5	117.5	117.6	117.9	0.6
C(1)-C(2)-C(3)	117.7	119.1	117.1	118.5	118.1	0.8
C(2)-C(3)-C(4)	123.7	123.0	125.4	122.9	123.7	1.0
C(3)-C(4)-C(5)	120.1	120.9	119.6	121.8	120.6	0.8
C(4)-C(5)-C(6)	115.1	114.8	114.2	113.0	114.3	0.8
C(5)-C(6)-C(1)	124.2	124.4	126.0	125.5	125.0	0.7
C(6)-C(1)-C(14)	121.6	120.7	120.7	120.0	120.7	0.6
C(2)-C(1)-C(14)	119.4	121.8	121.7	122.4	121.3	1.1
C(1)-C(2)-C(13)	122.8	119.1	123.2	121.0	121.5	1.6
C(3)-C(2)-C(13)	119.4	121.6	119.8	120.4	120.3	0.8
C(3)-C(4)-C(12)	116.0	113.6	115.5	113.8	114.7	1.0
C(5)-C(4)-C(12)	123.9	125.3	124.9	124.4	124.6	0.5
C(4)-C(5)-C(7)	127.8	126.9	126.4	127.6	127.2	0.6
C(6)-C(5)-C(7)	116.8	118.0	118.9	119.2	118.2	0.9
C(5)-C(6)-O(1)	117.9	117.8	116.3	118.8	117.7	0.9
C(1)-C(6)-O(1)	117.9	117.6	117.7	118.7	118.0	0.4
C(5)-C(7)-C(8)	112.4	108.8	112.2	110.9	110.7	1.5
C(5)-C(7)-C(10)	106.8	109.4	108.0	108.5	108.2	0.9
C(5)-C(7)-C(11)	114.1	115.7	117.3	116.3	115.9	1.2
C(8)-C(7)-C(10)	109.7	110.5	108.2	110.2	109.7	0.9
C(8)-C(7)-C(11)	107.9	104.7	105.1	103.9	105.4	1.5
C(10)-C(7)-C(11)	105.7	107.8	105.6	106.9	106.5	1.4
C(7)-C(8)-C(9)	114.6	115.8	115.3	114.0	114.9	0.7
C(8)-C(9)-O(2)	109.5	110.5	110.0	109.5	109.9	0.4

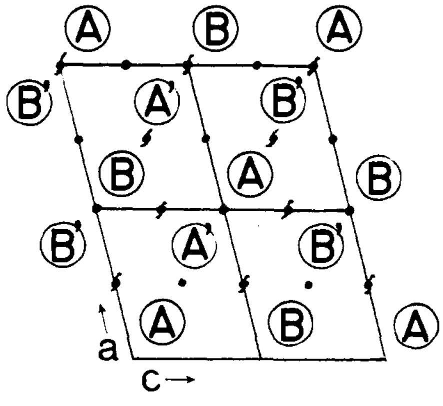


Figure 5. A possible scheme for packing in the disordered structure (an explanation of Figure 4). Four unit cells are outlined. The symbol ● is used to indicate a center of symmetry and the symbol ƒ is used for a twofold screw axis. Molecule A is located at x, y, z; A′ at $\bar{x}, \bar{y}, \bar{z}$; B at $x, \frac{1}{2} - y, z$; and B′ at $\bar{x}, \frac{1}{2} + y, \bar{z}$. An "averaged" unit cell has molecules A and B superimposed and related by a mirror at $y = \frac{1}{4}$, similarly for A′ and B′.

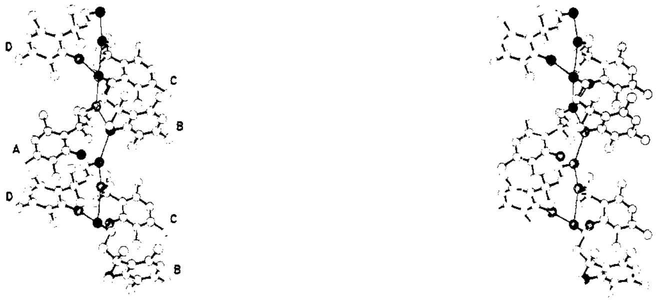


Figure 6. Stereodiagram of diol molecules A, B, C, and D showing the continuous chain of hydrogen bonds parallel to the *a* axis. The *c* axis is horizontal.

diol. Each OH group except O(1A) participates in intermolecular hydrogen bonding as illustrated in Figure 6. There is a continuous chain of OH···OH ···OH bonds including some O(1) and all O(2) type hydroxyl groups which is parallel to the *a* axis. The molecules A, B, C, and D are disposed in a roughly helical fashion about the hydrogen-bonded chain forming a column parallel to the *a* axis. The lengths of the OH···O bonds are shown in Table VI. They vary between 2.76 and 2.94 Å, a normal range of values, except for O(1A)···O(2C) which is 3.20 Å. Although atoms O(1A) and O(2C) are oriented in a manner which would be appropriate for hydrogen bond formation, since the position of the H atom on O(1A) was

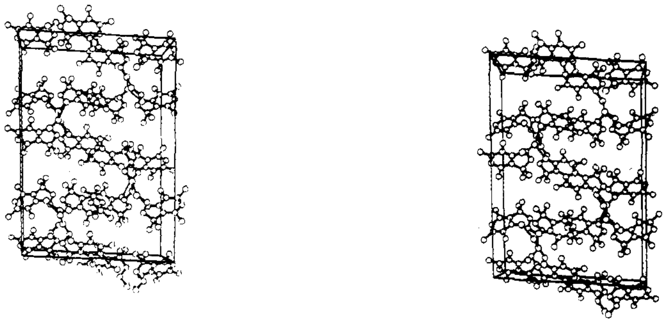


Figure 7. Stereodiagram of the contents of a unit cell of the diol. The directions of the axes are: *b* ↑, *c* →, and *a* perpendicular to the plane of the page and down.

Table VI. Hydrogen Bonds in a Crystal of Pentamethyl-*o*-hydroxyhydrocinnamyl Alcohol

Donor	Acceptor[a]	Length, Å
O(2A)	O(2B)	2.85
O(1B)	O(2A)	2.82
O(2B)	O(2C)	2.94
O(1C)	O(2A)	2.76
O(2C)	O(2D)	2.76
O(1D)	O(2B)	2.76
O(2D)	O(1B)	2.68
(O(1A)	O(2C)	3.20)

[a] Most of the H atoms on the donor O atoms were not located in the experiment and one of two possible hydrogen bonding schemes is presented. The only difference between the two schemes is the interchange of the acceptor with the donor in some cases.

not found experimentally and since the O(1A)···O(2C) distance is longer than the values ordinarily observed for hydrogen bonding, it is not possible to state whether a weak bond exists between these atoms.

The packing of the molecules in the unit cell is illustrated in Figure 7. This view shows the columns of molecules containing the OH···OH···OH chains perpendicular to the plane of the page. There are four columns per unit cell. The phenyl groups in molecules in adjacent columns are stacked parallel to each other along the *a* direction and in a herringbone fashion along the *b* direction. Aside from the hydrogen bonds, the nearest approaches between molecules are: C(14B)···O(2D), 3.24 Å; C(9B)···O(1D), 3.29 Å; C(9B)···O(2A), 3.29 Å; and C(8B)···O(2A), 3.38 Å.

Discussion

The restriction of conformational freedom by alkylation and the accompanying increase by a factor of 10^{11} in the rate of lactonization[2] can be discussed in the context of the results described above. The unsubstituted *o*-hydroxyhydrocinnamic acid most probably is planar and assumes the extended conformation VII. Crystal data for VII are not available; however, the extended conformation is the preferred form for hydrocarbon chains, either free or attached to ring systems. The pentamethyl-*o*-hydroxyhydrocinnamic acid, by analogy to the structure of pentamethyl-*o*-hydroxyhydrocinnamyl alcohol, Figure 1b, most probably has the conformation VIII where the C atoms of the CH_3 groups on C(4) and C(7) are approximately coplanar with the ring and the torsional angle about the C(6)–C(5)–C(7)–C(8) is near 55° as compared to 180° for the extended form in the unmethylated acid. The

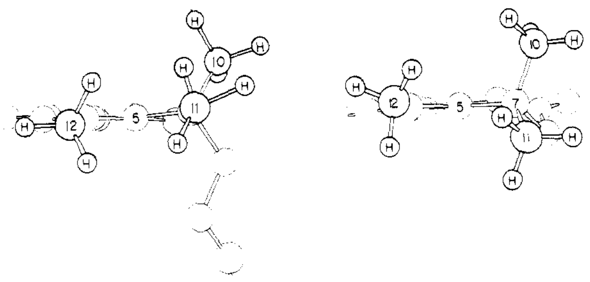
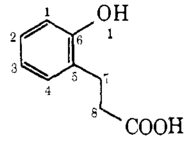
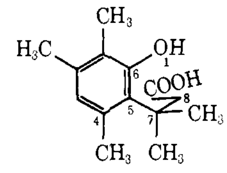


Figure 8. A comparison of the orientation of the methyl groups in the "trialkyl lock" in the diol and in the lactone. The diagrams were drawn using the experimentally determined coordinates for the atoms. The planes of the phenyl rings are perpendicular to the plane of the paper. The illustrations in this paper were drawn by a computer using a program prepared by C. K. Johnson (C. K. Johnson, ORTEP, ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965).



VII



VIII

folded conformation VIII approximates that present in the lactone, Figure 1a, where the C(6)–C(5)–C(7)–C(8) torsional angle is 36°. The O(1)···C(8) distance in the lactone is 2.42 Å as compared to ~2.8 Å in VIII and ~4.3 Å in VII. (It is 2.81, 2.91, 2.74, and 2.79 Å for the four molecules of the diol.) In the order for the lactone to form from VIII, there need be only a rotation about C(7)–C(8) of ~120°, whereas two rotations are required for lactone formation from VII, ~120° about C(5)–C(7) and ~120° about C(7)–C(8). Apparently, the elimination of one rotation by the restricted conformation imposed by the "trialkyl lock" is sufficient to greatly enhance the rate of reaction.

Intramolecular hydrogen bonds were not found in the crystal of the diol. *A priori*, one would imagine that the presence of an intramolecular hydrogen bond would bring the reacting groups even in closer proximity for a more favorable reaction rate. Inspection of a molecular model for VIII with the conformation of the diol for all atoms except the COOH groups shows that there does not exist a combination of rotations about the C(7)–C(8) and C(8)–C(9) bonds which would bring the carbonyl oxygen into a position favorable for the formation of a hydrogen bond with the OH on the ring without interference from the hydrogen atoms of the C(10) methyl group.

The conformational restriction imposed by the methylation has been attributed to the "trialkyl lock." Nmr data on the lactone show a single, very sharp signal for the two CH_3 groups on C(7)[7] implying that their electronic environments are the same, or in other words, that in solution they are equally disposed about the methyl group on C(4). This conformation is verified by the crystal structure analysis in that C(10) and C(11) are on either side of C(12), although not equally disposed. Figure 8 contains a diagram of the lactone drawn from the experimentally determined coordinates of all the atoms (including the H atoms) and viewed end

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[7] L. A. Cohen, private communication.

on so as to show the orientation of the three CH_3 groups with respect to each other. The torsional angles C(4)–C(5)–C(7)–C(10) and C(4)–C(5)–C(7)–C(11) are −94 and +45°, respectively. A comparison with a similar view of the diol (the coordinates for molecule B were used) shows an interesting occurrence; namely, that both CH_3 groups on C(7) are on the *same* side of C(12) as compared to *opposite* sides for the lactone. This means that the barrier to rotation is not as severe as previously supposed. It is possible for the C(11) and C(12) methyl groups to slip past each other. The substantial enlargement of angles C(5)–C(4)–C(12) and C(5)–C(7)–C(11) in both the diol and the lactone over the idealized value of 120° allows enough space for the slippage. The H atoms on C(10) and C(11) are eclipsed with respect to each other. In the diol, the H atoms on C(12) are eclipsed with respect to those on C(11), whereas in the lactone, the C(12) methyl group has rotated so that the H···H distances between CH_3 groups are roughly equidistant. Thus, a rotation about the C(5)–C(7) bond appears to generate a rotation of the C(12) methyl group by gear-like action.

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Polynucleotides. XVI.[1] Oligomers of 8,2′-Anhydro-8-mercapto-9-(β-D-arabinofuranosyl)adenine 5′-Monophosphate

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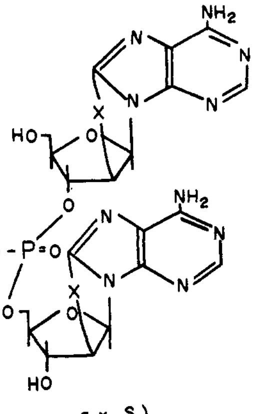
Abstract: N^6-Benzoyl-8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)adenine 5′-phosphate (III) was subjected to polymerization using dicyclohexylcarbodiimide in pyridine. The resulting polynucleotides (pA^s)$_n$ were isolated and purified by means of DEAE–cellulose column chromatography, paper chromatography, and paper electrophoresis. Chain lengths were obtained by phosphate analyses of the polymer and its dephosphorylated product formed by alkaline phosphatase digest. All these polynucleotides had CD spectra of similar profile, which had a trough at 277–288 nm and two peaks at around 264 and 222 nm, respectively. Based on the similarity of these spectra with that of A^{s}pA^s previously obtained, the same conformations were assigned to the polymers, *i.e.*, stacked with a left-handed screw axis. The magnitude of Cotton effect [θ] and uv absorbance increased with increasing chain length and reached a plateau at five to six nucleotide units. While [θ] of the pentamer decreased to 62% from 0 to 80°, ε decreased only 15% from 10 to 90°. These phenomena may imply that, owing to a restricted rotation of bases around the glycosidic linkage in each nucleotide unit, the destacking of bases was inhibited to a certain degree.

Recently, we synthesized a dinucleoside monophosphate (Ia) having two 8,2′-anhydro-8-mercapto-9-(β-D-arabinofuranosyl)adenine (8,2′-*S*-cycloadenosine, A^s) residues linked together with a 3′–5′ phosphodiester linkage.[2,3] We found that compound Ia had a highly stacked conformation with left-handed screw axis by means of its uv, nmr, and CD spectra. In this connection, we also synthesized compound Ib, in which 8,2′-S linkages of Ia were substituted by O linkages.[4] With compound Ib a left-handed stacked conformation was also predicted. In this connection, a versatile method for the specific tosylation of 8-bromoadenosine 5′-monophosphate (BrAMP) and the cyclization to give 8,2′-*S*-cycloadenosine 5′-monophosphate (pA^s) (II) was found.[5] In this paper we report the polymerization of compound II by means of dicyclohexylcarbodiimide (DCC) and properties of the resulting polynucleotides having chain lengths of two to ten nucleotide units.



I (a. X=S)
(b. X=O)

Synthesis of the Polynucleotides. To obtain a suitable substrate for the polymerization reaction, we first attempted to synthesize the N^6-dimethylamino-

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(1) Part XV of this series: M. Ikehara and M. Hattori, *Biochim. Biophys. Acta*, in press.

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(5) M. Ikehara and S. Uesugi, *Tetrahedron Lett.*, 713 (1970); *Tetrahedron*, **28**, 3687 (1972).