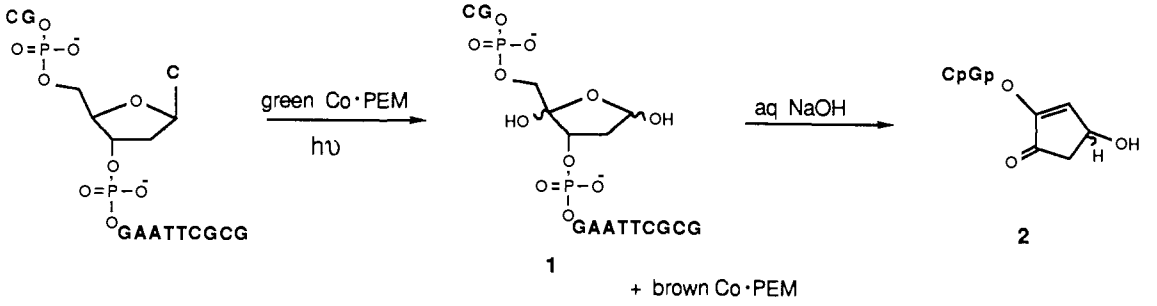
Table I. Quantitative Analysis of Products Formed during the Photoirradiation of Green and Brown Co·PEM Complexes in the Presence of d(CGCGAATTCGCG)[a]

	cytosine, μM	thymine, μM	adenine, μM	dGMP,[b] μM	dodecamer consumed,[c] μM	green complex consumed, μM	brown complex, μM
green Co·PEM	58	15	9	42	67	149	125
brown Co·PEM	8			6	39		248
Fe·PEM[d]	59[e]	12[e]	5[e]				

[a] The 40-μL reaction mixture contained 1 mM d(CGCGAATTCGCG),[c] 50 mM sodium cacodylate (pH 7.0), and 250 μM Co·PEM. After irradiation (Transilluminator TL 33, 5-cm distance) for 1 h at 0 °C (aerobic conditions), analysis was effected by reverse-phase HPLC.[8a] [b] Quantitated as deoxyguanosine after successive treatments of the reaction mixture with base (0.1 M NaOH, 60 °C, 2 min) and alkaline phosphatase. [c] 83 μM dodecanucleotide was used. [d] Control reaction carried out in 60 μL of 50 mM sodium cacodylate (pH 7.0) using 1 mM d(CGCGAATTCGCG) and 300 μM Fe^{II}·PEM. The reaction was carried out for 15 min at 0 °C and then analyzed as above. [e] Base + base propenal.

Scheme II. Plausible Scheme for Formation of Alkali-Labile Lesion **1** and Free Base with Concomitant Conversion of Green Co^{III}·BLM to Brown Co^{III}·BLM



of diastereomers **2** (Figure 1). The absence of cytosine propenal and 2′-deoxycytidylyl(3′→5′)(2′-deoxyguanosine 3′-(phospho-2″-*O*-glycolate)) suggests that no C-4′ hydroperoxide intermediate was formed;[7a,d,e,8a,10,11] while the chemical transformation(s) leading to the observed products (Scheme I) are not established unequivocally by the present study, C-4′ hydroxylation of the deoxyribose moieties of C_3 and C_{11} would clearly suffice to produce the observed products.

Also studied was the photoirradiation of brown Co^{III}·PEM in the presence of d(CGCGAATTCGCG). This complex was much less efficient than the green complex in mediating degradation of the dodecanucleotide, in spite of the fact that the green and brown complexes of Co^{III}·BLM would be expected to saturate the strong oligonucleotide binding sites under these experimental conditions (Table I).[3c] Degradation of (CGCGAATTCGCG) by green Co^{III}·PEM was accompanied by conversion of the green complex to brown Co^{III}·PEM;[12] analogous degradation by the brown complex did not result in any detectable change in the amount of brown Co^{III}·PEM present at the conclusion of the experiment. Under anaerobic conditions, d(CGCGAATTCGCG) was degraded to the same extent by green Co^{III}·PEM, producing oligonucleotide degradation products and brown Co^{III}·PEM in the same yields observed in the presence of O_2. Further, the quantum yield for the conversion of green Co^{III}·PEM to brown Co^{III}·PEM increased from 1.4×10^{-5} to 6.2×10^{-5} when d(CGCGAATTCGCG) was present.[13] These results suggest strongly that conversion of the green hydroperoxide complex to the brown aquo complex[5] may be associated with ribose C-4′ hydroxylation. Photochemical conversion of green PEM·Co^{III}–OOH to an active oxygen species, perhaps by scission of the O–O bond, could plausibly lead to the observed hydroxylation and production of brown PEM·Co^{III}–OH_2 (Scheme II). Consistent with this suggestion, brown PEM·Co^{III} would be unable to degrade DNA unless it were first converted to green PEM·Co^{III}–OOH, e.g., by photoreduction of Co^{III} to Co^{II} and reoxidation by O_2.[14]

Acknowledgment. We thank Dr. C.-H. Chang for samples of brown and green Co^{III}·BLM A_2. This work was supported at Kyoto University by a Grant-in-Aid for Scientific Research (No. 61065003, 61123005, and 62607003) from the Ministry of Education, Japan, and the Asahi Glass Foundation; and at the University of Virginia and University of California by PHS Grants CA38544 and CA16861, respectively. Generous support from Nihon Kayaku and Yamasa Shoyu Co., Inc., is also gratefully acknowledged.

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(12) This was verified by HPLC analysis and a 400-MHz ^1H NMR spectrum of the newly formed brown complex following HPLC purification.

(13) The quantum yield measurement was carried out with 366-nm light using phenylglyoxylic acid (ϕ = 0.72 at 365 nm) as an actinometer (Kuhn, H. J.; Defoin, A. *EPA News Letter* **1986**, *26*, 23).

(14) Reduction of brown Co^{III}·PEM with $NaBH_4$ under aerobic conditions led to the formation of green Co^{III}·PEM (20% yield), suggesting that part or all of the oligonucleotide-modifying activity observed with brown Co^{III}·PEM could be due to its conversion to green Co^{III}·PEM under the reaction conditions.

Tautomers of Cytosine by Microwave Spectroscopy

Ronald D. Brown,* Peter D. Godfrey, Donald McNaughton, and Anthony P. Pierlot

Department of Chemistry, Monash University Clayton, Victoria 3168, Australia

Received October 12, 1988

The relative stability of tautomers of the pyrimidine bases uracil, thymine, and cytosine is of fundamental importance to the structure and functioning of nucleic acids, the occurrence of certain tautomers being suggested as a possible mechanism of spontaneous mutation.[1] Recent developments in the technique of microwave spectroscopy[2] have made it feasible to identify the most stable tautomers of these bases. In our previous studies of uracil[3] and

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Table I. Derived Spectroscopic Parameters for Cytosine[a,b]

	C_α	C_β	C_γ
A, MHz	3871.547 (9)	3951.72 (4)	3848.109 (6)
B, MHz	2024.930 (3)	2008.890 (4)	2026.253 (5)
C, MHz	1330.306 (1)	1332.456 (2)	1327.972 (2)
Δ, μÅ^2	−0.22	−0.18	−0.18
no. of lines	55	31	28

[a] Transition frequencies are available from the authors upon request. [b] The inclusion of centrifugal distortion does not improve the RMS error of the fit and does not significantly change the values of the rotational constants.

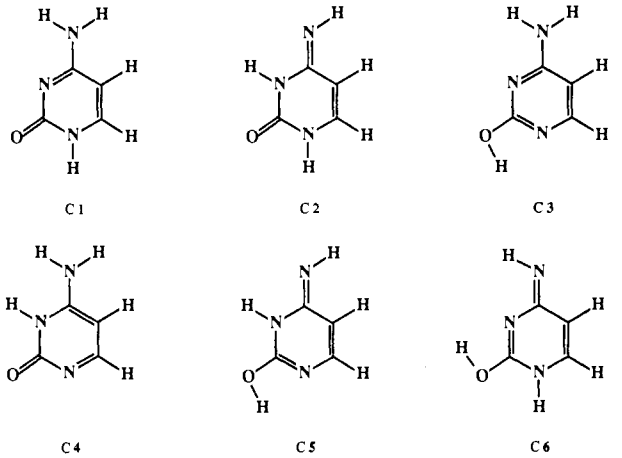

Figure 1. The six possible protomeric tautomers of cytosine.

thymine[4] we established that the diketo tautomers predominate, and we were unable to detect any others, in harmony with the circumstances prevailing in aqueous solution. However, in the case of cytosine there is debate as to the order of stability of tautomers in the gas phase.[5] In our recently developed Stark-modulated spectrometer, microwave measurements on a seeded supersonic beam of cytosine in argon expanded through a 550-μm diameter heated (295 °C) nozzle proved successful in detecting three distinct tautomeric species. The microwave spectrum of the heterocycle has been observed at adequate S/N in the vicinity of 60 GHz. The spectroscopic parameters for these species are presented in Table I. A number of lines of similar intensity to those detected for cytosine were also observed. However, these unassigned lines did not fit the pattern expected for other cytosine tautomers or a vibrational satellite and so probably originated from a decomposition product.

The value of the inertial defect of each of the cytosine tautomers is that expected for an essentially planar cyclic molecule bearing substituent groups in which at most one or two hydrogens are somewhat out-of-plane (as would be the case for a pyramidal amino substituent, for example). As the errors involved in calculating structures by the ab initio SCFMO method are systematic, scaling factors based on similar molecules where experimental and theoretical results are available can be applied to correct for these systematic errors.[6] Our previous studies of uracil and thymine and our more recent study of adenine[7] have shown that the rotational constants from geometries optimized using ab initio 3-21G basis SCF calculations, with subsequent scaling of the rotational constants by 0.985 for *A* and 0.995 for *B* and *C*, gives agreement with the experimentally derived rotational constants to within 15 MHz. On the basis of this degree of agreement between theory and experiment we infer by comparison with the scaled rotational constants derived from the 3-21G optimized geometries[5] (Table II) that the observed tautomers are C_α = **1**, C_β = **3a**, and C_γ = **2** and that tautomers **3b**, **4**, **5**, and **6** have not been detected in the gas phase. This contrasts with the detection of tautomers **1** and **4** but not tautomer **3** in aqueous solution[8] and only tautomer **1** in the crystal phase,[9] whilst only tautomers **1** and **3** were observed by matrix isolation techniques.[10]

Table II. Observed and Predicted Parameters for the Tautomers of Cytosine

	A, MHz[a]	*B*, MHz[a]	*C*, MHz[a]	μ_a/D	μ_b/D	rel *E*, kJ mol^{-1}	
C1	3871	2021	1332	4.7	5.4	0.0[b]	0.0[c]
C2	3837	2036	1334	1.2	5.2	1.7[b]	5.8[c]
C3a[d]	3946	2004	1333	3.2	1.8	15.9[b]	1.0[c]
C3b[d]	3862	2029	1334	5.1	1.2	19.3[b]	
C4	3881	2004	1326	8.5	1.3	29.8[b]	29.7[c]
C5	3876	2015	1330	0.8	1.6	76.2[b]	
C6	3836	2020	1327	4.0	2.1	94.8[b]	
$C\alpha$[e]	3872	2025	1330	s	s	0 (2)	
$C\beta$[e]	3952	2009	1332	s	w	0 (2)	
$C\gamma$[e]	3848	2026	1328		s	6 (2)	

[a] Derived from the 3-21G optimized geometries;[5] *A* scaled by 0.985, *B* and *C* by 0.995. [b] Relative energies determined from the 3-21G optimized geometries.[5] [c] From a single-point calculation on the 3-21G optimized geometry using a 6-31G* basis and many body-perturbation theory to second order to determine the electron correlation energy.[12] [d] The a and b tautomers refer to the OH group being cis and trans, respectively, with respect to the N_1–C_2 bond. The geometries for these two tautomers given in ref 5 were identical, apart from the OH dihedral angle and so were reoptimized at the 3-21G level,[11] **3b** being somewhat different to the reported structure. [e] Experimental results where s and w refer to strong and weak, respectively, for the spectrum type observed.

The identification of tautomers based on rotational constants alone is not entirely conclusive. However the tautomer C_α exhibits both an a-type and a b-type spectrum with similar intensities which is in accordance with the predicted values of the dipole moment components (Table II) which we have derived by ab initio 3-21G basis MO calculations.[11] This contrasts with our observations of an a-type and a much weaker b-type spectrum for C_β and of only a b-type spectrum for C_γ. Taken together with the other predicted dipole moment components for the other possible tautomers these observations confirm our identification of the three observed tautomers. The change in rotational constants and dipole moment components observed for the three assigned species is sufficiently large to discount the possibility of confusion with a vibrational satellite spectrum. We see no evidence for the presence of splittings arising from internal rotation or NH_2 inversion in our spectrum. Even if our rotational constants are "effective" constants containing a small contribution from either or both of these effects, the small changes involved would not affect our conclusions drawn from the comparison of experimental and calculated rotational constants. Taking into account the different dipole moment components, the intensity differences between the spectra for the three tautomers observed indicates that C_α and C_β must have similar abundances in the gas phase, while the abundance of C_γ is somewhat lower, approximately one quarter of that of the other tautomers. Thus if we assume that the tautomeric equilibrium has been established at the temperature of the nozzle, then tautomer **C2** must be approximately 6 (2) kJ/mol higher in energy than tautomers **C1** and **C3a**. A summary of the results obtained for the three observed species is included in Table II for comparison with the predicted results.

The detection of three tautomers in the gas phase is in excellent agreement with the predictions[12] from single-point energy cal-

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culations carried out on the 3-21G optimized geometries using a 6-31G* basis and many-body perturbation theory to second order to calculate the electron correlation energy (Table II). However, the agreement between theory and experiment is not as good at the 3-21G level[5] (Table II) with the relative energy of tautomer **3** predicted to be so high that it would be unobservable in the gas phase.

Acknowledgment. This work was supported by the Australian Research Grants Scheme.

Registry No. C1, 71-30-7; **C2**, 66398-98-9; **C3**, 66460-21-7.

Supplementary Material Available: Three tables of observed and calculated transition frequencies used to derive the data in Table I (10 pages). Ordering information is given on any current masthead page.

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Vinyl Group Rearrangement in the Enzymatic Cyclization of Squalenoids: Synthesis of 30-Oxysterols

Julio C. Medina, Richard Guajardo, and Keith S. Kyler*

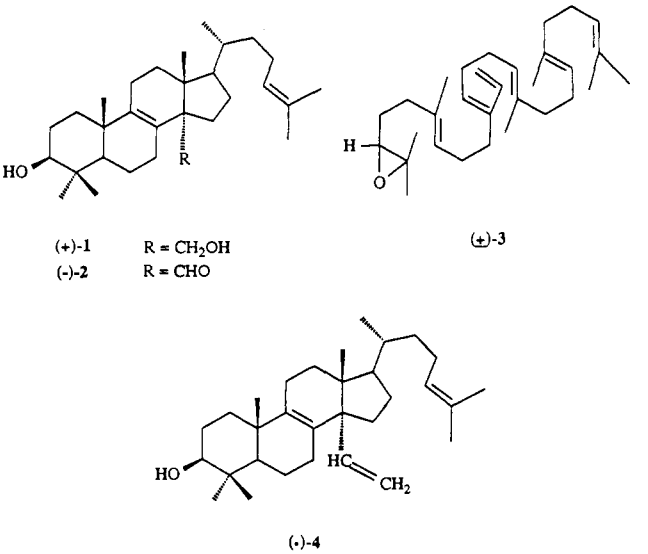
Department of Chemistry, University of Miami
Coral Gables, Florida 33124

Received October 26, 1988

There is currently an intense search for agents which inhibit HMG-CoA reductase due to their potential use as hypocholesteremic drugs.[1] The tentative identification of 30-hydroxylanosterol (**1**) and its corresponding aldehyde **2** in mevalonate-treated cell cultures suggested that they may be natural receptor-mediated feedback inhibitors of HMG-CoA reductase.[2] This view is supported by studies which have shown that the 24,25-dihydro derivatives of **1** and **2** strongly suppress HMG-CoA reductase activity[3] presumably at the level of transcription that involves binding of the sterol to a specific, intracellular receptor.[3b,4]

We wish to report the first synthesis of the putative natural oxysterols (+)-**1** and (-)-**2** where the key step involves the enantioselective enzymatic cyclization of the internally functionalized substrate **3** using bakers' yeast to obtain the lanostatriene (-)-**4**. Moreover, the enzymatic conversion of the C-10 vinylic substrate **3** to **4** is the first demonstration of *the remarkable ability of the oxidosqualene-lanosterol cyclase to rearrange a substituent other than a hydrogen or methyl group* along with the normal sequence of migrations which generates the natural lanosterol skeleton.

The substrate for the enzymatic reaction was constructed by the convergent sequence shown in Scheme I.[5] The anion from methyl 2-[(bis(trifluoroethyl)phosphono]acetate[6] was alkylated with homogeranyl iodide[7] to afford ester **5** which was converted,



via the standard terminal epoxidation procedure,[8] to the epoxy ester **6**. Oxidation of the farnesylic alcohol **7**[9] to the corresponding aldehyde **8** followed by the stereoselective coupling[6] of **8** to the anion from ester **6** and $KN(TMS)_2$/18-crown-6 gave a 58% yield of the α,β-unsaturated ester **9** after chromatographic separation of a 6.5:1 mixture of Z/E-isomers. Reduction of **9** using AlH_3[10] in THF afforded the epoxy allylic alcohol **10**[11] which was transformed to the aldehyde **11** by PDC[12] in DMF. Lastly, condensation of aldehyde **11** with 1.5 equiv of $Ph_3P{=}CH_2$ produced the desired vinylic substrate **3**.

The enzymatic cyclization of the substrate to the desired lanostane intermediate involved the anaerobic incubation of 1.00 g of (±)-**3** and 14 g of Triton X-100 with 1.5 L of ultrasonicated bakers' yeast homogenate (150 g of yeast in 0.10 M phosphate buffer prepared as previously described[13,14]) at 23 °C for 48 h to give, after extractive workup with ether and silica gel chromatography, 0.310 g of (-)-**4** ($[\alpha]_D^{23} = -47.3°$,[15] 62% conversion based on one enantiomer of (±)-**3**) as the only new sterol product. Control incubations using a boiled enzyme homogenate failed to produce any new sterol products. Since the ^1H NMR spectrum[16] of **4** displayed chemical shift values for the C-18,19,21,28, and 29 methyls and the C-20 proton (1.44 ppm) consistent with those in lanostane sterols[17] but not the unrearranged dammarane skeleton (C-20-H, 2.42 ppm),[17,18] we inferred that **4** also possessed the Δ^8-olefinic bond. This assignment was ultimately confirmed by converting **4** to the known 24,25-dihydro analogues of (+)-**1** and (-)-**2**.

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(16) 400 MHz ^1H NMR ($CDCl_3$) δ 0.75 (s, 3 H), 0.81 (s, 3 H), 0.88 (d, J = 7 Hz, 3 H), 0.99 (s, 3 H), 1.01 (s, 3 H), 1.59 (s, 3 H), 1.69 (s, 3 H), 3.23 (dd, J = 10.8, 4 Hz, 1 H), 4.88 (d, J = 16.5 Hz, 1 H), 4.93 (d, J = 10.5 Hz, 1 H), 5.08 (t, J = 7 Hz, 1 H), 5.76 (dd, J = 16.5, 10.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) 15.3, 16.5, 17.4, 18.4, 18.6, 19.2, 21.4, 24.8, 25.6, 26.7, 27.5, 27.8, 28.3, 29.4, 31.1, 35.6, 36.0, 36.1, 37.2, 38.8, 43.0, 50.2, 50.4, 56.7, 78.8, 111.8, 125.1, 129.8, 130.9, 137.0, 143.2.

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