Table I. Energetic Predictions for Singlet and Triplet Cycloheptatrienylidene[a]

basis set	wave function	total energy (hartrees)	rel energy (kcal/mol)
minimum	triplet SCF	-265.147 35	-15.3
minimum	singlet SCF	-265.110 89	7.6
minimum	singlet TCSCF	-265.122 94	0.0
DZ	triplet SCF	-268.363 88	-4.7
DZ	singlet SCF	-268.348 13	5.2
DZ	singlet TCSCF	-268.356 34	0.0
DZ + d	triplet SCF	-268.468 65	1.8
DZ + d	singlet SCF	-268.458 80	8.0
DZ + d	singlet TCSCF	-268.471 52	0.0

[a] Each total energy reported here corresponds to a completely optimized molecular structure of C_7H_6.

Chapman[4] and of Kuzaj, Lüerssen, and Wentrup.[5]

The earlier molecular structure optimizations of RSV[1] could be critized in that they were carried out at the minimum basis set (MBS) self-consistent-field (SCF) level of theory. In the present research both structures were optimized by using a much larger double ζ plus d function (DZ + d) basis set,[6] designated C(9s5p1d/4s2p1d), H(4s/2s). The triplet state was described at the single-configuration SCF level of theory and the singlet state at the two-configuration (TC) SCF level of theory.[7]

The predicted structures of singlet and triplet cycloheptatrienylidene are seen in Figures 1 and 2. Differences with respect to the minimum basis (MBS) structures of RSV[1] are substantive but not qualitative in nature. For example, for the triplet structure, the DZ + d SCF C-C distances to the carbene carbon are 1.427 Å, or 0.033 Å less than the earlier MBS SCF prediction. At an intermediate level of theory, DZ SCF for the triplet state and DZ TCSCF for the singlet, we find in the present research that both structures are predicted to be genuine minima—that is, all 3 (13) - 6 = 33 vibrational frequencies are real.

The theoretical energetic predictions are summarized in Table I. At the highest structurally optimized level of theory presented there the singlet energy falls below the triplet by 1.8 kcal/mol. For CH_2, the simplest carbene, the analogous DZ + d SCF/TCSCF level of theory predicts a singlet-triplet separation of 12.3 kcal,[8] while the experimental value (in accord with the highest levels of theory) is ΔE(S-T) = 9.1 kcal.[9] If the analogy with methylene is valid, one would expect the exact value of ΔE(S-T) to be about 5 kcal for cycloheptatrienylidene.

The above supposition is confirmed by higher level theoretical studies. For example when the basis set for the carbene carbon is extended to C(9s5p2d/7s4p2d), total energies of -268.477 47 (singlet TCSCF) and -268.471 90 hartrees (triplet SCF) are obtained, yielding a singlet-triplet splitting of 3.5 kcal/mol. Going back to the original DZ + d basis set, configuration interaction (CI) wave functions including all single and double excitations were also determined. The corresponding energies are -269.174 65 (singlet two-reference CISD) and -269.170 15 (triplet CISD), yielding a singlet-triplet splitting of 2.8 kcal. Adding the two corrections (1.7 kcal for basis set, 1.0 kcal for correlation effects) to the results in Table I yields ΔE(S-T) = 4.5 kcal/mol.

Given the clear theoretical prediction that cycloheptatrienylidene has a singlet ground state, why is an EPR spectrum observed in the laboratory? The simplest explanation would appear to be that *the triplet state is lower in energy than the singlet at the former's equilibrium geometry*. This hypothesis has been confirmed at the DZ + d triplet SCF/singlet TCSCF level of theory. At the triplet equilibrium geometry, the singlet energy lies 6.7 kcal/mol higher. Thus, although the singlet state is indeed the true planar ground state (as predicted earlier[1]), the triplet state minimum is well separated geometrically (the singlet and triplet C-carbene C-C angles differ by 13.8°; analogous singlet and triplet bond distances differ by as much as 0.045 Å) and has a relatively long lifetime with respect to intersystem crossing.

Acknowledgment. This research was supported by the United States National Science Foundation, Division of Chemistry, Chemical Physics Program, Grant CHE-821875. We thank Professor William H. Miller for helpful discussions.

(6) Huzinaga, S. *J. Chem. Phys.* **1965**, *42*, 1293. Dunning, T. H. *J. Chem. Phys.* **1970**, *53*, 2823.

(7) For an early use and justification of this triplet SCF/singlet TCSCF procedure, see: O'Neil, S. V.; Schaefer, H. F.; Bender, C. F. *J. Chem. Phys.* **1971**, *55*, 162. For a more recent discussion in the context of the simplest "aromatic carbene", see: Lee, T. J.; Bunge, A.; Schaefer, H. F. *J. Am. Chem. Soc.* **1985**, *107*, 137.

(8) Bauschlicher, C. W.; Schaefer, H. F.; Bagus, P. S. *J. Am. Chem. Soc.* **1977**, *99*, 7106.

(9) Schaefer, H. F. *Science (Washington, DC)* **1986**, *231*, 1100.

Structure of FK506: A Novel Immunosuppressant Isolated from *Streptomyces*

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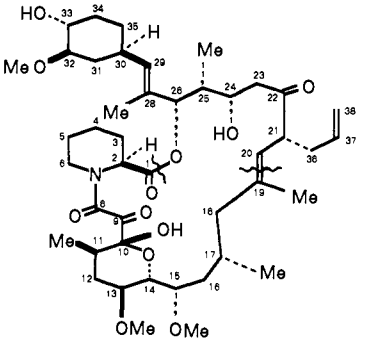
Considerable attention has recently been focused on the immunosuppressants represented by ciclosporins[1] because of their usefulness in bone marrow and organ transplantations. In the course of search for such immunosuppressive agents in our laboratories, FK506 (**1**), a novel 23-membered macrolide lactone, was isolated from *Streptomyces tsukubaensis* no. 9993. Herein we report the structural elucidation of this natural product.

FK506 (**1**) was isolated as colorless prisms from MeCN:[2] $C_{44}H_{69}NO_{12}$ (SIMS and elemental analysis[3]); mp 127-129 °C; $[\alpha]_D$ -84.4° (*c* 1.02, $CHCl_3$). The IR spectrum ($CHCl_3$) showed the presence of hydroxy groups (3700, 3600, 3550 cm^{-1}), carbonyl groups (1750, 1730, 1710 cm^{-1}), and an amide group (1650 cm^{-1}). The ^{13}C NMR spectrum ($CDCl_3$) revealed that **1** exists as an



1

equilibrium mixture of two isomers in solution (ca. 3:1 in $CDCl_3$). A detailed analysis of the spectrum[4] with the aid of the DEPT technique revealed all the carbon signals which are assignable to

(1) For a review on ciclosporins, see: *Ciclosporin, Progress in Allergy 38*; Borel, J. F., Ed.; Karger: New York, 1986.

(2) Kino, T.; Hatanaka, H.; Hashimoto, M.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.*, in press.

(3) SIMS, *m/z* 804 (M + 1); elemental analysis. Anal. Calcd for $C_{44}H_{69}NO_{12}\cdot H_2O$: C, 64.29; H, 8.71; N, 1.70. Found: C, 64.20; H, 8.86; N, 1.72.

(4) Spectral data for FK506 (**1**) and its degradation products (**2**, **3**, **6**, **7**, and **9**) are given in the Supplementary Material.

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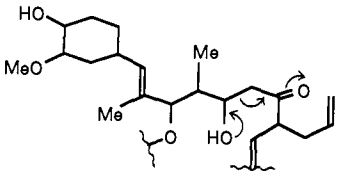
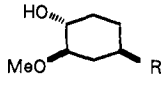
two ketones, one lactone (or ester), one amide, one vinyl, two trisubstituted olefins, one hemiketal (or ketal), three O-methyls, and five C-methyls, the remainder being 12 methylenes and 13 methines.

Partial structure A was suggested by the following experimental data. Ozonolysis of **1** (O_3/CH_2Cl_2, −78 °C) followed by reductive



A

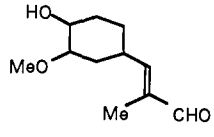


2 R=CHO

3 R=CH_2OH

and alkaline workup (1. Me_2S; 2. 1 N NaOH) gave aldehyde **2** (EIMS m/z 158 (M^+)).[4] Reduction of **2** with $NaBH_4$ (EtOH) gave the corresponding alcohol **3**, an inspection of whose ^1H NMR spectrum[4] with the aid of the COSY experiment presented the structure **3** for this compound and hence the structure **2** for the aldehyde. The structure of alcohol **3** was finally confirmed by identification with an authentic sample.[5]

Hydrolysis of **1** with 1 N NaOH (dioxane) produced α,β-unsaturated aldehyde **4** (EIMS m/z 198 (M^+): δ_H ($CDCl_3$) 1.78



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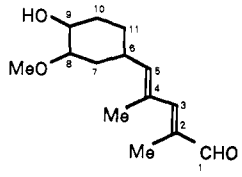

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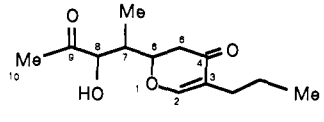
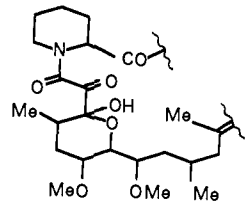


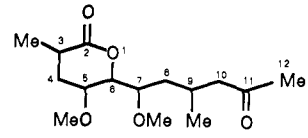
6

(d, J = 1.0 Hz, 3 H); δ_c ($CDCl_3$) 9.3 (q)), while treatment of **1** with NaH (THF, reflux) provided dienal **5** (EIMS m/z 238 (M^+): δ_H ($CDCl_3$) 2.00 (d, J = 1.3 Hz, 3 H), 1.95 (d, J = 1.1 Hz, 3 H); δ_c ($CDCl_3$) 16.2 (q), 10.7 (q)), establishing that two of the five Me groups in **1** are located at C-2 and C-4 in **5**.[6] Ozonolysis of the dihydro derivative of **1**, derived by catalytic reduction of the vinyl group in **1** (H_2 (1 equiv)/PtO_2/MeOH), gave, after reductive and alkaline workup, dihydropyrone **6** (EIMS m/z 240 (M^+)), whose structure was deduced on the basis of its spectroscopic data,[4] establishing the linkage of the propyl group (corresponding to the allyl group in **1**) to C-3 and the bonding of the hydroxy and dihydropyrone ring oxygens to C-8 and C-6, respectively, in **6**. The chemical evidence described above thus leads to the partial structure A.

The partial structure B was derived on the following grounds. Acid hydrolysis of **1** (6 N HCl, reflux) gave L-pipecolic acid, which was identified by comparison with an authentic sample on HPLC.[7] The direct ozonolysis of **1** as described above also gave lactone **7** (SIMS m/z 287 (M + 1)), whose structure was assigned from



B



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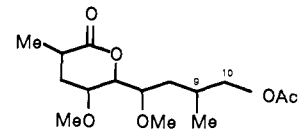
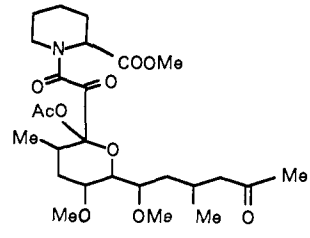


8

its NMR data including the COSY and C–H correlation experiments[4] except for the Me group tentatively assigned to be linked to C-9: the possibility remained that it is linked to C-10. Conversion of **7** to compound **8** (mCPBA/CH_2Cl_2), however, shifted the resonance of methylene protons (C-10) downfield to δ 4.00 (dd, J = 10.7, 5.6 Hz, 1 H) and 3.92 (dd, J = 10.7, 6.4 Hz, 1 H), confirming the validity of the structure **7** for the ozonolysis product.[8]

After alkaline treatment of **1** (1 N NaOH as described above) followed by methylation (CH_2N_2/Et_2O) and acetylation (Ac_2O/pyr), the reaction mixture was subjected to ozonolysis to give, after reductive workup, compound **9**, whose structural assignment was made by comparison of its ^{13}C NMR data[4] with those of **7** and methyl *N*-acetylpipecolate.[9] These chemical data thus postulate the partial structure B.



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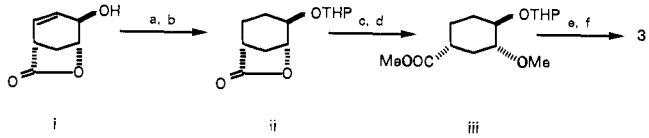
A reasonable connection of the partial structure A and B via lactone and olefin linkages (as shown by the wavy line in **1**) leads

(5) Alcohol **3** was synthesized as follows. The starting material i was prepared according to the literature: Bartlett, P. A.; McQuaid, L. A. *J. Am. Chem. Soc.* **1984**, *106*, 7854.



i ii iii

[a] 2,3-DHP/PPTS/CH_2Cl_2. [b] H_2/10%Pd–C/EtOH. [c] MeONa/MeOH. [d] MeI/NaH/THF. [e] $LiAlH_4/Et_2O$. [f] HCl/MeOH.

(6) The formation of **4** or **5** can be rationalized by retroaldol cleavage, followed by further retroaldol or dehydration reactions.

(7) HPLC: column, CHIRALPAK WH (DAICEL CHEMICAL) (4.6 × 250 mm); eluent, 0.25 mM solution of $CuSO_4$ in H_2O; flow rate, 1.2 mL/min; temperature, 50 °C; retention time, 12.5 min (authentic samples: L-pipecolic acid, 12.5 min; D-pipecolic acid, 9.1 min).

(8) The lactone carbonyl in **7** would be formed by an abnormal ozone oxidation of the masked α,β-diketo function. For similar abnormal ozone oxidations, see, e.g.: Deslongchamps, P.; Moreau, C. *Can. J. Chem.* **1971**, *49*, 2465.

(9) Methyl *dl-N*-acetylpipecolate was prepared from *dl*-pipecolic acid by esterification (MeOH/$SOCl_2$) followed by acetylation (Ac_2O/pyr): for the ^{13}C NMR data, see the Supplementary Material.

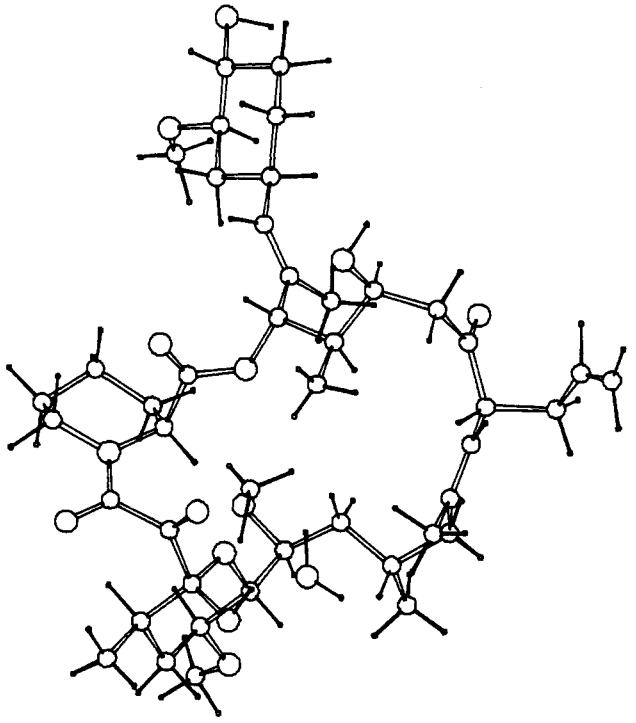


Figure 1. A perspective drawing of the X-ray model of FK506 (**1**). The water molecule is included.

to the full structure of FK506. The geometry of the two trisubstituted olefins in **1** was assigned to both to be E on the basis of the upfield resonations of the Me groups bonded to these double bonds (19-Me, δ_c 15.8; 27-Me, δ_c 13.9). Since several attempts to assign the stereochemistry of the other functional groups were unsuccessful, an X-ray analysis was performed on crystalline FK506 itself (Figure 1),[10] establishing the relative stereochemistry as depicted in **1**. The absolute configuration was determined by the fact that **1** contains L-pipecolic acid (see above). The tautomeric equilibration of **1** in solution might be associated with a restricted rotation of the amide bond within the macrolide ring.[11,12]

FK506 represents a new class of macrolide lactones with amino acid and hemiketal-masked α,β-diketoamide functionalities incorporated in a 23-membered ring.[13] The activity of FK506 was considerably greater than that of cyclosporin A in various immunosuppression assays.[14]

(10) Crystal data for **1** ($C_{44}H_{69}NO_{12}\cdot H_2O$, M = 804.0): orthorhombic; space group $P2_12_12_1$; unit cell a = 10.939 (1) Å, b = 15.878 (1) Å, c = 27.184 (1) Å; v = 4721.0 Å^3; Z = 4; Dx = 1.131 g·cm^{-3}. Intensities were measured on a Rigaku AFC-5RU diffractometer by using graphite-monocromated Cu Kα radiation (λ = 1.5418 Å). Of 4484 independent reflections with $2\theta <$ 130°, 4249 were used for structure determination. The structure was determined by direct methods (RANTAN) and successive Fourier syntheses and block-diagonal least-squares. The final R factor, based on the used reflections, was 0.071.

(11) In comparison of the ^{13}C NMR signals of the major and minor isomers, the most significant differences in chemical shift were observed at C-2 and C-6. In the major isomer, C-2 resonated by 4.2 ppm to lower fields than in the minor isomer, while C-6 was observed by 4.7 ppm to upper fields, suggesting that in the major isomer the amide bond is in cis conformation in accord with the result of the X-ray crystal analysis.

(12) The ^{13}C NMR spectrum in solid state revealed that FK506 exists as one conformer (cis amide conformation): see the Supplementary Material.

(13) The closest literature analogue that contains these functionalities is rapamycin, which has been described as an antifungal antibiotic: Findlay, J. A.; Radics, L. *Can. J. Chem.* **1980**, *58*, 579.

(14) The exceptional activity of FK506 will be reported separately. (a) Kino, T.; Hatanaka, H.; Miyata, S.; Inamura, N.; Yajima, T.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Ochiai, T. *J. Antibiot.*, in press. (b) Inamura, N.; Nakahara, K.; Kino, T.; Goto, T.; Aoki, H.; Yamaguchi, I.; Kohsaka, M.; Ochiai, T. *Transplantation*, in press. (c) Ochiai, T.; Nakajima, K.; Nagata, M.; Hori, S.; Asano, T.; Isono, K. *Transplantation*, in press. (d) Ochiai, T.; Nagata, M.; Nakajima, K.; Suzuki, T.; Sakamoto, K.; Enomoto, K.; Gunji, Y.; Uematsu, T.; Goto, T.; Hori, S.; Kenmochi, T.; Nakagouri, T.; Asano, T.; Isono, K.; Hamaguchi, K.; Tsuchida, H.; Nakahara, K.; Inamura, N.; Goto, T. *Transplantation*, in press.

Supplementary Material Available: Spectral data (IR, ^{1}H NMR, and ^{13}C NMR) for **1**, **2**, **3**, **6**, **7**, and **9** and additional X-ray crystallographic data for **1** (9 pages). Ordering information is given on any current masthead page.

Molecular Recognition: Hydrogen Bonding and Stacking Interactions Stabilize a Model for Nucleic Acid Structure

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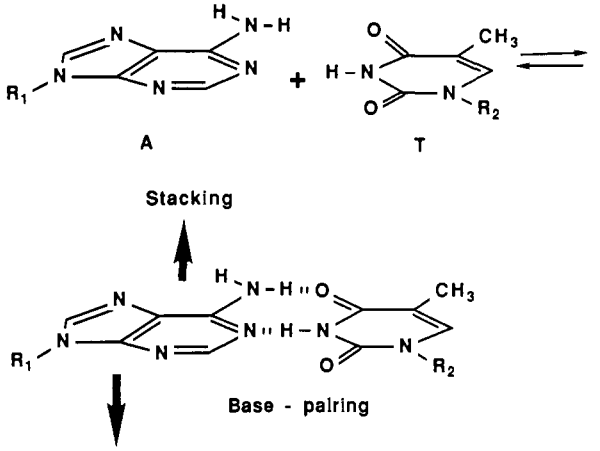
The classical form of molecular recognition is the base pairing within nucleic acids as formulated by Watson and Crick.[1] The complementary hydrogen bonding surfaces shown in eq 1 for



(1)

adenine (A) and thymine (T) provide a vehicle for information transfer, while stacking interactions between adjacent base pairs provide additional stability for the helical structure.[2] The hydrogen bonding aspects of eq 1 have been examined in detail by Rich[3] and Hammes[4] with use of derivatives of A and T in the noncompeting solvent $CDCl_3$, while the stacking of individual bases in H_2O was observed by Chan.[5] Here, we introduce a model system in which both forces can operate simultaneously.

The new models, e.g., **1**, are designed in accord with the principles of molecular recognition[6] and feature stacking and hydrogen bonding surfaces that converge on the substrate from perpendicular directions. Moreover, their bulk reduces the dimerization (self-recognition) that is generally observed[3] in addition to eq 1. The scaffolding for the new structures is provided by derivatives of Kemp's triacid[7] **2**, in which the U-shaped relationship that exists between any two carboxyl functions is enforced by the equatorial methyl groups. Sublimation of **2** or its successive treatment with $(CF_3CO)_2O$ and water gives the anhydride acid[7] **3a**. With NH_4OH **3a** gives the imide acid[8] **3b** (mp > 280 °C) from which the acid chloride **3c** (mp 171 °C) can be obtained with $SOCl_2$. The new amides are obtained by acylation of the aromatic amines **4a–e** with **3c**. In addition, the methyl ester **3d**

(1) Watson, J. D.; Crick, F. H. C. *Nature (london)* **1953**, *171*, 737–8.

(2) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; Chapter 6.

(3) Kyogoku, Y.; Lord, R. G.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 250–257. Iwahaski, H.; Kyogoku, Y. *J. Am. Chem. Soc.* **1977**, *99*, 7765.

(4) Hammes, G. C.; Park, A. C. *J. Am. Chem. Soc.* **1968**, *90*, 4151–57.

(5) Chan, S. I.f Schweitzer, M. P.; Ts'o, P. O. P.; Helmkamp, G. K. *J. Am. Chem. Soc.* **1964**, *86*, 4182. Schweitzer, M. P.; Chan, S. I.; Ts'o, P. O. P. *J. Am. Chem. Soc.* **1965**, *87*, 5241.

(6) Rebek, J., Jr. *Science (Washington, DC)* **1987**, *235*, 1478–1484.

(7) Kemp, D. S.; Petrakis, K. S. *J. Org. Chem.* **1981**, *46*, 5140–43.

(8) All new compounds were characterized by 300-MHz PMR, 75-MHz ^{13}C NMR, and FTIR spectroscopy. Elemental analyses were either within 0.3% of calculated combustion values or within 0.001 of calculated mass spectral values.

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