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THERMAL REARRANGEMENT OF THE IMMUNOSUPPRESSANT FK-506

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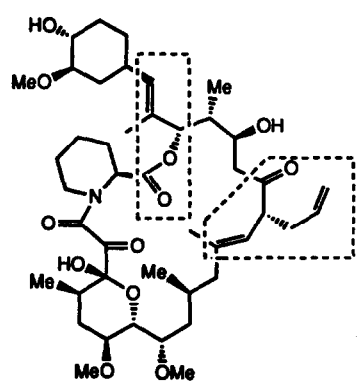
Abstract: Treatment of FK-506 in xylene at reflux results in a [3,3] sigmatropic rearrangement of the allylic ester portion of the molecule to give a new ring expanded macrolide.

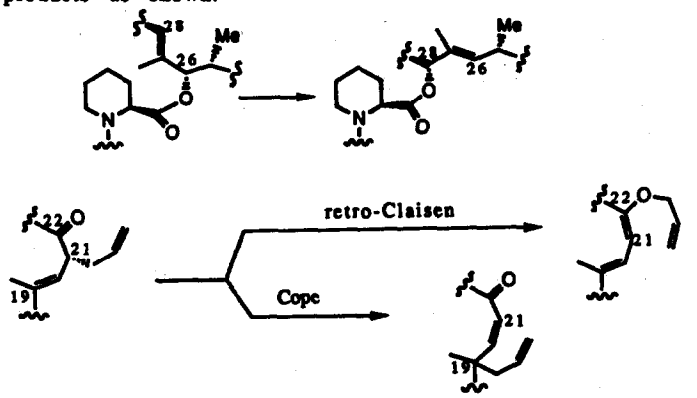
FK-506 **1**, a novel 21-membered macrolide isolated from *Streptomyces tsukubaensis* (no. 9993),[1] has been shown recently to possess immunosuppressive activity at much lower dosage than the current therapeutic agent cyclosporin A[2]. The potential clinical utility of this powerful agent in bone marrow and organ transplantations has stimulated many diverse approaches toward the total synthesis[3] of this intriguing tetracyclic, masked tricarbonyl macrolide. We have been interested in modifications of the natural product in order to gain a clearer understanding of which structural and conformational features are important for immunosuppressive activity. During the course of our efforts we examined the thermal stability of FK-506, since this molecule contains two structural units (highlighted in **1**) which have potential for a thermal [3,3] sigmatropic rearrangement as shown below.



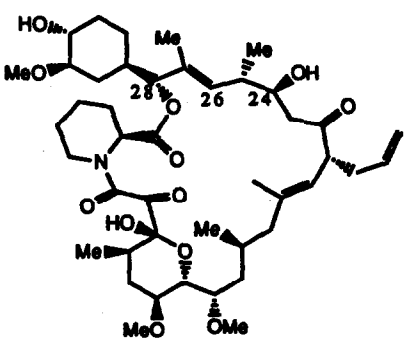
1

In practice, FK-506 was quite inert in both benzene and toluene at reflux for 24 h. However, upon treatment in xylene at reflux under nitrogen, FK-506 underwent a slow thermal

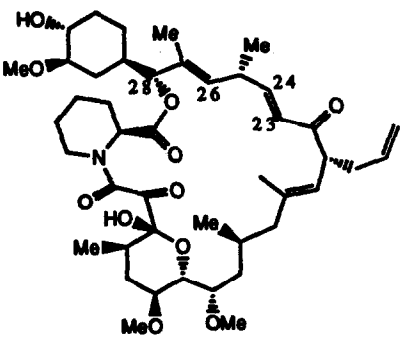
reaction to give a new product (24 h, 45%) which had the same m/e as FK-506 but differed significantly in its ^{1}H NMR spectrum. Both the allylic ester and the substituted 1,5-hexadiene can be envisioned to undergo a formal [3,3] sigmatropic reaction[4] to give rearranged products as shown.



The structure of this new macrolide was initially assigned from NMR studies. Proton and 2D ^{1}H NMR suggested that the carbon framework of C19 through C21 was intact. A double irradiation experiment indicated that the C25 methine proton located at 2.6 ppm was next to a vinylic proton at 5.25 ppm suggesting structure **2**, the rearranged allylic ester, as the newly formed macrolide. Additionally, the fact that FK-520 (C21-ethyl homolog of FK-506 which lacks the structural elements required for Cope and retro-Claisen rearrangements) gave the corresponding ring expanded product under similar experimental conditions is also supportive of an allylic ester rearrangement.



2

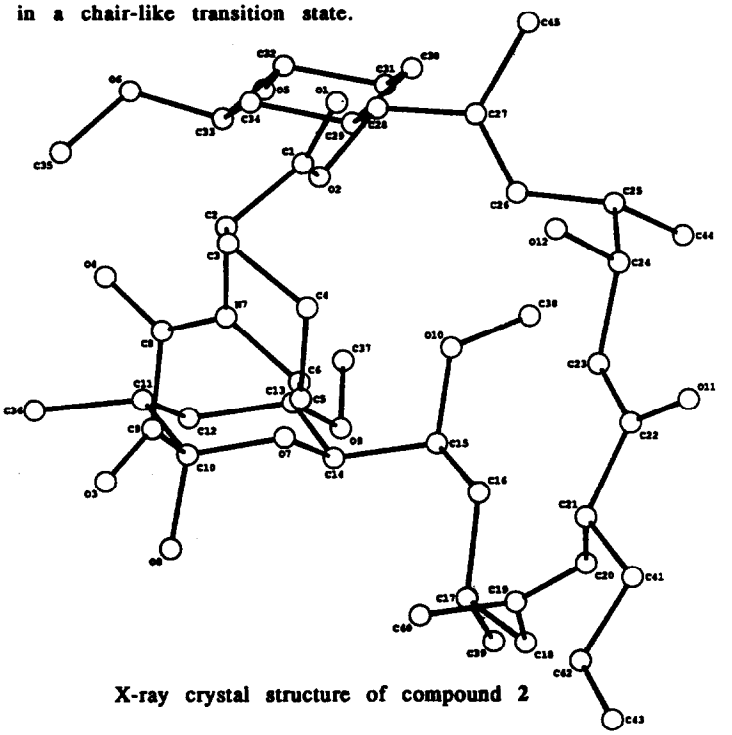


3

It is well known that allylic esters can undergo [3,3] sigmatropic rearrangement in the presence of transition metal catalysts under mild conditions to give highly regio- and stereoselective products in good yield[5] whereas the thermal rearrangement usually requires very high temperatures and often gives complicated product mixtures in low yield[4]. However, the X-ray crystal structure of FK-506 and the fact that treatment of $\Delta^{23,24}$-

FK-506[6] under the same conditions did not give compound 3 illustrate that the π–orbitals of the lactone carbonyl and the C27,C28 double bond in 1 are held in a precise alignment which favors a [3,3] sigmatropic rearrangement at relatively low temperature. On the other hand, the flexible allylic side-chain of the 1,5-hexadiene portion evidently is not suitably constrained to undergo a Cope or retro-Claisen rearrangement at the same low temperature.

Comparison of the ^{13}C spectrum of compound 2[7] with FK-506 also provided support for the formation of rearranged allylic ester. The product contained the same number of sp^2 and C-O moieties as in FK-506, but with chemical shift changes in the regions assigned to C25-C28 carbons. A downfield shift of 6 ppm for the methyl group at C25 combined with the near-equivalent chemical shift of the C22-carbonyl in the product and in FK-506 (213 ppm) eliminated the possible 1,5-hexadiene Cope rearrangement product and the retro-Claisen structure. Finally, the X-ray crystal structure[8] of this new macrolide 2 confirmed its structure as depicted and showed the stereochemistry at the double bond between C27 and C26 with respect to C28 and C25 to be *trans*, consistent with the thermal rearrangement of FK-506 occurring in a chair-like transition state.



X-ray crystal structure of compound 2

Extended treatment in hot xylene resulted in dehydration of 2 to form its $\Delta^{23,24}$ analog (compound 3) without a trace of Cope and retro-Claisen rearrangement products. Isolation of a small amount of this material at 24 h and increasing amounts (40%) at 36 h with no

evidence for $\Delta^{23,24}$-FK-506 being formed in the reaction suggested that the dehydration occurs after the rearrangement. This was confirmed by isolation of the same dehydrated product **3** after heating **2** in xylene at reflux. The dehydro analog **3** (compound exists as a 4:3:*cis:trans* mixture of rotomers in $CDCl_3$) showed the same signals in the C26-28 region as **2** with additional signals at 6.8 (dd, J=10 and 2.5 Hz) and 6.1 (d, J=10 Hz) ppm characteristic of an α,β-unsaturated ketone and consistent with known chemistry on FK-506[6].

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5. Overman, L.E. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 579; Subsquent to our work, a Lewis acid catalyzed allylic ester rearrangement was observed in acyclic variants of FK-506, see: Askin, D.; Reamer, R.A.; Joe. D.; Volante, R.P.; Shinkai, I. *J. Org. Chem.*, submitted.
6. $\Delta^{23,24}$-FK506 was prepared by acid-catalyzed dehydration of FK-506, see: Okuhara, M.; Tanaka, H.; Goto, T.; Kino. T. and Hatanaka, H. U.S. Patent No. 4,879,366 (1990).
7. Compound **2** exists as a 3:2 (*cis:trans*) mixture of rotamers in $CDCl_3$. mp 138-140°C. ^{13}C NMR chemical shifts of compound **2**: (100.6 MHz, $CDCl_3$) δ 213.2, 212.2, 197.1, 192.0, 168.9, 165.9, 164.9, 139.3, 137.1, 135.6, 135.2, 134.3, 133.5, 128.5, 127.9, 123.3, 123.2, 16.9, 116.5, 98.3, 18.0, 84.4, 84.3, 81.5, 80.5, 75.7, 75.4, 73.7, 73.5, 72.4, 71.7, 71.5, 71.3, 57.7, 57.3, 56.6, 56.4, 56.35, 56.25, 52.5, 52.1, 51.1, 49.8, 49.4, 47.0, 44.9, 44.0, 39.0, 38.9, 37.9, 37.8, 37.7, 36.8, 35.6, 34.6, 33.7, 33.4, 32.7, 32.0, 30.9, 30.8, 29.8, 28.8, 27.3, 26.8, 26.6, 26.3, 25.8, 25.7, 24.9, 24.0, 20.8, 20.4, 19.6, 18.3, 17.7, 17.3, 17.2, 16.3, 16.2, 5.8, 15.4, 15.3.
8. Crystal structure details for compound **2** are available upon request.

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