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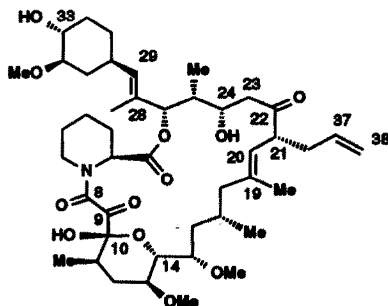
MODIFICATION OF THE IMMUNOSUPPRESSANT ASCOMYCIN (21-ETHYL-FK506) AT THE C19-C20 DOUBLE BOND

K.Baumann[a], A.J.F.Edmunds[a*1], M.A.Grassberger[a], G.Schulz[a], W. Schuler[b], and G. Zenke[b]

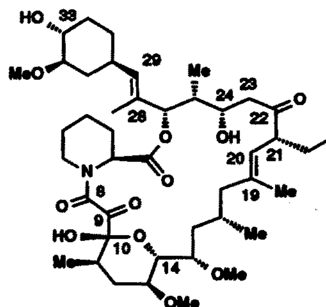
[a]*Sandoz Forschungsinstitut, Brunner Strasse 59, A-1235, Vienna, Austria*
[b]*Sandoz Pharma AG, CH-4002, Basel, Switzerland.*

***Summary:** Selective epoxidation of the C19-C20 double bond of ascomycin (the 21-ethyl anologue of FK506) has been accomplished. The resultant epoxide was opened by hydroxy nucleophiles and the impact of the modifications on the biological activities determined.*

The novel macrolides FK506 (1)[2] and its 21-ethyl analogue (ascomycin, 2)[3] are currently of great interest, due to their novel structure and potent immunosuppressive activity *in vitro*[4] and *in vivo*[5]. Several papers have recently been published regarding the reactivity of the tricarbonyl functionality (C8-C10)[6], the region ultimately involved in binding to the major cytosolic receptor (FKBP, FK506 binding protein)[7]. However, only limited chemical modifications of the right-half of the molecule(s) (C14-C23, the effector domain) have thus far been reported[8, 9], and little is known about the impact of such modifications on biological activities[9b]. In this paper we report our contributions to this area in which we selected the C19-C20 double bond of ascomycin as a target for modification.



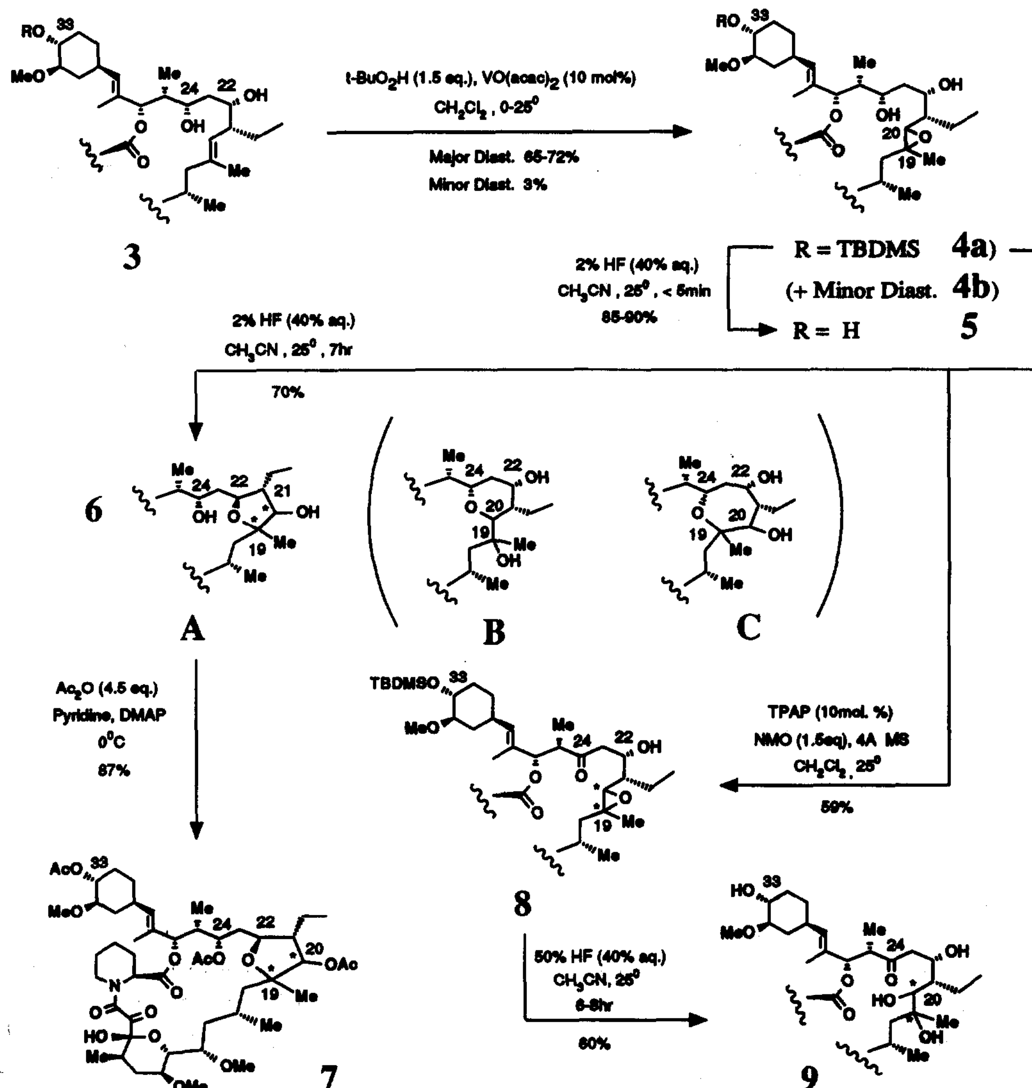
FK506 1



Ascomycin 2

In order to regioselectively epoxidise the C19-C20 double bond of 2, one of two approximately equivalent electron rich double bonds in the molecule, we elected 33-O-TBDMS-22(S)-dihydro-ascomycin (3)[10] as the starting material: The 22-hydroxy group, as part of a homoallylic alcohol could then be used to direct oxygen delivery to the C19-C20 double bond. Thus, epoxidation of 3 under Sharpless conditions[11] led to the corresponding C19-C20 epoxide (4) in excellent yield and with high diastereoselectivity[12,13,14] (Scheme 1). Removal of the TBDMS group of 4a (major diastereomer) under controlled conditions (2% aqueous HF in CH_3CN, < 5min) gave high yields of the corresponding deprotected compound 5, whereas a slightly more polar product was formed (70% isolated yield) when the reaction was allowed to run for 7hr. The ^1H NMR of this compound (6) was extremely complex due to the presence of amide bond rotamers (two distinct sets of NMR signals): The ^{13}C NMR spectrum on the other hand could be explained[15] by an intramolecular epoxide ring opening, resulting in a downfield shift of the C-19 signal by nearly 28ppm.

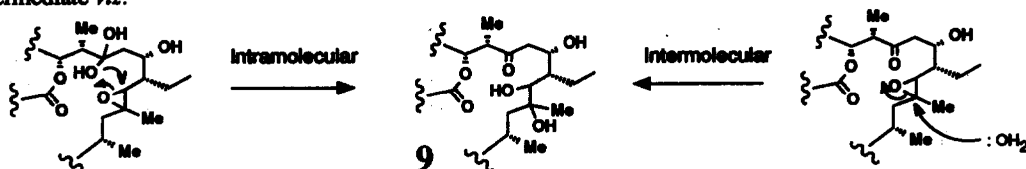
However, as there was no clear assignment of the signals of C-22 and C-24, it was impossible to distinguish between the oxygen heterocyclic structures A, B, and C. The NMR analysis was simplified by acetylation of the free hydroxy groups of 6 as the resulting triacetate 7 (87% isolated yield) exists in $CDCl_3$-solution mainly as a single rotamer (i.e. one distinct set of NMR signals). With the aid of spin decoupling experiments[16] the signals in the ^{1}H-NMR spectrum for the protons in positions 20 to 25 could then be assigned unambiguously: H-20: 5.28ppm (d, J=8Hz); H-21: 1.97ppm (m); H-22: 3.55ppm (t, J=9Hz); H-23a: 1.68ppm (dd, J=10Hz, J=14Hz); H-24: 4.87ppm (m); H-25: 1.97ppm (m).



Scheme 1. * Denotes diastereomeric purity, absolute configuration unknown

This ruled out structures B and C and showed that 6 contains a tetrahydrofuran structure, resulting from the attack of the 22-hydroxy group at the more highly substituted carbon atom of the protonated epoxide.

Having ascertained that the 22-hydroxy function of 4a was involved in an acid catalysed intramolecular epoxide opening, and bearing in mind that an extremely facile benzilic acid rearrangement of the tricarbonyl functionality can occur under aqueous basic conditions[6b], we envisaged that alternative nucleophilic opening of the epoxide (e.g. to the corresponding diol) would only be possible under acidic conditions if the 22-hydroxy function of 4a was first removed, for example by oxidation to carbonyl. Various oxidation methods were therefore attempted and always resulted in the preferential oxidation of the 24-hydroxy group, with Ley's catalytic peruthenate procedure[17] giving the highest yield (59% isolated) of mono-oxo product 8[18]. When treated with strong acid this compound proved to be remarkably stable, and reaction at the epoxide could only be achieved with higher concentrations of HF (1:1 of 40% aqueous HF and acetonitrile). The product isolated (60% yield) was suprisingly shown to be the single diol diastereomer (9)[19] which must arise either from an intermolecular attack of water at C19, or *via* intramolecular opening of a ketal-hydroxy intermediate *viz:*



Scheme 2.

The lack of C22-hydroxy β-eliminated product, and the inability of this group to attack the epoxide intramolecularly, is strong evidence that oxidation at the C24 position leads to a conformational change which removes the 22-hydroxy function from the proximity required for attack at C19.

The biological activities of compounds 3, 5, 6, and 9 in the macrophillin binding assay[20] (MBA) and the *in vitro* assays for immunosuppression (IL-2 Reporter Gene Assay[21] and in the Mixed Lymphocyte Reaction[22]) are shown below.

Subst. No.	Derivative of Ascomycin	MBA[1]	MLR[2]	IL-2 RGA[3]
1	FK-506	1	1	1
2	Ascomycin	0.6	2	1.8
3	22(S)-dihydro-	4	>1000	233
5	22(s)-dihydro-19,20-epoxy-	9	>890	>920
6	22(S)-dihydro-19,24-epoxy-20-hydroxy-	0.5	>890	>920
9	22(S)-dihydro-19,20-dihydroxy-24-oxo-	2	190	59

[1] MBA = Macrophillin Binding Assay [IC-50 / IC-50 FK506]* [2] MLR = Mixed Lymphocyte Reaction [IC-50 / IC-50 FK506]*
[3] IL-2 RGA = IL-2 Reporter Gene Assay [IC-50 / IC-50 FK506]*
* Relative IC-50 values

The results shown in the table are in full accord with the dual-domain model of Schreiber [7,23,24]. Thus, while modifications in the effector domain have little impact on binding to FKBP (MBA assay), the lack of activity in the cellular assays for immunosuppression show however that they do evidently have an impact on the interaction of the compound/FKBP complex with the putative target protein-phosphatase, calcineurin[25].

References and Notes

1) Current Address, Sandoz Agro AG, CH-4002, Basel, Switzerland.
2) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T; Taga, T. *J. Am. Chem. Soc.*, **1987**, *109*, 5031.
3) a) Arai, T.; Koyoma, Y.; Suenaga, T.; Honda, H. *J. Antibiot.*, **1962**, 231. b) Inamine, E.S.; Wicker, L.S.; Chen, S.T.; Arison; B.H. EP 349061, **1990**.

4) Kino,T.; Inamura, N.; Sakai, F.; Nakahara, K.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Ochiai, T. *Transplantation Proceedings*, **1987**, *19* (5, Suppl.6), 36-39.
5) Starzl, T.E.; Todo, S.; Fung, J.; Demetris; A. J.; Venkataraman, R.; Jain, A. *The Lancet*, **1989**, *2*, 1000.
6) a) Fisher, M.J.; Chow, K.; Villalobos, A.; Danishefsky, S.J. *J. Org. Chem.*, **1991**, *56*, 2900. b) Askin, D.A.; Reamer, R.A.; Jones, T.K.; Volante, R.P.; Shinakai, I. *Tetrahedron Lett.*, **1989**, 671 and Askin, D. A.; Reamer, A.; Daisy Joe, R.P.; Volante, R.P.; Shinakai, I. *Tetrahedron Lett.*, **1989**, 6121. c) Donald, D.K.; Cooper, M.E.; Furber, M.; Wells, E.; Hutchinson, R.; Black, F.M. *Tetrahedron Lett.* **1991**, 1375. b) Nussbaumer, P.; Grassberger, M. A.; Schulz, G. *Tetrahedron Lett.*, **1992**, 3845.
7) Schreiber, S.L. *Science*, **1991**, *251*, 283., and references cited therein.
8) Edmunds, A.J.F.; Baumann, K.; Schulz, G.; Grassberger, M.A. *Tetrahedron Lett.*, **1991**, 7039.
9) For some semi-synthetic and synthetic analogues of FK506 containing the binding region with different effector domains see: a) Goulet, M.T.; Hodkey, D.W. *Tetrahedron Lett.*, **1991**, 4627. b) Schreiber, S.L; Somers, P.K; Wandless, T.J. *J. Am. Chem. Soc.*, **1991**, *113*, 8045.
10) Grassberger, M.A.; Fehr, T.; Horvath, A.; Schulz, G. *Tetrahedron*, **1992**, *48*, 413.
11) Sharpless, K.B.; Michaelson, R.C. *J. Am. Chem. Soc.*, **1973**, *95*, 6136.
12) The diastereoselectivity (4a:4b) was at least 24:1 based on ratios of isolated pure diastereomers. The minor diastereomer 4b could be isolated chromatographically in large scale reactions (30g).
13) Selected ^{1}H NMR data for 4a (main diastereomer): (250MHz in $CDCl_3$) *ca* 2:1 mixture of rotamers: Diagnostic are the oxirane protons (H-20) at 2.550 (main rotamer) and 2.600ppm (minor rotamer) which appeared as doublets with a coupling of 9Hz to H-21. In the ^{1}H NMR spectrum of the isolated minor diastereomer 4b ($CDCl_3$, *ca* 2:1 mixture of rotamers) the corresponding doublets appeared at 2.580 (main rotamer) and 2.640ppm (minor rotamer) both with a coupling of 9Hz to H-21. Selected ^{13}C NMR data for 4a ($CDCl_3$, *ca* 2:1 mixture of rotamers): 46.5 and 46.9 (C-18); *ca* 57ppm (C-19, overlapping with a methoxy signal and the signal of C-2); 61.1 and 60.2 (C-20); 44.9 and 45.3 (C-21); 132.9 and 131.8 (C-28); 129.1 and 128.5ppm (C-29).
14) We have also found that homo-allylic epoxidation of the FK506 analogue of 3 gives regioselective C19-C20 epoxidation with similar yield and stereoselectivity.
15) Selected ^{13}C NMR data for 6 ($CDCl_3$, mixture of rotamers): 84.9 and 85.6 (C-19); 79.0 and 78.3; 77.6 and 78.0; 75.7 and 76.2; 75.3 and 75.4; 73.6 and 73.7; 72.3 and 71.3; 70.6 and 70.9ppm, (C-20, C-22, C-24, C-26, C-13, C14 and C15).
16) The signal at 5.28ppm (d, J=8Hz) coupled with the signals at 2.01ppm (m, H-21) and 3.55ppm (t, J=9Hz, H-22) while the signal at 5.20ppm coupled only to the signal at 1.97ppm (m, H-25); The multiplet at 4.87ppm showed coupling to the signals at 1.97ppm (m, H-25) and geminal protons at C-23 (1.68ppm, dd, J=10 and 14Hz, H-23a; 1.48ppm, dd, J=10 and 14Hz, H-23b). The position of the acetate groups follows from the downfield shift of the adjacent proton signals. With the knowledge of the spin decoupling experiments the signals at 5.28ppm and 4.87ppm could thus be unambiguously assigned to H-20 and H-24, respectively (the signals at 4.68ppm (ddd, J=5,10, and 11Hz) and at 5.20ppm(s) being assigned respectively to H-32 and H-26), enabling clear distinction between the structures A, B, and C.
17) Griffith, W.P.; Ley, S.V. *Aldrichimicia Acta* **1990**, *23*, 13.
18) Spin decoupling experiments and selected NMR data for 8: ^{1}H NMR (250MHz, $CDCl_3$+10% CD_3OD): 2.80ppm (d, J=9Hz, H-20) couples with H-21 at 1.17ppm; 4.32ppm (br d, J=11Hz, H-22) couples with H-21, H-23a, and H-23b; 3.25ppm (dd, J=11 and 19Hz, H-23a); 2.55ppm (br d, J=19Hz, H-23b); 5.32ppm (d, J=4Hz, H-26) couples with H-25 at 2.98ppm. That the oxidation occurs at the 24-position and not the 22-position follows from the chemical shift of H-21 and H-25 as decoupling experiments show that it is the H-25 proton signal that is shifted down field due to the presence of an adjacent carbonyl.
19) Selected NMR data for 9: (250MHz, $CDCl_3$); 5.12 (d, J=9Hz, H-29); 4.13 (q, J=6Hz, H-22); 3.99 (t, J=7Hz, H-20); 2.07 (m, H-21); 2.70 (dd, J=4 and 16Hz, H-23a); 2.60ppm (dd, 6 and 16Hz, H-23a).
20) MBA (ELISA-type competition assay): Binding of biotinylated recombinant human macrophilin-12 to immobilised FK506 (FK506 coupled covalently via a spacer at its 33-position to bovine serum albumine) in the presence of test substance. BSA-FK506-bound immunophilin is assessed by incubation with streptavidin-alkaline phosphatase and determination of phosphatase activity (p-nitrophenylphosphate).
21) IL-2 RGA: Jurkat clone, stably transfected with an IL-2 promoter/lac z (β-galactosidase) construct; stimulation by PHA/PMA (1μg/ml/20ng/ml;16h); readout β-galactosidase activity (methylumbelliferyl-galactoside).
22) MLR: 2-Way murine MLR (CBA vs BALB/c) in serum-free CG-medium (5 days, 37^{0}).
23) Baumann, G. *Médecine/Sciences*, **1992**, *8*, 366
24) McKeon, F. *Cell*, **1991**, *66*, 823.
25) Liu, J.; Farmer, J.D.; Schreiber, S.L. *Cell*, **1991**, *66*, 1.

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