Preparation of Sulfhydryl Cyclosporin A†

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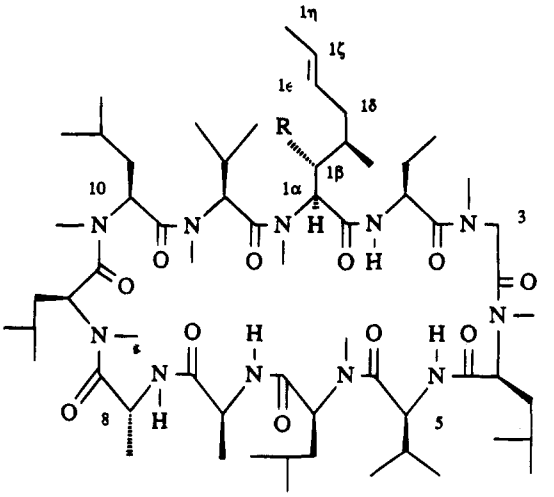
Since its discovery, cyclosporin A (**1**), the active ingredient of Sandimmune, has been the subject of over 20 000 publications (for a brief overview, see ref 1a,b). When we initiated the current investigation, the role of the free hydroxy group of cyclosporin A (**1**) had not been examined adequately, since derivatives at that position were available in limited numbers only. Modifications included acetyl-cyclosporin A[2] and more recently O-alkylated cyclosporin A derivatives.[3] In this context, we were aiming for the replacement of the (secondary) hydroxy group of cyclosporin A (**1**) by e.g. a sulfhydryl group.

Our work was initiated following a communication[4] describing the replacement of various hydroxy groups by sulfhydryl groups in the presence of Lawesson's[5] reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-phosphetane 2,4-disulfide]. Since then, results of the treatment of cyclosporin A with the same reagent have been published.[6] According to that report, a solution of the components was kept at room temperature for extended periods of time for the conversion of selected amides to thioamides. We, however, have employed slightly different reaction conditions and were rewarded with a hitherto unknown cyclosporin derivative. This work was carried out in parallel to our own transformations of acetylated cyclosporins to yield acetylated cyclosporin mono and bis thioamides.[1a,b] Following completion of this work, others have demonstrated[7] through X-ray analysis that cyclosporin A, following its complexation with cyclophilin A, was forced to assume a conformation differing from the conformation of cyclosporin A found in nonpolar solvents[8] as well as in the solid state.[9] This new conformation is characterized by a novel intramolecular hydrogen bridge between the hydroxy group and the carbonyl group of amino acid 4 (MeLeu, see Figure 1). This type of hydrogen bridge was not observed prior to the addition of cyclophilin A.



1: R = OH
2: R = SH
3: R = SAc

Figure 1.

A solution of cyclosporin A (**1**) in toluene was heated to reflux for 30 min in the presence of Lawesson's reagent. The crude product consisted of a complex mixture of substances containing sulfur (see also ref 6). Careful chromatography on silica gel with water saturated ethyl acetate produced a pure fraction, albeit in low yield, with an $R_f = 0.27$ (see Experimental Section). We were able to crystallize this material from ether/hexane. The designated structure **2** was based on analytical and spectral data obtained for this compound. Its fast atom bombardment spectrum (FAB) showed a molecular ion of 1218.7 $[MH]^+$ mass units, an increment of 16 mass units relative to the starting material. This higher molecular weight indicated that one of the twelve oxygen atoms of cyclosporin A (**1**) had been replaced by a sulfur atom. The proton NMR spectrum of compound **2** revealed no significant shift differences for either the NH signals of the four non methylated amides or the *N*-methyl groups of the seven *N*-methylated amides. It was therefore safe to assume that the observed molecular weight of **2** was due to the replacement of the secondary alcohol, located on the MeBmt, by a sulfhydryl group. Additional support for this hypothesis was gained from the fragmentation pattern of **2** as revealed by the mass spectrum. A small signal at 1184.7 mass units indicated the loss of the sulfhydryl group. An even smaller signal at 1089.6 mass units, due to a retro thioaldol reaction, was seen as additional evidence. The same fragment was also present in the mass spectrum of cyclosporin A (**1**) itself.

Structure **2** was fully supported by an in-depth spectral analysis (COSY, ROESY). These spectra attested to the presence of a singular compound free of isomers. A comparison of the correlated proton and ^{13}C NMR spectra obtained for the sulfur containing cyclosporin with spectra obtained for cyclosporin A, indicated that the most significant shifts were registered for the signals due to the carbon and hydrogen atoms at the β position of the amino acid 1 (MeBmt). The largest *upfield* shift observed for this carbon atom amounted to more than 28 ppm (from 74.74 to 46.01 ppm). Furthermore, an *upfield* shift[10] of 0.35 ppm (from 3.83 to 3.48 ppm) was observed for the signal due to the hydrogen atom at-

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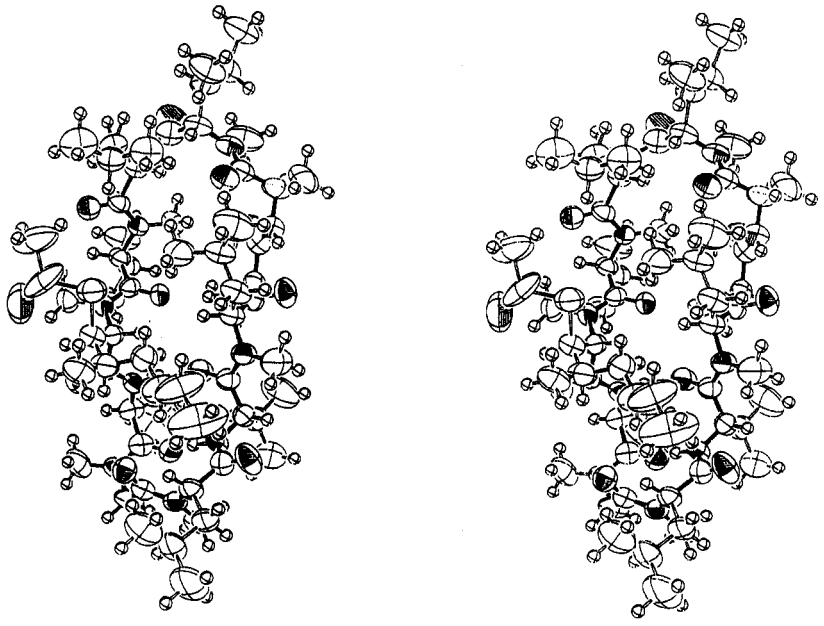

Figure 2. Stereo ORTEP diagram of compound **3**, showing the molecular conformation and thermal ellipsoids of the non hydrogen atoms. The bonds of the peptidic backbone are black filled; the *S*-acetyl is near the the middle left hand side of the structure.

tached to the same carbon atom. These observations led us to conclude that, in solution, the thiol **2** should have a conformation that closely resembles the conformation known to be the most stable for cyclosporin A (**1**) in solution[8] as well as in the solid state.[9] These similarities between educt and product were taken as an indication that this transformation might have taken place with retention of configuration. Additional work seemed to be appropriate.

In order to corroborate the absolute stereochemistry of the novel thiol **2**, it was decided to perform an X-ray analysis. Since satisfactory crystals could not be obtained from this material, the acetyl derivative **3** was prepared and crystals suitable for X-ray analysis were grown. The results of this study[12] secured in every detail the absolute stereochemistry of the thiol acetate **3** and therefore, that of the thiol **2**. The replacement of the secondary hydroxy group of cyclosporin A (**1**) by a sulfhydryl group, giving **2** with retention of configuration during the reaction with Lawesson's reagent was thus confirmed. During the preparation of this manuscript the transformation of (*S*)-(−)-1-phenylethanol to (*S*)-(−)-1-phenylethanethiol (with retention of configuration) in the presence of Lawesson's reagent was reported.[13]

The sulfhydryl-cyclosporin **2** turned out to be quite stable and was not susceptible to oxidative processes when exposed to air. It was stored without special precautions over an extended period of time. This is in sharp contrast to the instability of [D-cysteine]^8-cyclosporin[1b] when exposed to the same medium.

In a direct comparison[14] in our in vitro test models (Mishell-Dutton [MD], mixed lymphocyte reactions [MLR] and cyclophilin binding [Cyph]), the sulfhydryl compound **2** was estimated to be approximately 40 [MD, Cyph] to 100 [MLR] times less active than cyclosporin A (**1**).

Experimental Section

General. Thin layer chromatography (TLC) was carried out on glass plates coated with silica gel F-60 (E. Merck). The plates were developed in ethyl acetate saturated with water. For column chromatography silica gel 60 (230−400 mesh ASTM) E. Merck, Darmstadt, was used. High pressure liquid chromatography (HPLC) analyses were carried out on a Merck-Hitachi HPLC instrument using a RP-18 reverse phase column at 75 °C in combination with a UV detector for monitoring purposes at 204 nm. As mobile phase, mixtures of acetonitrile and water were used. The amount of water might vary between 15 and 40%. The aqueous phase contained 1 mL of 85% phosphoric acid per 3.7 L. Proton and ^{13}C magnetic resonance (NMR) spectra of the new compounds were obtained in deuterated chloroform solution on a Bruker-360 spectrometer and are recorded in δ values relative to TMS (tetramethylsilane) as internal standard. The corresponding values for cyclosporin A (2.70) were added for convenience. For the complete spectra of cyclosporin A, see ref 5b. Fast atom bombardment mass spectra (FAB MS) were measured on a VG 70-SE high resolution mass spectrometer. Melting points are not corrected.

[(2S,3R,4R,6E)-3-Sulhydryl-4-methyl-2-(methylamino)-6-octenoic acid]^1-cyclosporin (2). A mixture of cyclosporin A (**1**) (6 g, 5 mmol) and Lawesson's reagent[5] (1.2 g, 3 mmol) in toluene (150 mL) was heated to reflux for 30 min. The cold solution was diluted with ether, washed with water and then with brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give the crude product (7.0 g). This was chromatographed on silica gel with ethyl acetate, saturated with water, as eluent. One pure fraction (R_f = 0.27; 550 mg; yield 9%) was crystallized from ether/hexane; yield 200 mg (4%); mp 220−221 °C; m/z calcd for $C_{62}H_{111}N_{11}O_{11}S$: 1217.9, found: 1218.7 $[MH]^+$; $[\alpha_D] = -202.0°$ (c = 0.390 in MeOH); ^{1}H-NMR[15] **0.81 (0.73)**, 0.84 (0.85), 0.85 (0.85), 0.87 (0.89), 0.89 (0.85), 0.90 (0.97), 0.91 (0.81), 0.94 (0.93), 0.94 (0.97), 0.96 (1.01), 0.96 (0.93),

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(12) The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

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(14) We are indebted to Mr. P. Hiestand (MD, MLR) and Dr. Valerie Quesniaux (Cyph) for performing these tests.

(15) Chemical shifts assigned to amino acid 1 (MeBmt) are indicated in boldface.

0.97 (0.89), 0.99 (1.01), 1.10 (1.22), 1.24 (1.26), 1.25 (1.26), 1.34 (1.34), 1.34 (1.34), 1.35 (1.62), 1.37 (1.98), 1.45 (1.42), 1.45 (1.49), 1.56 (1.38), **1.59 (1.62)**, 1.64 (1.62), **1.77 (1.70)**, **1.80 (1.62)**, 1.87 (1.78), 1.88 (1.74), 1.97 (1.98), 2.00 (2.16), **2.08 (2.38)**, 2.13 (2.08), 2.16 (2.10), 2.45 (2.42), 2.68 (2.70), 2.69 (2.70), 3.08 (3.11), 3.18 (3.11), 3.18 (3.19), 3.23 (3.27), **3.36 (3.51)**, 3.47 (3.39), **3.48 (3.83)**, 4.43 (4.51), 4.63 (4.71), 4.71 (4.67), 4.85 (4.83), 4.88 (5.04), 5.10 (5.15), 5.10 (5.08), **5.25 (5.36)**, 5.26 (4.99), **5.31 (5.36)**, **5.33 (5.48)**, 5.41 (5.32), 5.68 (5.68), 7.22 (7.17), 7.56 (7.48), 7.78 (7.68), 8.29 (7.96); ^{13}C-NMR[15] 10.0 (9.93), 15.33 (16.07), **17.57 (17.96)**, 17.77 (18.19), **18.34 (16.76)**, 18.60 (18.48), 18.73 (18.75), 19.56 (19.81), 19.95 (20.26), 21.23 (21.18), 21.94 (21.86), 23.03 (21.93), 23.03 (23.38), 23.54 (23.49), 23.54 (23.85), 23.80 (23.87), 23.80 (23.74), 24.76 (24.55), 24.76 (24.70), 24.76 (24.90), 25.47 (25.06), 29.58 (29.65), 25.60 (25.40), 28.94 (29.05), 29.58 (29.81), 30.16 (29.83), 31.25 (31.17), 31.25 (31.32), 31.38 (31.53), **31.96 (33.97)**, **32.60 (35.99)**, **34.39 (35.63)**, 35.80 (35.99), 37.02 (37.41), 39.14 (39.04), 39.14 (39.40), 40.75 (40.73), 44.66 (45.20), **46.01 (74.74)**, 47.87 (48.30), 48.45 (48.69), 48.77 (48.86), 49.99 (50.37), 54.48 (55.31), 54.74 (55.39), 55.13 (55.51), 57.05 (57.54), 58.01 (57.93), **58.01 (58.75)**, **126.12 (126.32)**, **129.33 (129.68)**, 168.8–173.3 [11 C=O].

Thiol Acetate 3. The solution of thiol **2** (1.4 g, 1.1 mmol) in a mixture of pyridine (15 mL), acetic acid anhydride (15 mL) and 4-(dimethylamino)pyridine (60 mg, 0.5 mmol) was kept at room temperature overnight. The mixture was evaporated under reduced pressure and chromatographed on silica gel. Fractions containing pure product only (900 mg, 62%) were crystallized from ether; yield 690 mg (48%); mp 244–245 °C; *m/z* calcd for $C_{64}H_{113}N_{11}O_{12}S$: 1259.9; found: 1260.4 $[MH]^+$, 1284.8 $[M - CH_3COSH]^+$; $[\alpha_D] = -217.2°$ ($c = 0.267$ in MeOH); NMR (partial listing) 1.27, 1.33, 1.58, 2.36, 2.67, 2.68, 3.10, 3.15, 3.21, 3.23, 3.33, 7.28, 7.50, 7.87, 8.38.

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Supplementary Material Available: Copies of 360 MHz ^{1}H NMR spectra of **2** and **3** (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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