Peptide Coupling Reactions of the Mycotoxin Sporidesmin: Synthesis of Sporidesmin Hemisuccinates, X-ray Crystal Structure of Sporidesmin A 11-Hemisuccinate, and Its Coupling with N^{α}-*t*-Boc-L-lysine Methyl Ester

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Coupling reactions of the potent mycotoxin sporidesmin A were studied for the development of immunogenic protein conjugates. Acylation of sporidesmin D and sporidesmin A with benzoic anhydride in the presence of 4-(dimethylamino)pyridine and triethylamine led to the 10b,11-dibenzoates and 10b-benzoates. Succinoylation of sporidesmin A with succinic anhydride under similar conditions led to a mixture of the 10b,11-dihemisuccinate and the 10b- and 11-hemisuccinates. The structure of sporidesmin A 11-hemisuccinate was confirmed by X-ray crystallography. Sporidesmin A 11-hemisuccinate was coupled to N^{α}-*t*-Boc-lysine methyl ester in buffered aqueous dimethylformamide using tributylamine and isobutyl chloroformate activation.

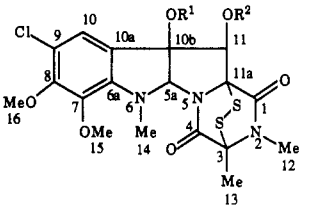
INTRODUCTION

Since the beginning of the century the New Zealand livestock industry has been troubled by a disease known as facial eczema (Mortimer and Ronaldson, 1983). Following many years of investigation it was shown (Thornton and Percival, 1959) that facial eczema was associated with a strain of the fungus *Pithomyces chartarum*, and from cultures of this organism an active substance named sporidesmin was isolated (Synge and White, 1959).

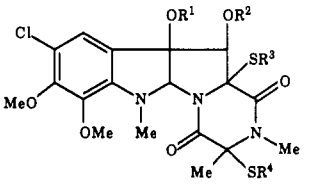
Chemical studies (Ronaldson et al., 1963; Hodges et al., 1963) and X-ray crystallographic analysis (Fridrichsons and Mathieson, 1965; Beecham et al., 1966) established that the substance now designated sporidesmin A (sdm A) was an aryl dithiodioxopiperazine derivative possessing structure **1a**.

In connection with immunological studies directed toward an ELISA for sdm A and immunophysiological control of facial eczema (Gallagher et al., 1987), we required procedures for the coupling of sdm A (**1a**) and some related analogues to amino acids, as model substrates for coupling to proteins.

Ronaldson (1978) has described an alkylation procedure that opened the disulfide bridge of **1a** to afford mixtures of secosporidesmin esters, including the methyl mercaptosecosporidesmin *S*-acetates **2a** and **2b**, which were in turn coupled in transacylation reactions with the ϵ-amino groups of poly(L-lysine), bovine serum albumin, and rabbit serum albumin. However, this procedure disrupted the comparatively reactive disulfide linkage and altered the molecular conformation of the dithiodioxopiperazine ring system. The sensitivity of sdm A and a number of other related mycotoxins to concomitant degradation during synthetic transformations (Ronaldson, 1978; Halder et al., 1980; Nagarajan, 1984) has been mainly attributed to the presence in these molecules of the disulfide linkage and the dithiodioxopiperazine ring system (Taylor, 1971; Munday, 1989). We considered it desirable for our immunochemical approach that this ring system should be retained intact in the coupled species.



(1a) $R^1 = R^2 = H$
(1b) $R^1 = COC_6H_5$, $R^2 = H$
(1c) $R^1 = COCH_2CH_2COOH$, $R^2 = H$
(1d) $R^1 = H$, $R^2 = COCH_2CH_2COOH$
(1e) $R^1 = R^2 = COCH_2CH_2COOH$
(1f) $R^1 = COCH_2CH_2COOMe$, $R^2 = H$
(1g) $R^1 = H$, $R^2 = COCH_2CH_2COOMe$
(1h) $R^1 = R^2 = COCH_2CH_2COOMe$
(1i) $R^1 = H$, $R^2 = COCH_2CH_2CONH(CH_2)_4CH(COOMe)NHCOOC(Me)_3$



(2a) $R^1 = R^2 = R^4 = H$, $R^3 = CH_2COOMe$
(2b) $R^1 = R^2 = R^3 = H$, $R^4 = CH_2COOMe$
(2c) $R^1 = R^2 = H$, $R^3 = R^4 = Me$
(2d) $R^1 = COC_6H_5$, $R^2 = H$, $R^3 = R^4 = Me$
(2e) $R^1 = R^2 = COC_6H_5$, $R^3 = R^4 = Me$

It might be expected that the secondary 11-hydroxyl group in sdm A (**1a**) would be more readily derivatized than the tertiary 10b-hydroxyl group. However, molecular models and X-ray crystallographic data reveal that in **1a** the hydrogen-bonded secondary 11-hydroxyl group is more sterically congested than the tertiary 10b-hydroxyl

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group. The upper face tertiary hydroxyl group is comparatively unhindered, other than for a single 1,3-pseudodiaxial interaction with the 11a-sulfur atom. Ronaldson et al. (1963) reported that acetylation of sdm A (**1a**) using acetic anhydride proceeds to afford the sdm A 10b,11-diacetate in high yield and that attempts to prepare a monoacetate were not productive. We anticipated that the use of a comparatively bulky reagent could lead to the stereoselective acylation of either the 10b- or the 11-hydroxyl groups and that this might provide a route to a variety of haptens.

We chose to investigate the reactions of sdm A (**1a**) and sdm D (**2c**) with benzoic anhydride, as a model for acylation. We also report the results of a succinoylation procedure leading to hemisuccinates suitable for coupling primarily with the lysine ϵ-amino groups of proteins or peptides, without disruption of the disulfide linkage and the dithiodioxopiperazine ring system of the parent mycotoxin. Finally, the model coupling of the hemisuccinate adduct with N^{α}-*t*-Boc-protected lysine was studied.

MATERIALS AND METHODS

Spectroscopy. NMR spectra in $CDCl_3$ were determined at 89.6 MHz (^1H) and at 22.5 MHz (^{13}C) using a JEOL FX-90Q spectrometer. Chemical shifts are reported relative to TMS. The multiplicities of ^{13}C NMR signals (s, t, d, or q) were established using the INEPT and/or SEFT sequences. The two-dimensional double quantum filtered COSY spectrum of sdm A was determined at 300 MHz on a Bruker AC-300 instrument.

Low-resolution electron impact (EI) mass spectra were determined on a Varian MAT CH-5 or a Kratos MS80RFA instrument. High-resolution mass measurements were made using the Kratos instrument. Sample introduction was by direct probe, source temperature 250 °C. Liquid secondary ion mass spectra (LSIMS) were recorded on the Kratos instrument using a xenon fast atom beam and a thioglycerol matrix. For ions containing Cl, only the ^{35}Cl ion is given, although in all cases appropriate ^{37}Cl isotope containing ions were observed.

Chromatography. Thin-layer chromatography (TLC) was conducted on Merck Kieselgel 60 F254 Art. 5554 or 5729 plates which were visualized under a UV lamp at 254 nm.

Analytical and semipreparative high-pressure liquid chromatographies (HPLC) were performed using Waters 6600 pumps with a gradient solvent programmer and a Perkin-Elmer LC 75 UV detector, coupled to a Waters RCM-500 unit with a silica Radpac column or an RCM C_{18} column. Preparative HPLC was carried out using a Waters Prep-500 system and a silica cartridge.

Reagents. The purities of benzoic and succinic anhydrides (Sigma) were confirmed by TLC and IR. N^{α}-*t*-Boc-N^{ϵ}-benzyloxycarbonyl-L-lysine was obtained from Beckman Instruments Co.

Benzoylation of Sdm D (2c) and Sdm A (1a). A solution of benzoic anhydride (58 mg, 257 μmol), sdm D (**2c**) (23 mg, 46 μmol), and 4-(dimethylamino)pyridine (DMAP) (10 mg) in triethylamine (70 μL) and dichloromethane (0.5 mL) was stirred for 2 h at room temperature, during which time the reaction was followed by HPLC analysis of aliquots withdrawn from the reaction mixture on a Waters C_{18} column using CH_3OH–H_2O as eluent (4:1). Benzoic acid eluted first followed by sdm D, benzoic anhydride, and two slower peaks. The reaction mixture was poured into $CHCl_3$ (50 mL) and washed with 0.2 M HCl (3 × 50 mL). Evaporation of $CHCl_3$ afforded material which when purified by semipreparative HPLC afforded sdm D 10b,11-dibenzoate (**2e**) (22 mg).

The reaction was repeated with less benzoic anhydride (13 mg, 58 μmol). These conditions afforded unchanged sdm D (**2c**) (18 mg), sdm D 10b-benzoate (**2d**) (5 mg), and sdm D 10b,11-dibenzoate (1 mg).

Sdm D (**2c**): ^1H NMR (90 MHz) 1.86 (s, Me), 2.32 (s, SMe), 2.40 (s, SMe), 3.06 (s, NMe), 3.36 (s, NMe), 3.79 (s, OMe), 3.86 (s, OMe), 4.65 (s), 5.30 (s), 7.08 (s, ArH); ^{13}C NMR (22.6 MHz) 14.6 (q), 15.4 (q), 25.1 (q), 28.9 (q), 41.4 (q), 60.4 (q), 60.9 (q), 68.7 (s), 72.6 (s), 80.1 (d), 89.5 (s), 95.7 (d), 119.8 (s), 120.6 (d), 124.3 (s), 140.6 (s), 145.5 (s), 163.9 (s), 166.9 (s).

Sdm D 10b-benzoate (**2d**), a waxy solid: mp 84–88 °C; ^1H NMR (90 MHz) 1.86 (s, Me), 2.31 (s, SMe), 2.41 (s, SMe), 3.09 (s, NMe), 3.50 (s, NMe), 3.83 (s, OMe), 3.90 (s, OMe), 5.14 (s), 5.85 (s), 7.07 (s, ArH), 7.54 (m, 3 × ArH), 8.07 (m, 2 × ArH); MS (EI, 30 eV) *m/z* 77 (34), 105 (100), 122 (10), 240 (28), 345 (34), 375 (5.1), 438 (2.8), 559 (3.5), 607 (11, M^+). (Found *m/z* 607.1203; $C_{27}H_{30}O_7N_3ClS_2$ requires 607.1214.)

Sdm D 10b,11-dibenzoate (**2e**), a waxy solid: mp 66–69 °C; ^1H NMR (90 MHz) 1.88 (s, Me), 2.29 (s, SMe), 2.58 (s, SMe), 3.01 (s, NMe) 3.53 (s, NMe), 3.59 (s, OMe), 3.81 (s, OMe), 6.05 (s), 6.57 (s), 6.80 (s, ArH), 7.58 (m, 6 × ArH), 8.06 (m, 2 × ArH); ^{13}C NMR (22.6 MHz) 14.6 (q), 15.6 (q), 26.1 (q), 28.8 (q), 41.0 (q), 60.0 (q), 60.8 (q), 70.0 (s), 77.3 (s), 77.5 (d), 91.7 (d), 93.3 (s), 119.0 (d), 119.9 (s), 122.4 (s), 128.2 (2 × d), 128.7 (2 × d), 128.9 (s), 129.1 (2 × d), 129.5 (s), 129.8 (2 × d), 133.3 (d), 133.8 (d), 140.7 (s), 146.2 (s), 151.4 (s), 162.6 (s), 163.9 (s), 164.7 (s), 166.1 (s); MS (EI, 30 eV) *m/z* 252 (67), 373 (100), 390 (45), 494 (11), 542 (10), 663 (44), 711 (37, M^+). (Found *m/z* 711.1457; $C_{34}H_{34}O_8N_3ClS_2$ requires 711.1476.)

Benzoylation of sdm A (**1a**) (23 mg, 49 μmol), with benzoic anhydride (13 mg, 58 μmol), followed by semipreparative HPLC as described above, afforded sdm A 10b-benzoate (**1b**) (9 mg), a waxy solid: mp 126–129 °C; ^1H NMR (90 MHz) 2.06 (s, Me), 3.09 (s, NMe), 3.46 (s, NMe), 3.87 (s, OMe), 3.90 (s, OMe), 5.08 (s, H-11), 5.09 (s, 11-OH), 5.94 (s, H-5a), 7.04 (s, ArH), 7.50 (m, 3 × ArH), 8.04 (m, 2 × ArH); ^{13}C NMR (22.6 MHz) 18.1 (q), 27.2 (q), 39.2 (q), 60.7 (q), 60.9 (q) 73.7 (s), 75.9 (s), 80.5 (d), 91.4 (d), 94.7 (s), 119.6 (s), 120.1 (d), 121.4 (s), 128.7 (2 × d), 129.4 (s), 129.9 (2 × d), 133.9 (d), 140.5 (s), 146.1 (s), 151.8 (s), 164.3 (s), 164.8 (s), 166.2 (s); MS (EI, 30 eV) *m/z* 77 (29), 105 (100), 210 (11), 240 (41), 345 (69), 391 (2.1), 513 (1.5), 577 (1.8, M^+). (Found *m/z* 577.0740; $C_{25}H_{24}O_7N_3ClS_2$ requires 577.0744.)

Succinoylation of Sdm A (1a). A solution of succinic anhydride (640 mg, 6.34 mmol), sdm A (**1a**) (1000 mg, 2.11 mmol), and DMAP (400 mg) in triethylamine (2 mL) and dichloromethane (20 mL) was stirred for 30 min at room temperature, during which time the progress of the reaction was followed by TLC analysis of aliquots withdrawn from the reaction mixture. TLC analysis with EtOAc–$CHCl_3$–HOAc (12:8:1) as eluent indicated the partial conversion of sdm A into two lower R_f products. The reaction mixture was poured into $CHCl_3$ (300 mL) and washed with 0.2 M HCl (3 × 500 mL). Evaporation of the $CHCl_3$ afforded material (1050 mg) that was purified by preparative HPLC on a silica column using a six-step gradient using CH_3CN–CH_2Cl_2–HOAc mixtures (4:96:1, 1.5 L, 6:94:1, 1 L; 7:93:1, 1 L; 10:90:1, 1 L; 13:87:1, 1 L; and 16:84:1, 1 L). Three fractions were obtained: unchanged sdm A (**1a**) (693 mg), sdm A 10b-hemisuccinate (**1c**) (76 mg), and sdm A 11-hemisuccinate (**1d**) (166 mg). Repetition of the reaction using sdm A (**1a**) (25 mg, 53 μmol), DMAP (5.4 mg), DCM (500 μL), and TEA (50 μL) with a greater molar excess of succinic anhydride (67 mg, 670 μmol) for 4 h, followed by HPLC purification as described above, gave sdm A 10b,11-dihemisuccinate (**1e**) (29 mg). NMR and mass spectral characterization of the hemisuccinates used the corresponding methyl esters, which were prepared by methylation with an ethereal solution of diazomethane.

Sdm A 10b-hemisuccinate, mp 116–118 °C, was a glassy solid. Sdm A 10b-hemisuccinate methyl ester (**1f**): ^1H NMR (90 MHz) 2.05 (s, Me), 2.67 (s, 4 H, 2 × CH_2), 3.06 (s, NMe), 3.37 (s, NMe), 3.68 (s, COOMe), 3.82 (s, OMe), 3.88 (s, OMe), 4.87 (s, H-11), 5.01 (s, 11-OH), 5.74 (s, H-5a), 7.02 (s, Ar H); MS (EI 30 eV) *m/z* 101 (12), 115 (10), 168 (15), 226 (34), 241 (100), 355 (20), 456 (0.9), 523 (2.1), 587 (1.2, M^+). (Found *m/z* 587.0763; $C_{23}H_{26}O_9N_3ClS_2$ requires 587.0799.)

Sdm A 11-hemisuccinate was a glassy solid, mp 164–166 °C (with decomposition). Sdm A 11-hemisuccinate methyl ester (**1g**): ^1H NMR (90 MHz) 2.07 (s, Me), 2.26 and 2.32 (m, 4 H, 2 × CH_2), 3.04 (s, NMe), 3.25 (s, 10b-OH), 3.35 (s, NMe), 3.63 (s, COOMe), 3.83 (s, OMe), 3.89 (s, OMe), 5.38 (s, H-11), 5.91 (s, H-5a), 6.96 (s, Ar H); MS (EI 30 eV) *m/z* 101 (100), 115 (40), 159 (50), 192 (55), 241 (30), 279 (24), 391 (47), 409 (3.8), 523 (0.9), 587 (0.5, M^+). (Found *m/z* 587.0730; $C_{23}H_{26}O_9N_3ClS_2$ requires 587.0799.)

Sdm A 10b,11-dihemisuccinate dimethyl ester (**1h**), a glassy solid: ^{1}H NMR (90 MHz) 2.06 (s, Me), 2.26 and 2.32 (m, 4 H, 2 × CH_2), 2.69 (s, 4 H, 2 × CH_2), 3.03 (s, NMe), 3.40 (s, NMe), 3.64 (s, COOMe), 3.69 (s, COOMe) 3.85 (s, OMe), 3.89 (s, OMe), 5.81 (s), 6.13 (s), 6.81 (s, Ar H); MS (EI 30 eV) *m/z* 101 (100), 115 (30), 160 (20), 192 (18), 256 (20), 373 (15), 506 (1), 637 (0.7), 701 (0.1, M^+). (Found *m/z* 701.1076; $C_{28}H_{32}O_{12}N_3ClS_2$ requires 701.1116.)

Preparation of N^α-*tert*-Butyloxycarbonyllysine Methyl Ester. N^α-*t*-Boc-N^ϵ-benzyloxycarbonyl(Cbz)-L-lysine was methylated according to the procedure of Slotin et al. (1977). The Cbz group was reduced at 25 °C using 10% Pd on carbon with cyclohexadiene in acetic acid (Felix et al., 1978) to yield N^α-*t*-Boc-lysine methyl ester: ^{1}H NMR (90 MHz) 1.38 (s, 3 × Me), 3.69 (s, COOMe), 9.0 (br m, 3 × NH); ^{13}C NMR (22.6 MHz) 22.3 (t), 27.1 (t), 28.3 (3 × t), 31.6 (t), 37.0 (t), 52.2 (q), 53.4 (d), 80.0 (s), 155.7 (s), 173.2 (s).

Coupling of the Hemisuccinate (1c) with N^α-*t*-Boc-lysine Methyl Ester. A solution of sdm A 11-hemisuccinate (**1d**) (40 mg) in dry dimethylformamide (1.5 mL), tributylamine (40 μL), and isobutyl chloroformate (16 μL) was stirred for 20 min at 4 °C. The solution was then slowly added to a stirred solution of N^α-*t*-Boc-lysine methyl ester (40 mg) in dimethylformamide (0.5 mL) and an aqueous sodium phosphate buffer (0.2 M, pH 9, 2 mL). After 3 h, the reaction mixture was extracted with chloroform (50 mL). After washing with 0.2 M HCl (3 × 50 mL), the chloroform extract afforded material which when separated by semipreparative HPLC on a Waters silica Radpak column using CH_2Cl_2–CH_3CN–HOAc (16:4:1) as eluent gave unreacted sdm A 11-hemisuccinate (**1d**) (12 mg) and sdm A 11-hemisuccinate N^α-*t*-Boc-lysine methyl ester (**1i**) (29 mg), a glassy solid: mp 78–80 °C; ^{1}H NMR (90 MHz) 1.40 (br s, 3 × Me), 1.2–2.4 (m, 6 × CH_2), 3.01 (Me), 3.30 (Me), 3.73 (2 × Me), 3.78 (Me), 3.85 (Me), 5.40 (s, 11-H), 7.00 (s, Ar H); ^{13}C NMR (22.6 MHz) 18.5 (q), 22.6 (t), 27.3 (q), 28.3 (3 × q), 28.8 (t), 29.7 (t), 31.2 (t), 32.4 (t), 39.0 (t), 39.3 (t), 52.3 (q), 53.3 (d), 60.9 (q), 61.0 (q), 74.6 (s), 76.1 (s), 78.9 (d), 89.4 (s), 94.2 (d), 118.9 (s), 119.9 (d), 124.1 (s), 140.1 (s), 144.4 (s), 151.7 (s), 155.6 (s), 163.4 (s), 163.8 (s), 170.5 (s), 171.1 (s), 173.2 (s); MS (LSIMS) *m/z* 240 (100), 241 (60), 287 (71), 343 (18), 392 (60), 652 (0.71), 684 (0.51), 716 (0.88), 816 (0.57) (MH^+).

X-ray Crystal Structure of Sdm A 11-Hemisuccinate (1d). Suitable crystals of sdm A 11-hemisuccinate (**1d**) were obtained by crystallization from hexane–ethyl acetate. A needle-like crystal ca. 0.4 × 0.1 × 0.1 mm was used on a Nicolet XRD P3 diffractometer with Mo Kα radiation (λ = 0.71069 Å). Crystal data: $C_{22}H_{24}ClN_3O_9S_2$, M = 574.032, orthorhombic, space group $P2_12_12_1$, a = 9.195(3), b = 14.214(4), c = 20.826(7) Å, U = 2721.9 Å^3 (from 25 unique high-angle reflections), Z = 4, D_c = 1.40 g cm^3, $F(000)$ = 1192 electrons, μ(Mo Kα) = 3.4 cm^{-1}, T = 150 K.

A total of 3530 unique reflections were collected using a θ–2θ scan technique, in the range $0 < 2\theta < 55°$. After data reduction, this afforded 2968 reflections with $I > 2\sigma(I)$. No absorption corrections were deemed necessary. The structure was solved by direct methods using the MULTAN-78 program package (Main et al., 1978); this revealed the majority of the heavy atoms, while subsequent difference maps generated using the SHELX-76 program package (Sheldrick, 1976) located the remaining heavy atoms. In the final cycles of full matrix least-squares refinement the sdm A ring carbon and nitrogen atoms, except C(1) and N(2), were assigned isotropic temperature factors, other heavy atoms were refined anisotropically, and the hydrogen atoms, except those of the succinate group, were included in calculated positions with a common temperature applied for each type (CH, CH_2, or CH_3). The succinate side chain was found to be disordered from C(19) outward and was refined with tied occupancy factors for C(19)/C(19′), C(20)/C(20′), O(20)/O(20′), and O(20a)/O(20′a) which converged to 0.62/0.38 for the major/minor orientations, respectively. The comparatively large U_{11} value (0.15) determined for C(18), and the unrealistically long C(18)–C(19′) bond (1.81 Å) indicated that C(18) was also disordered; however, the structure refinement did not satisfactorily differentiate between the major/minor positional coordinates of this atom. Convergence gave a final R = 0.067, R_w = 0.070 for the weighting scheme $w = [\sigma^2(F_o) + 0.001F_o^2]^{-1}$. Refined atom coordinates, bond lengths, and bond angles are given in Tables I, II, and III, respectively. Tables of heavy atom thermal parameters, calculated hydrogen atoms positions, and F_o/F_c data have been deposited as supplementary material.

Figure 1 is a perspective view of the adduct, showing the crystallographic numbering scheme. The absolute configuration shown in Figure 1 was assumed by comparison (Beecham

Table I. Fractional Coordinates of Heavy Atoms of Sporidesmin A 11-Hemisuccinate

	x/a	*y/b*	*z/c*	sof[a]
C(1)	0.3205 (6)	0.6915 (4)	0.2601 (3)	
C(3)	0.1183 (7)	0.5965 (4)	0.2922 (3)	
C(4)	0.1737 (6)	0.5121 (4)	0.2530 (2)	
C(5a)	0.4128 (5)	0.4436 (3)	0.2171 (2)	
C(6a)	0.5375 (5)	0.4267 (3)	0.1213 (2)	
C(7)	0.5762 (6)	0.4063 (4)	0.0576 (2)	
C(8)	0.7219 (6)	0.4202 (4)	0.0391 (3)	
C(9)	0.8233 (6)	0.4533 (4)	0.0838 (3)	
C(10)	0.7862 (6)	0.4740 (4)	0.1460 (3)	
C(10a)	0.6411 (6)	0.4612 (4)	0.1642 (3)	
C(10b)	0.5699 (5)	0.4830 (3)	0.2266 (2)	
C(11)	0.5481 (5)	0.5907 (3)	0.2389 (2)	
C(11a)	0.3975 (6)	0.5987 (3)	0.2681 (2)	
C(12)	0.0875 (7)	0.7714 (4)	0.2754 (2)	
C(13)	−0.0476 (7)	0.5928 (4)	0.2994 (4)	
C(14)	0.3081 (6)	0.3378 (4)	0.1343 (3)	
C(15)	0.4940 (9)	0.2800 (4)	−0.0074 (3)	
C(16)	0.748 (1)	0.4895 (6)	−0.0612 (4)	
C(17)	0.6422 (8)	0.7090 (4)	0.1713 (3)	
C(18)	0.629 (2)	0.7562 (6)	0.1061 (5)	
C(19)	0.761 (2)	0.8271 (7)	0.1000 (6)	0.62
C(19′)	0.468 (2)	0.715 (2)	0.0647 (9)	0.38
C(20)	0.913 (2)	0.7903 (9)	0.1009 (6)	0.62
C(20′)	0.315 (2)	0.730 (2)	0.0872 (8)	0.38
N(2)	0.1727 (5)	0.6848 (3)	0.2693 (3)	
N(5)	0.3185 (5)	0.5210 (3)	0.2390 (2)	
N(6)	0.3977 (5)	0.4215 (3)	0.1486 (2)	
O(1)	0.3866 (4)	0.7639 (3)	0.2493 (3)	
O(4)	0.0983 (4)	0.4470 (2)	0.2365 (2)	
O(7)	0.4760 (4)	0.3766 (3)	0.0136 (2)	
O(8)	0.7615 (5)	0.4043 (3)	−0.0238 (2)	
O(10b)	0.6414 (4)	0.4499 (2)	0.2823 (2)	
O(11)	0.5455 (4)	0.6379 (3)	0.1783 (2)	
O(17)	0.7315 (6)	0.7308 (3)	0.2102 (3)	
O(20)	1.009 (1)	0.8495 (6)	0.1194 (6)	0.62
O(20′)	1.233 (2)	0.663 (1)	0.0597 (7)	0.38
O(20a)	0.947 (1)	0.7099 (7)	0.0861 (6)	0.62
O(20′a)	0.280 (1)	0.7901 (9)	0.1209 (6)	0.38
Cl(9)	1.0043 (2)	0.4657 (1)	0.0591 (1)	
S(3)	0.1909 (2)	0.5729 (1)	0.3756 (1)	
S(11a)	0.4124 (2)	0.5874 (1)	0.3575 (1)	

[a] Site occupancy factor for disordered atoms.

Table II. Bond Lengths (Angstroms) for Sporidesmin A 11-Hemisuccinate

C(1)–C(11a)	1.506 (7)	C(10b)–C(11)	1.564 (7)
C(1)–N(2)	1.376 (7)	C(10b)–O(10b)	1.414 (6)
C(1)–O(1)	1.217 (7)	C(11)–C(11a)	1.518 (7)
C(3)–C(4)	1.538 (8)	C(11)–O(11)	1.430 (6)
C(3)–C(13)	1.533 (8)	C(11a)–N(5)	1.454 (6)
C(3)–N(2)	1.433 (7)	C(11a)–S(11a)	1.875 (5)
C(3)–S(3)	1.890 (6)	C(12)–N(2)	1.465 (7)
C(4)–N(5)	1.369 (7)	C(14)–N(6)	1.477 (6)
C(4)–O(4)	1.207 (6)	C(15)–O(15)	1.450 (7)
C(5a)–C(10b)	1.562 (7)	C(16)–O(16)	1.445 (8)
C(5a)–N(5)	1.474 (6)	C(17)–C(18)	1.52 (1)
C(5a)–N(6)	1.467 (6)	C(17)–O(11)	1.354 (8)
C(6a)–C(7)	1.405 (7)	C(17)–O(17)	1.195 (9)
C(6)–C(10a)	1.394 (7)	C(18)–C(19)	1.58 (2)
C(6a)–N(6)	1.408 (6)	C(19)–C(20)	1.49 (2)
C(7)–C(8)	1.407 (8)	C(20)–O(22)	1.28 (2)
C(7)–O(7)	1.367 (6)	C(20)–O(22a)	1.23 (2)
C(8)–C(9)	1.400 (8)	S(3)–S(11a)	2.082 (2)
C(8)–O(16)	1.379 (7)	C(18)–C(19′)	1.81 (3)
C(9)–C(10)	1.372 (8)	C(19′)–C(20′)	1.50 (3)
C(9)–Cl(9)	1.750 (6)	C(20′)–O(20′)	1.34 (2)
C(10)–C(10a)	1.398 (7)	C(20′)–O(20′a)	1.15 (2)
C(10a)–C(10b)	1.489 (7)		

Table III. Bond Angles (Degrees) for Sporidesmin A 11-Hemisuccinate

C(11a)-C(1)-O(1)	121.8 (5)	C(11)-C(10b)-O(11)	104.6 (4)
C(11a)-C(1)-N(2)	112.8 (5)	C(10b)-C(11)-C(11a)	104.8 (4)
N(2)-C(1)-O(1)	125.3 (5)	C(10b)-C(11)-O(11)	108.6 (4)
C(4)-C(3)-C(13)	110.8 (5)	C(11a)-C(11)-O(11)	107.7 (4)
C(4)-C(3)-N(2)	113.0 (5)	C(1)-C(11a)-C(11)	116.7 (4)
C(13)-C(3)-N(2)	114.2 (5)	C(11)-C(11a)-N(5)	103.4 (4)
S(3)-C(3)-C(4)	103.5 (4)	C(1)-C(11a)-S(11a)	102.7 (4)
S(3)-C(3)-C(13)	104.8 (5)	C(11)-C(11a)-S(11a)	109.0 (3)
S(3)-C(3)-N(2)	109.7 (4)	S(11a)-C(11a)-N(5)	112.7 (3)
C(3)-C(4)-N(5)	111.3 (5)	C(18)-C(17)-O(11)	112.0 (7)
C(3)-C(4)-O(4)	124.0 (5)	C(18)-C(17)-O(17)	123.2 (7)
N(5)-C(4)-O(4)	124.7 (5)	O(11)-C(17)-O(17)	124.8 (7)
C(10b)-C(5a)-N(5)	103.7 (4)	C(17)-C(18)-C(19)	107.0 (9)
C(10b)-C(5a)-N(6)	106.8 (4)	C(18)-C(19)-C(20)	119.6 (9)
N(5)-C(5a)-N(6)	114.0 (4)	C(19)-C(20)-O(20)	114 (1)
C(7)-C(6a)-N(6)	127.0 (4)	C(19)-C(20)-O(20a)	124 (1)
C(10a)-C(6a)-C(7)	120.3 (5)	O(22)-C(20)-O(20a)	120 (1)
C(10a)-C(6a)-N(6)	112.6 (4)	C(1)-N(2)-C(3)	116.9 (5)
C(6a)-C(7)-C(8)	118.1 (5)	C(1)-N(2)-C(12)	118.8 (5)
C(6a)-C(7)-O(15)	121.8 (5)	C(3)-N(2)-C(12)	121.4 (5)
C(8)-C(7)-O(15)	120.1 (5)	C(4)-N(5)-C(5a)	124.7 (4)
C(7)-C(8)-C(9)	120.0 (5)	C(4)-N(5)-C(11a)	117.9 (4)
C(7)-C(8)-O(16)	119.2 (5)	C(5a)-N(5)-C(11a)	113.8 (4)
C(9)-C(8)-O(16)	120.7 (5)	C(5a)-N(6)-C(6a)	107.1 (4)
C(8)-C(9)-C(10)	122.3 (5)	C(5a)-N(6)-C(14)	114.9 (4)
C(8)-C(9)-Cl(9)	118.2 (4)	C(6a)-N(6)-C(14)	118.1 (4)
C(10)-C(9)-Cl(9)	119.5 (4)	C(11)-O(11)-C(17)	115.8 (4)
C(9)-C(10)-C(10a)	117.7 (5)	C(7)-O(15)-C(15)	114.7 (5)
C(6a)-C(10a)-C(10)	121.7 (5)	C(8)-O(16)-C(16)	110.6 (5)
C(6a)-C(10a)-C(10b)	109.4 (4)	S(3)-S(11a)-C(11a)	96.7 (2)
C(10)-C(10a)-C(10b)	128.9 (5)	S(11a)-S(3)-C(3)	99.3 (2)
C(5a)-C(10b)-C(10a)	102.8 (4)	C(17)-C(18)-C(19')	110.3 (9)
C(5a)-C(10b)-C(11)	104.7 (4)	C(18)-C(19')-C(20')	124 (2)
C(5a)-C(10b)-O(10b)	114.5 (4)	C(19')-C(20')-O(20')	106 (2)
C(10a)-C(10b)-O(10b)	116.3 (4)	C(19')-C(20')-O(20'a)	124 (2)
C(10a)-C(10b)-C(11)	113.7 (4)	O(20')-C(20')-O(20'a)	128 (2)

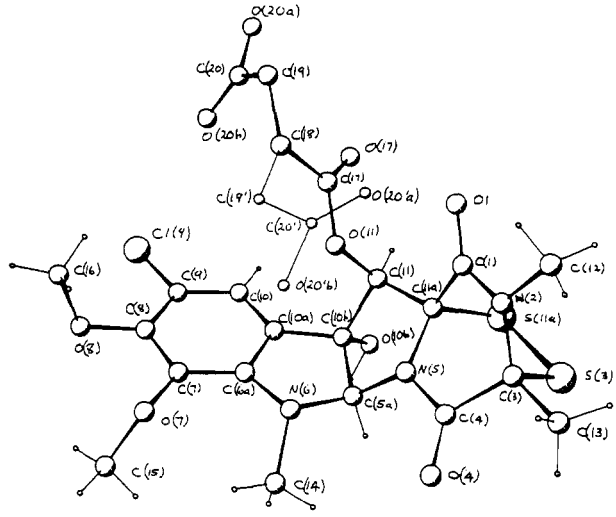
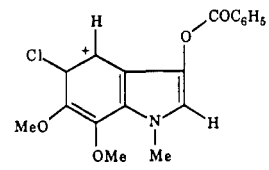


Figure 1. Perspective view of sporidesmin A 11-hemisuccinate, showing the atom numbering scheme. Disordered side-chain atoms are also shown.

et al., 1966) with those of an earlier assignment of sdm derivatives, since refinement of the enantiomorph did not lead to a significantly different R value.

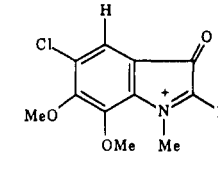
DISCUSSION

Because of the greater stability of sdm D in comparison to that of sdm A (in the former substance the comparatively reactive disulfide linkage has been stabilized by cleavage and alkylation), we chose to use sdm D for our initial investigation of the esterification reactions of the 10b- and 11-hydroxyl groups of sdm-type molecules. Thus, we observed that benzoylation of sdm D using benzoic anhydride did not proceed in the absence of a catalyst; however, in the presence of DMAP the reaction proceeded cleanly and without difficulty to afford the dibenzoate (**2e**). After a reduction in the molar ratio of benzoic anhydride to sdm D in the reaction, a monobenzoate was obtained. The mass spectrum of the monobenzoate included ions of m/z 345 and 240 for which structures a and b, respectively, are proposed, indicative of 10b substitution. Accordingly, the monobenzoate was assigned structure **2d**.



m/z 345

ion a

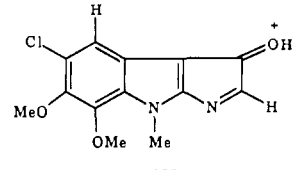


m/z 240

ion b

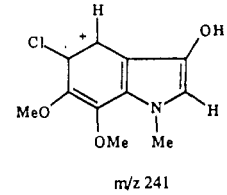
Reaction of sdm A under similar reduced-molar-ratio conditions also afforded a monobenzoate, which was assigned structure **1b**. Ions of m/z 345 and 240, also indicative of 10b substitution, appeared in the mass spectrum of this compound, while the ^{1}H NMR spectrum of the monobenzoate included a signal (5.09 ppm) attributable to the hydroxyl proton of the 11-OH group. In sdm A the equivalent 11-OH signal (a weakly coupled doublet, J = 0.8 Hz), occurred at 4.87 ppm, while the 10b-OH signal (a broadened singlet, $W_{1/2}$ = 3.5 Hz) occurred at 3.17 ppm. The two-dimensional double quantum filtered COSY spectrum of sdm A confirmed that H-11 was coupled to 11-OH and H-11 was also ^4J coupled to H-5a.

Encouraged by the stereoselective reaction of the 10b-hydroxyl group of sdm D and sdm A using a bulky derivatization reagent, we then investigated the succinoylation of sdm A. As with the benzoates, derivatization of sdm A (**1a**) with succinic anhydride did not proceed in the absence of a catalyst; however, in the presence of DMAP and an excess of succinic anhydride, mainly disubstitution was observed. Monosubstitution was achieved by limiting the reaction time and the amount of succinic anhydride. This afforded a mixture of unchanged sdm A and two hemisuccinates, which were separated by preparative HPLC on a silica column. The methyl ester of the first eluting hemisuccinate gave a ^{1}H NMR which included a signal attributable to the hydroxyl proton of the 11-OH group (5.01 ppm). The mass spectrum included a prominent ion at m/z 355 which is attributed to the fragment from ring C cleavage analogous to ion a from the 10b benzoate. A weaker ion at m/z 279 has the proposed structure c. These observations are consistent with the



m/z 279

ion c



m/z 241

ion d

assignment of structure **1c**, corresponding to 10b-succinyl substituted sdm A, to the first eluting hemisuccinate. On the other hand, the ^{1}H NMR spectrum of the second eluting isomer included a signal at 3.25 ppm attributable to the hydroxyl proton of the 10b-OH group. The base peak

in the mass spectrum was m/z 101, protonated succinic anhydride, arising by intramolecular ion rearrangement. Elimination of monomethyl succinic acid leads to the strong peak at m/z 391. The mass spectrum included an ion of m/z 241 for which structure d has been proposed for the analogous ion in the mass spectrum of sdm A and related compounds possessing an unsubstituted 10b-hydroxyl group (Shannon, 1963). The occurrence of an ion of m/z 241 in the spectrum of the 10b-substituted isomer can be attributed to intramolecular elimination of COC_2H_4-$COOCH_2$ from the ion of m/z 355. These observations are consistent with the assignment of structure **1d** to the second eluting hemisuccinate.

These conclusions were unequivocally confirmed by an X-ray crystallographic analysis of the second eluting hemisuccinate. Solution of the X-ray crystallographic data set as described under Materials and Methods established the major hemisuccinate to be 11-substituted. Disorder was observed in the orientation of the hemisuccinate side chain; Figure 1 depicts the conformation established for the major (62%) conformer. Heavy atom coordinates appear in Table I, while bond lengths and bond angles appear in Tables II and III, respectively.

Molecules of sdm A 11-hemisuccinate pack together with hydrogen bonding between adjacent molecules via the tertiary 10b-hydroxyl group and the oxygen of the C-2 keto group (O-2). The absence of hydrogen-bonding interactions involving the terminal carbonyl group of the succinate moiety explains the ease with which disorder can occur in this side chain. The geometry of the sdm A segment of the molecule is very similar to that found in an early X-ray analysis (Fridrichsons and Mathieson, 1965), the only significant difference being the transoid configuration of the two aryl methoxyl groups found in the present study, compared to the cisoid arrangement observed in the original study. Otherwise, bond angles and bond lengths agree surprisingly well with those determined in the previous low-resolution analyses and with those reported by Przybylska and Gopalakrishna (1974) for sdm G.

The 11-hemisuccinate (**1d**) was reacted with N^{α}-*t*-Boc-lysine methyl ester to yield the adduct **1i**. Adduct formation was initially demonstrated by TLC (single non-polar product spot possessing an R_f value greater than that of sdm A) with a positive ninhydrin reaction (peptide link). Subsequently, ^1H and ^{13}C NMR and mass spectral data verified adduct formation. Thus, the ^1H and ^{13}C NMR spectrum of **1i** included signals attributable to an intact sdm A skeleton, a hemisuccinate entity, and an N^{α}-*t*-Boc-lysine group. The LSIMS mass spectrum of the adduct gave a weak ion pair at m/z 816/818, consistent with the protonated molecular ion of the adduct **1i**. Further high mass ions corresponded to loss of *t*-Boc (m/z 716) and subsequent loss of the disulfide bridge (m/z 684, 652). Lower mass fragments (m/z 392, 241, 240) confirmed the presence of a sdm A nucleus. Ions were also observed for the cleavage at the side-chain carbonyls (m/z 343, succinyl-N^{α}-*t*-Boc-lysine methyl ester; m/z 287, N^{α}-*t*-Boc-lysine methyl ester).

CONCLUSIONS

Suitable mild benzoylation conditions using sdm D as a model for sdm A have been optimized and then subsequently applied to sdm A benzoylation. This approach avoided side reactions, and acylation using the bulky benzoic anhydride molecule resulted in the largely sequential regioselective acylation of the 10b-hydroxyl followed by 11-hydroxyl groups of sdm A and sdm D. It was unnecessary to use this strategy to produce haptens resulting from both 10b and 11 acylations as succinoylation of sdm A resulted in an approximate balance between the expected greater chemical reactivity of the secondary 11-hydroxyl group over the tertiary 10b-hydroxyl group and the greater accessibility to succinic anhydride of the 10b-hydroxyl over the 11-hydroxyl group. Thus, two desirable acylations were obtained by using reaction conditions that limited production of the dihemisuccinate.

The sdm A 11-hemisuccinate hapten has been coupled to the ϵ-amino group of N^{α}-*t*-Boc-lysine methyl ester, by the mixed anhydride method, under a normal set of protein coupling conditions for hydrophobic haptens, without any accompanying loss of integrity in the sdm A moiety, thus opening routes to protein coupling and immunological studies.

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Supplementary Material Available: Further data for the X-ray crystal structure including a table of thermal parameters, a table of fractional coordinates and thermal parameters for hydrogen atoms, and a figure showing the numbering of atoms for **1d** (4 pages); listings of observed and calculated structure factors (17 pages). Ordering information is given on any current masthead page.

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