The Chemistry of Amphotericin B. Synthesis of 13,14-Anhydro Derivatives

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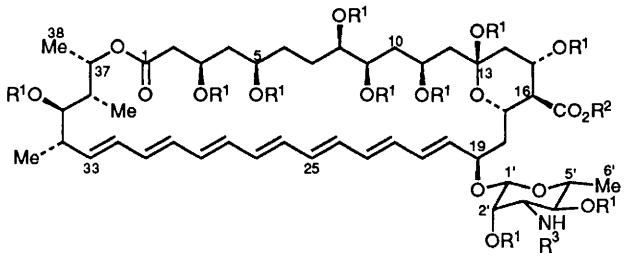
Pertrimethylsilylation of *N*-acetylamphotericin B methyl ester (**2**) using trimethylsilyl trifluoromethanesulphonate results in anomeric elimination to give the 13,14-anhydro derivative (**5**) rather than the reported 13-*O*-trimethylsilyl ether (**3**).

The polyene macrolide antibiotics are an important class of natural products, many of which show significant bioactivity.[1] One such compound, amphotericin B (AmB, **1**), isolated from *Streptomyces nodosus*,[2] is the drug of choice for the parenteral treatment of many serious, deep-seated fungal infections despite having severe side-effects. In addition, it is the only complex polyene whose molecular structure and absolute stereochemistry have been determined using *X*-ray crystallography.[3]

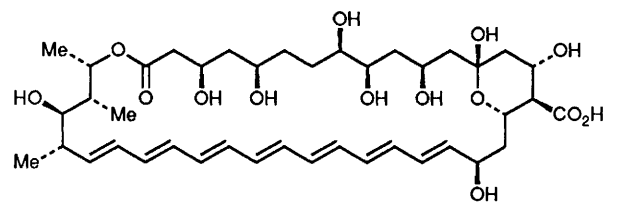
Recent publications have described, for the first time, the total synthesis of AmB (**1**)[4] and the corresponding aglycone, amphoteronilide B (**4**).[5] The aglycone (**4**) was also obtained from AmB (**1**) by the controlled degradation of the persilylated derivative (**3**)[6] and reported to be identical with the synthetic material.[5] Similar procedures have been used by other workers in this area.[7]

In connection with synthetic studies on polyene antibiotics the preparation of the pertrimethylsilylated derivative (**3**) was undertaken. Accordingly, following the described procedure,[6] *N*-acetylamphotericin B methyl ester (NAcAmE, **2**) was treated with an excess of trimethylsilyl trifluoromethanesulphonate in the presence of 2,6-lutidine to give one major product (purity *ca.* 90% by normal phase HPLC,† TLC,^{1}H NMR), thought to be the persilylated derivative (**3**), as reported. The isolated material proved to have limited chromatographic stability on silica but rapid, medium-pressure column techniques‡ facilitated the removal of reagent residues and gave the product which was shown to be ≥95% pure. The analytical data of this compound (NMR, IR, UV, $[\alpha]_D^{20}$) were essentially the same as those previously reported.[6] We found that the use of [$^2H_6$]acetone as ^{1}H NMR solvent (rather than $CDCl_3$) simplified the spectrum somewhat, especially in the region δ 6.5—3.0 (see Figure 1). All the mycosamine sugar proton signals were clearly visible, including a one-proton doublet (*J* 1.0 Hz) at δ 4.55 (H-1′).

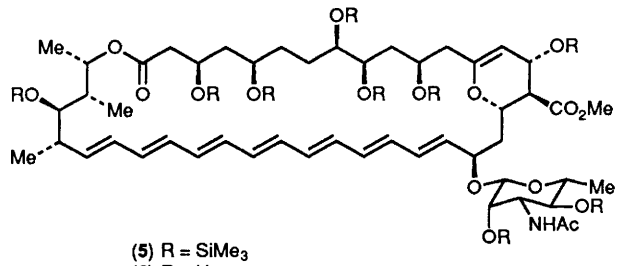
Further spectroscopic analysis of the isolated material, however, showed several discrepancies with the proposed structure (**3**) (see Table 1). The ^{13}C NMR spectrum showed signals at δ 102.8 and 98.9, chemical shift values characteristic for C-13 and C-1′ respectively,[8] but neither were due to quaternary carbon atoms. The methine carbon signal at δ 98.9 correlated with the proton resonance at δ 4.55 and was thus assigned as C-1′, but it was apparent that the resonance due to the quaternary C-13 must appear elsewhere in the spectrum. There was a low-field, quaternary carbon signal evident at δ 153.5 in addition to the ester, lactone, and acetamide carbonyl carbon resonances at δ 173.4, 170.9, and 169.3 respectively.



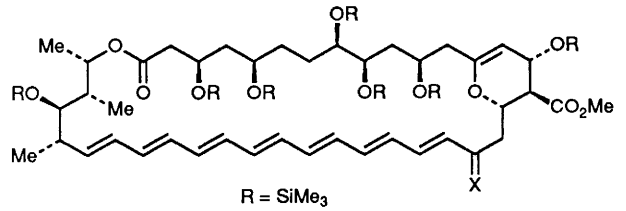
(**1**) Amphotericin B (AmB) $R^1 = R^2 = R^3 = H$
(**2**) (NAcAmE) $R^1 = H$, $R^2 = Me$, $R^3 = Ac$
(**3**) $R^1 = SiMe_3$, $R^2 = Me$, $R^3 = Ac$



(**4**) Amphoteronilide B



(**5**) $R = SiMe_3$
(**6**) R = H



$R = SiMe_3$

(**7**) X = O
(**8**) X = H, OH

† Waters RCM 8 × 10 with Resolve 5 μ silica cartridge; eluant: 20% ethyl acetate in n-hexane; flow rate: 2 ml/min; detection wavelength: 405 nm; retention time: 7.4 min.

‡ Merck silica gel 60 < 230 mesh, Medcalf Hyflow pump, 5—10% ethyl acetate in n-hexane.

Table 1. NMR data for (**5**).[a]

Carbon	δ_C[b]	δ_H[c]	Carbon	δ_C[b]	δ_H[c]
1	170.9	—	32	131.1	6.14
2	43.6	2.47	33	138.1	5.61
3	67.3	4.18	34	41.5	2.42
4	47.7	1.72, 1.83	35	78.6[e]	3.66
5	71.1[d]	3.87	36	43.6	1.90
6	35.1	1.44—1.56	37	72.2	4.90
7	28.0	1.44—1.74	38	18.4	1.16
8	76.3[e]	3.63	39	11.9	0.96
9	72.4[d]	3.87	40	19.5	1.03
10	39.2	1.50, 1.95	1′	98.9	4.55
11	69.2	4.01	2′	72.9	3.88
12	42.3	2.12, 2.13	3′	55.8	3.98
13	153.5	—	4′	73.6	3.45
14	102.8	4.49	5′	74.7	3.31
15	68.4	4.68	6′	18.8	1.20
16	53.9	2.56	16-CO_2CH_3	173.4	—
17	72.9	4.20	16-CO_2CH_3	52.5	3.76
18	37.2	1.92, 2.02	3′-N*H*$COCH_3$	—	6.71
19	75.4	4.62	3′-NH$COCH_3$	169.3	—
20	135.8	5.89	3′-NHCOCH_3	23.4	1.96
21	—[f]	6.38			

[a] Recorded on a Bruker AM-400 spectrometer in [$^2H_6$]acetone. [b] Relative to [$^2H_6$]acetone at δ 29.8. [c] Relative to [$^2H_5$]acetone at δ 2.05. [d,e] Assignments may be interchanged. [f] C-21—C-31 gave rise to resonances at δ 134.3, 134.1, 133.9, 133.8, 133.6 (C-22), 133.3, 131.9, and 130.1. The $SiMe_3$ resonances were observed at δ_C 1.03, 1.00, 0.97, 0.88, 0.83, 0.62, and 0.16 and δ_H 0.160, 0.158, 0.154, 0.142, 0.136, 0.131, 0.118, 0.089, and 0.070. The protons on C-22—C-31 resonated in the range δ 6.11—6.49.

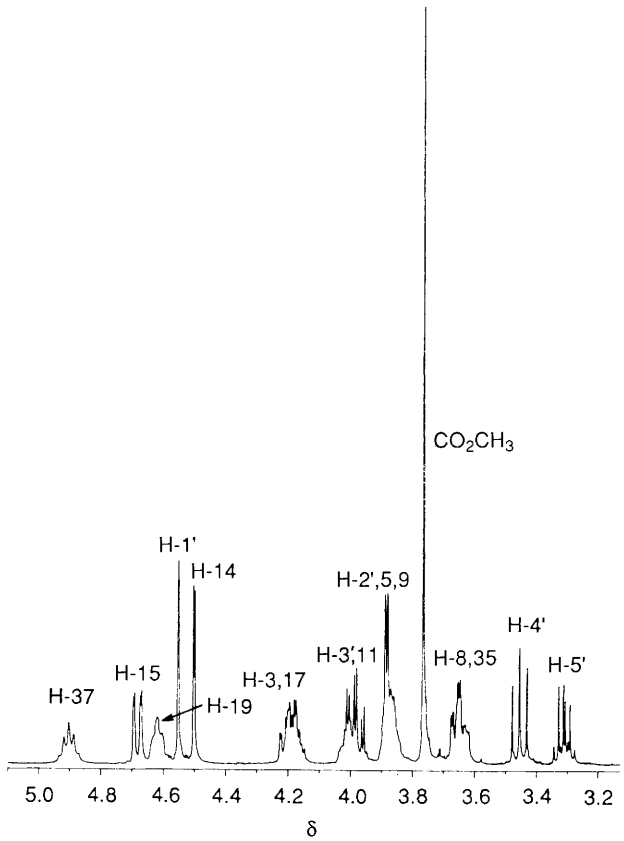


Figure 1. Portion of the ^1H NMR spectrum of (**5**) in [$^2H_6$]acetone measured at 400 MHz.

Furthermore, only seven of the expected eight methylene absorptions were apparent and the ^1H NMR spectrum indicated the presence of nine $SiMe_3$ groups. These data are clearly not consistent with the structure of the reported 13-*O*-trimethylsilylated derivative (**3**) and we conclude that the material isolated was the 13,14-anhydro compound (**5**). The quaternary carbon signal at δ 153.5 is thus due to C-13 while C-14, a shielded sp^2 carbon, gives rise to the signal at δ 102.8. The loss of one methylene resonance (C-14) is explained and, in the ^1H NMR spectrum, H-14 was found to resonate at δ 4.49 (d, *J* 1.6 Hz). Analysis of proton–proton connectivities showed that this proton was coupled to H-15, resonating at δ 4.68 (dd, *J* 1.6 and 8.8 Hz), which, in turn, was coupled to H-16 (δ 2.56, dd, *J* 8.8 and 10.9 Hz). The mass spectrum of (**5**) (VG ZAB 1F, positive ion FAB in 3-nitrobenzyl alcohol matrix, with and without sodium acetate) showed molecular ion peaks at *m/z* 1633 and 1610, corresponding to $[M + Na]^+$ and $M^{+\cdot}$.

The 13,14-anhydro derivative (**5**) is presumably formed after (bis)trimethylsilylation of the anomeric hydroxy group at position 13, elimination (involving the intermediacy of an oxonium species) and loss of one of the H-14 protons.[9]§

Desilylation of this material (**5**) with HF·pyridine reagent gave a product which had different chromatographic properties (TLC, reverse phase HPLC¶) compared to NAcAmE (**2**). We judge this compound to have structure (**6**) on the basis of NMR evidence.

We also confirmed that oxidative cleavage of the mycosamine sugar unit in (**5**) (2,3-dichloro-5,6-dicyanobenzoquinone, tetrahydrofuran)[10] gave heptaenone (**7**), which on

§ We have tried the silylation reaction under different conditions (solvent, base, temperature, relative proportions of reagents, and rate of addition of reagents) and have always obtained (**5**) as the major isolated product.

¶ Apex 5 μ ODS (250 × 4.6 mm column); eluant: 22% 0.05 M phosphate buffer (adjusted to pH 3.0 with phosphoric acid) in methanol;[11] flow rate: 1 ml/min; detection wavelength: 350 nm; retention times: (**2**) 8.1, (**6**) 6.5 min.

reduction ($NaBH_4$, MeOH) gave (**8**), both compounds retaining the C-13,14 double bond.‖

The authors gratefully acknowledge the excellent assistance provided by Mr. A. Greenlees. We thank Mr. G. Risbridger, Mr. M. Redrup, Dr. D. Bell and Dr. P. Skett for their mass spectrometry expertise, Mr. J. Tyler and Miss S. Stratford for certain NMR experiments and Dr. A. Taylor for helpful comments.

Received, 20th January 1990; Com. 0/00314J

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‖ We have found that a characteristic C-13 quaternary carbon signal is observed in the ^{13}C NMR spectra of all 13,14-anhydroamphotericin B derivatives at around δ 153. Compounds (**6**), (**7**), and (**8**) show absorptions at δ 152.8, 153.5, and 153.3 respectively.