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STRUCTURE OF TRICHOMYCIN A, A POLYENE MACROLIDE FROM STREPTOMYCES

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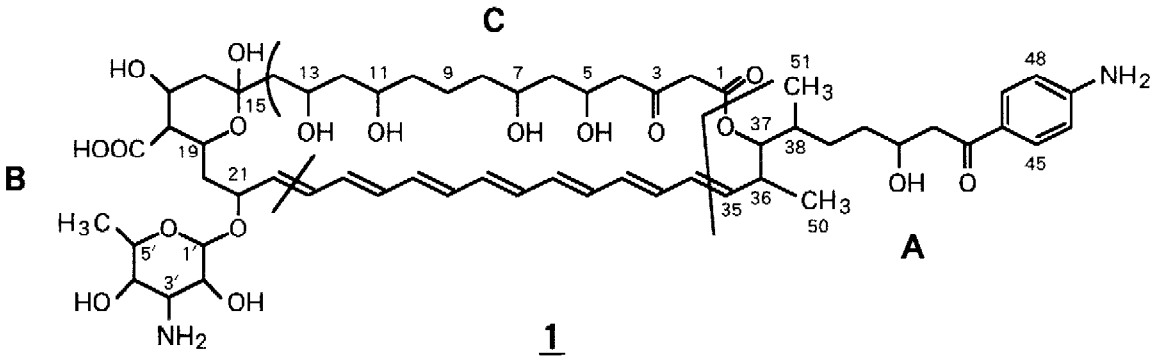
Summary: The structure of trichomycin A, a polyene macrolide of the aromatic heptaene subgroup, was determined on the basis of chemical and spectroscopic evidence.

The antibiotic trichomycin, presently used as a potent clinical drug for the treatment of vaginal infections, was first reported in 1952[1] and somewhat later recognized as a member of the family of antibiotics containing a heptaene chromophore[2]. Although the structure of trichomycin has long been the subject of chemical studies[3-6], its structure has not yet been clarified, because it is a mixture of closely related unstable compounds, and its poor solubility in water or organic solvents make separation extremely difficult. However, our recent studies on trichomycin indicated it to consist of more than seventeen closely related components and have made possible separation of the trichomycin complex into trichomycins A, B, C, D, E and F, using flash liquid chromatography and HPLC[7]. We report here the gross structure of the main component, trichomycin A 1.



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Pure 1 isolated from extracts of Streptomyces hachijoensis forms a fine yellow amorphous powder, mp >300° C and $[\alpha]_D^{20}$ +129.8° (c 0.1, DMF). Its molecular formula was determined as $C_{58}H_{84}N_2O_{18}$ (mw 1096) from elemental analysis and mass spectrum (FAB-MS in positive ion mode, m/z 1097 MH^+)). The UV-VIS spectrum with absorption maxima at 236, 343, 359 and 400 nm in pH 6.81 was typical of a heptaene. The presence of a β-ketolactone and/or a β-keto-ester (1734 cm^{-1}) and a ketone (1710 cm^{-1}) was recognized from its IR spectrum.

Trichomycin A 1 gave a ^{13}C-NMR spectrum[8] containing signals attributable to aliphatic ketone (δ 201.7), aromatic ketone (δ 196.2), carboxylic acid (δ 176.7), lactone (δ 166.3), hemiketal (δ 96.9, s) and acetal (δ 96.2, d). The ^{1}H- and ^{13}C-NMR spectra showed the presence of three methyl groups: (δ 0.83, d, J=6 Hz; δ 12.5), (δ 0.96, d, J=6 Hz; δ 16.4) and (δ 1.18, d, J=3 Hz; δ 17.8). The ^{1}H-NMR spectrum showed the presence of aromatic protons of an AB system: (δ 6.55, d, J=8 Hz) and (δ 7.66, d, J=8 Hz)[9].

Acetylation of 1 with Ac_2O gave the N,N'-diacetate 2, which was further treated with Ac_2O-pyridine to give an octa-O-acetate. Since 1 does not react rapidly with periodate, no hydroxyl group exists as vicinal glycol pair. Ozonolysis of 2 followed by alkaline hydrolysis gave the aldehyde 3[10]: $C_{19}H_{25}O_4$; mp 136-137° C; $[\alpha]_D^{25}$ -5.4° (c 1.6, MeOH). Extensive ^{1}H-NMR analysis of 1 and 3, referring ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectra of 1[11], made possible assignment of the C-35–C-51. The ^{1}H-^{1}H COSY spectrum of 1 also established the C-35–C-36 and C-37–C-1 connection: The allylic methine proton H-36 (δ 2.44, m) was coupled with both a terminal olefinic proton H-35 (δ 5.46, dd, J=10/16 Hz) and an acyloxycarbinyl proton H-37 (δ 4.64, d, J=9 Hz). These results are fully consistent with the partial structure A in 1.

The partial structure B was confirmed on the basis of a comparison of the ^{1}H- and ^{13}C-NMR characteristics of the two antibiotics, 1 and amphotericin B[12]. The pertinent ^{1}H- and ^{13}C-NMR data of both compounds are listed in Table 1. As these data are in good agreement to one another, it was concluded that the hemiketal was formed between C-15 and C-19, and mycosamine was attached at C-21.

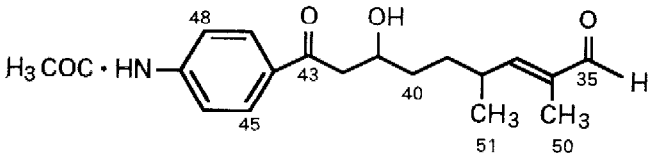

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TABLE 1. Chemical Shift Values for Trichomycin A and Amphotericin B.

	Trichomycin A*		Amphotericin B[12]	
Hydrogen/Carbon	Hydrogen	Carbon	Hydrogen	Carbon
14	δ 1.57 ppm	δ 46.2 ppm	δ 1.57 ppm	δ 46.4 ppm
15		96.9		97.0
16	1.15/1.86	44.1	1.15/1.86	44.0
17	3.98	65.5	4.04	65.4
18	1.86	57.9	1.91	57.6
19	4.15	65.5	4.19	65.2
20	1.85	36.7	1.55/2.13	36.8
21	4.40	74.8	4.38	74.6
22	5.85	133.8	5.75	—
18-COOH		176.7		176.6
1′	4.47	96.2	4.49	—
2′	3.72	68.0	3.65/3.74	68.3
3′	2.71	56.0	2.75	55.9
4′	3.13	70.0	3.12	70.2
5′	3.22	72.7	3.21	72.8
5′-CH_3	1.18	17.8	1.18	18.0

*in DMSO-d_6, 400 MHz

The partial structures A and B account for the elemental aggregate $C_{44}H_{60}N_2O_{12}$ of 1, leaving $C_{14}H_{24}O_6$, yet to be elucidated. Evidence for a β-ketoester moiety came from a compatible chemical shift of the methylene proton signal H-2 (δ 3.40/3.49, d, J=18 Hz) inserted between C-1 ester and C-3 carbonyl. The presence of the functionality was also suggested by IR and the appearance of a UV maximum at 289 nm in alkaline solution of 1, which disappear with the addition of acid.

The linkages of C-4—C-10 and C-11—C-14[13] were deduced by the ^1H-^1H COSY spectrum of 1, in which cross peaks were observed between the following pairs of protons; H-4 (δ 2.36/2.47, d, J=7/16 Hz) and H-5 (δ 3.71, m), H-5 and H-6 (δ 1.18, m), H-6 and H-7 (δ 3.54, m), H-7 and H-8 (δ 1.35, m), H-8 and H-9 (δ 1.13, m), and H-9 and H-10 (δ 1.50, m); H-11 (δ 3.85, m) and H-12 (δ 1.45, m), H-12 and H-13 (δ 4.23, m), and H-13 and H-14 (δ 1.57, m). Long-range ^1H-^1H coupling was observed between H-4 and H-6 in COSYRCT.

Trichomycin A 1 was thus formulated as shown in the figure.

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References and Notes

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7. Isolation of trichomycin A and related compounds will be reported elsewhere.
8. All NMR spectra were recorded on a Bruker WM-400 in DMSO-d_6 and/or plus D_2O.
9. The aromatic part: ^{13}C-NMR (DMSO-d_6) C-44 (δ 137.2 ppm, s), C-45 (130.4, d), C-46 (112.4, d) and C-47 (153.4, s).
10. 3: UV (MeOH) λ_{max} 223 nm (ε 29,000) and 286 (25,000); IR (KBr) 3400-3420 cm^{-1} broad, 1678, 1655, 1634, 1594, 1523, 1180, 880 and 830; ^{1}H-NMR (100 MHz, $CDCl_3$) δ 0.85 ppm (3H, d), 1.77-2.5 (7H, m), 2.27 (3H, s), 3.10 (1H, m), 6.07 (1H, d), 7.45 (2H, d), 7.87 (2H, d) and 9.10 (1H, s).
11. The part A: ^{1}H-NMR (DMSO-d_6) H-38 (δ 1.70 ppm, m), H-39 (1.21, m), H-40 (1.40, m), H-41 (3.85, m) and H-42 (2.72/2.90, ddd, J=4/8/16 Hz). ^{13}C-NMR (DMSO-d_6) C-36 (δ 38.2 ppm), C-37 (79.2), C-38 (37.3), C-39 (29.7), C-40 (32.8), C-41 (67.3) and C-42 (45.3).
12. A. Aszalos, A. Bax, N. Burlinson, P. Roller and C. McNeal, J. Antibiotics, 38, 1699-1713 (1985)
13. The part C: ^{13}C-NMR (DMSO-d_6) C-4 (δ 51.1 ppm), C-5 (67.5), C-6 (38.2), C-7 (70.6), C-8 (37.3), C-9 (22.1), C-10 (37.9), C-11 (71.0), C-12 (43.7) and C-13 (67.0).

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