Avermectins. Structure Determination

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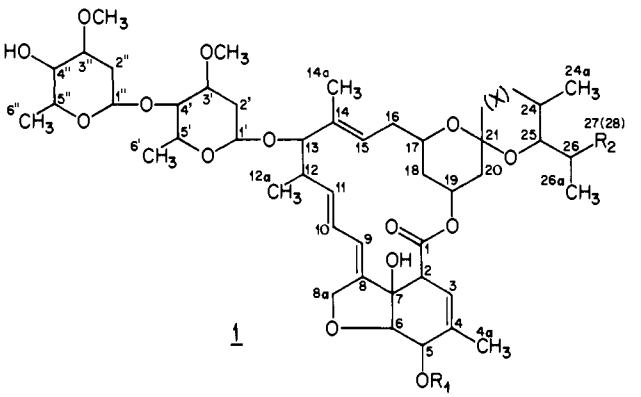
Abstract: The avermectins are a group of potent, broad-spectrum antiparasitic agents which appear to act by interference with invertebrate neurotransmission. They are α-L-oleandrosyl-α-L-oleandroside derivatives of pentacyclic 16-membered lactones (structure **1**), related to the milbemycins. Their characterization and structure determination, primarily by mass spectrometry and ^{13}C NMR spectroscopy, are described.

Parasitic diseases of animals and man constitute serious problems in many countries of the world, and the need for new, effective agents to treat these infections has been clearly stated.[1] Our laboratories recently reported the discovery of a group of natural products, the avermectins, which are effective against helminths and arthropods in doses as low as 10 μg/kg.[2] They are glycosidic derivatives of pentacyclic 16-membered lactones, but are devoid of the antibacterial properties associated with "macrolide" antibiotics. The avermectins do not inhibit protein synthesis but appear to act by interference with invertebrate neurotransmission.[3] In this paper we wish to report the structures of the avermectins.

The compounds were isolated by solvent extraction of the mycelia of *Streptomyces avermitilis* and first separated into four major (a) components by a series of silica gel chromatograms. Mass spectrometric analysis revealed the presence, in each of them, of 5-10% of a minor (b) homologue which could subsequently be separated from the major component by reverse-phase high-performance liquid chromatography.[2a] The eight components were designated A_{1a} through B_{2b}. A number of structural elements could be deduced from their mass spectral fragmentations and ^{13}C NMR absorptions which suggested a relationship with the milbemycins;[4] a detailed analysis of the data and investigation of the nature and attachment of a carbohydrate substituent which is not present in the milbemycin then resulted in the assignment of the α-L-oleandrosyl-α-L-oleandroside structures **1** for the eight compounds. The mass-spectral data and their interpretations are given in Table I and Scheme I. ^{13}C NMR assignments, listed in Table II, are based on general chemical shift considerations, patterns observed in proton coupled and single-frequency off-resonance decoupled experiments, and careful comparison of the four major structural variants of the series and their corresponding monosaccharides and aglycones. They are in agreement with the results of biosynthetic incorporations of ^{13}C-enriched precursors.[5]



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$R_1 = CH_3$: components A
$R_1 = H$: components B
$R_2 = C_2H_5$: components a
$R_2 = CH_3$: components b

X = —CH=CH— : components 1
X = —CH_2—CH(OH)— : components 2

Representative ^1H NMR data and their interpretation are given in the Experimental Section. X-ray crystallography confirmed the structures and established the remaining absolute stereochemical assignments as described in the accompanying paper.[6]

Comments

Branched butyl and propyl substituents at C25 of the avermectins replace methyl and ethyl substituents of the milbemycins. *sec*-Butyl groups are required by otherwise unassignable methyl triplets near δ 0.98 in ^1H NMR spectra of the a components and isopropyl groups by the absence of methyl triplets and the presence of a (sixth) methyl doublet near δ 1.1 in the spectra of the b components. Biosynthetic experiments[5] have shown that these substituents derive from L-isoleucine and L-valine, respectively.

The A components differ from the B components by a third methoxy signal in their NMR spectra. The diagnostic mass spectral fragments, o, are at *m/e* 275 and 261, respectively.

Milbemycins are hydroxylated at both C22 and C23 or at neither of these positions and not at C13. The avermectin aglycones are hydroxylated at C23 or contain a 22,23 double bond and are all hydroxylated at C13. Initial NMR data could not distinguish between hydroxylation at C16 and C13 but mass spectral fragment abundances, in particular those of e and f, favored the latter.

The mass spectral fragments *m/e* 289, p, q and s shown in Scheme I, indicated a disaccharide substituent consisting of isomeric or identical monomers. Acid-catalyzed methanolysis, yielding methyl α- and β-oleandroside in over 100 mol %, proved identical monomers. Of the two possible points of attachment

(1) Parasitology Supplement, *Nature* **1978**, *273*, 596-630.

(2) (a) Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, Georgia, October 1-4 1978. Avermectins. A new family of potent anthelmintic agents. Abstract No. 462: Producing Organism and Fermentation, R. W. Burg, B. M. Miller, E. E. Baker, J. Birnbaum, S. A. Currie, R. Hartman, Y. L. Kong, R. L. Monaghan, G. Olson, I. Putter, J. B. Tunac, H. Wallick, E. O. Stapley, R. Ōiwa, and S. Ōmura. Abstract No. 463: Isolation and Chromatographic Properties, T. W. Miller, L. Chaiet, D. J. Cole, L. J. Cole, J. E. Flor, R. T. Goegelman, V. P. Gullo, A. J. Kempf, W. R. Krellitz, R. L. Monaghan, R. E. Ormond, K. E. Wilson, G. Alber-Schönberg, and I. Putter. Abstract No. 464: Structure Determination, G. Albers-Schönberg, B. H. Arison, J. C. Chabala, A. W. Douglas, P. Eskola, M. H. Fisher, J. M. Hirshfield, K. Hoogsteen, A. Lusi, H. Mrozik, J. L. Smith, J. P. Springer, and R. L. Tolman. Abstract No. 465: Efficacy of the B_{1a} Component, J. R. Egerton, D. A. Ostlind, L. S. Blair, C. H. Eary, D. Suhayda, R. F. Riek, and W. C. Campbell. Full papers covering Abstracts No. 462, 463, and 465 have appeared in: *Antimicrob. Agents Chemother.* **15**, 361, 368, 372 (1979). (b) D. A. Ostlind, S. Cifelli, and R. Lang, *Vet. Rec.* **105**, 168 (1979).

(3) L. C. Fritz, C. C. Wang, and A. Gorio, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 2062-2066 (1979). (b) I. S. Kass, C. C. Wang, J. P. Walrond, and A. O. W. Stretton, *ibid.*, **77**, 6211-6215 (1980).

(4) Complete references are given in *J. Antibiot.*, **29**, Nos. 14-16, 35-42 (1976), Index of Compounds from Actinomycetes.

(5) G. Albers-Schönberg, A. W. Douglas, R. T. Goegelman, L. Kaplan, A. Kempf, and T. B. Tumac, in preparation.

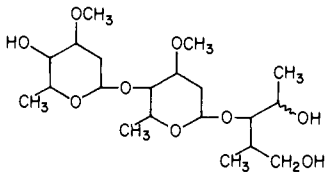
(6) J. P. Springer, B. H. Arison, J. M. Hirshfield, and K. Hoogsteen, *J. Am. Chem. Soc.*, following paper in this issue.

0002-7863/81/1503-4216$01.25/0

Table I. Most Characteristic Mass Spectral Ions (Experimental High-Resolution Values) of Avermectins

	A_{1a} $C_{49}H_{74}O_{14}$	A_{2a} (% of base peak)	empirical formula	A_{1b} $C_{48}H_{72}O_{14}$	A_{2a} $C_{49}H_{76}O_{15}$	A_{2b} $C_{48}H_{74}O_{15}$	B_{1a} $C_{48}H_{72}O_{14}$	B_{1b} $C_{47}H_{70}O_{14}$	B_{2a} $C_{48}H_{74}O_{15}$	B_{2b} $C_{47}H_{72}O_{15}$
M^+	886.5072	0.5		872	904	890	872	858	890	876
a	742.4229	0.5	$C_{42}H_{62}O_{11}$	728	760	746	728	714	746	732
b	598	0.5		584	616	602	584	570	602	588
c	580.3406	12	$C_{35}H_{48}O_7$	566.3265	598	584	566	552	584	570
d	456.2886	3		442	456	442	456	442	456	422
e		83			323.2225	309.2070			323	309
f	305.2120	62	$C_{19}H_{29}O_3$	291.1962	305	291	305	291	305	291
g		5	$C_{14}H_{23}O_3$		239.1637	225			239	225
h	221.1527	7	$C_{14}H_{21}O_2$	207	221.1527	207	221	207	221	207
i		20	$C_{13}H_{23}O_2$		211.1691	197.1542			211	197
k	193.1587	6	$C_{13}H_{21}O$	179.1429	193	179	193	179	193	179
l		16	$C_{11}H_{15}O_2$		179.1076	179			179	179
m	169.1226	7	$C_{10}H_{17}O_2$	155	169	155	169	155	169	155
n	111.0446	7	$C_6H_7O_2$	111	111	111	111	111	111	111
o	275.1292	10	$C_{16}H_{19}O_4$	275	275	275	261.1139	261	261	261
p	257.1382	2	$C_{13}H_{21}O_5$	257	257	257	257	257	257	257
q	145.0867	100	$C_7H_{13}O_3$	145	145	145	145	145	145	145
r	127.0754	59	$C_7H_{11}O_2$	127	127	127	127	127	127	127
s	113.0604	46	$C_6H_9O_2$	113	113	113	113	113	113	113
t	95.0496	26	C_6H_7O	95	95	95	95	95	95	95
u	87.0444	98	$C_4H_7O_2$	87	87	87	87	87	87	87

of the disaccharide to the aglycones,[7] the C7 and C13 hydroxy groups, the former could be ruled out. The data of Table II, supported by a deuterium exchange experiment,[8] indicated that C7 carries a free hydroxy group[9] (82.5 ± 0.1 ppm, s, in all compounds, with and without attached disaccharide). This was confirmed by ^1H NMR spectroscopy which for all compounds showed a sharp singlet for one exchangeable proton near δ 4.0 while all other hydroxy protons gave rise to doublets. Chemical proof for attachment of the saccharide at the C13 hydroxy group was obtained by ozonolysis of A_{2a} followed by sodium borohydride



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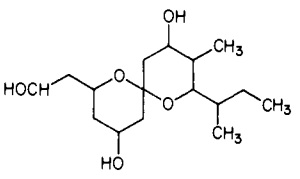

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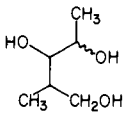

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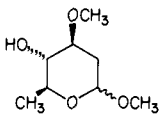

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reduction which gave compounds **2** and **3**; methanolysis of **2** again gave α- and β-methyl oleandroside and the two epimeric 2-methylpentanediols **4**.[10] Attachment of the disaccharide to the C13 hydroxy group also allowed the most consistent and logical overall interpretation of the ^{13}C NMR spectra as presented in Table II.

Stereochemistry

^1H NMR spectra of the natural products (c.f. Table III) showed only small coupling constants for the anomeric protons at C1′ and C1″ which for 2-deoxy sugars implies α-glycosidic linkages. The methanolysis product methyl oleandroside **5**, which, as stated, was isolated in over 100 mol% yield, consisted of a 6:1 mixture of the α and β isomers. The observed optical rotation $[\alpha]_D$ −97° of this product was equal to that calculated from reported values[11] for a 6:1 α/β mixture in the L series. This provided the basis for the assignment of absolute configurations at all asymmetric centers of the avermectins by X-ray crystallographic analyses.[6]

Experimental Section

Spectra were recorded on the following instruments: IR, Perkin-Elmer 421; UV, Cary 15; MS, Varian MAT-731; ^{13}C NMR, Varian CFT-20 and XL-100; ^1H NMR, Varian SC 300.

Isolation procedures, including separation of b from a components by HPLC, are described elsewhere.[2] Analyses described in this paper were carried out for the most part on samples for which relative peak heights at *m/e* 291 and 305 indicated 5–10% b components. These samples were purified, after a series of silica gel column chromatograms of mycelia extracts, by preparative TLC on prewashed SiO_2 HF 254 plates (Analtech) with repetitive development in hexane containing 5–10% isopropyl alcohol, followed by TLC in hexane–diethyl ether 1:1 (A_1 and A_2) or diethyl ether alone (B_1 and B_2). Approximate R_f values on SiO_2 GF 254 after two developments with hexane containing 15% isopropyl alcohol are: A_2, 0.49; A_2, 0.40; B_1, 0.30; and B_2, 0.21. Before elution, preparative plates were once developed in methylene chloride which does not move the compounds but removes residuals of the other solvents, in particular di-*tert*-butylcresol antioxidant of reagent grade diethyl ether. Products were eluted with methylene chloride–methanol 9:1 and the eluates evaporated, redissolved in methylene chloride, filtered through tight cotton plugs, again evaporated, and lyophilized from glass-distilled, VPC-quality benzene.

$A_{1a/b}$: UV (CH_3OH) 237 nm (ϵ 28 700), 243 (31 275), 252 (20 290); IR ($CHCl_3$) 3570, 2460, 3000, 2965, 2930, 2875, 2820, 1705, 1447, 1374, 1336, 1292, 1190, 1155, 1138, 1114, 1100, 1068, 1042, 1003, 980, 962, 940, 929, 910, 901, 890, 864, 818 cm^{-1} (sh not listed); $[\alpha]^{27}_D$ +68.5 ± 2° (*c* 0.77, $CHCl_3$).

$A_{2a/b}$: UV (CH_3OH) 237 nm (ϵ 28 800), 243 (31 740), 252 (20 425); IR ($CHCl_3$) 3500, 3004, 2964, 2936, 2875, 2825, 1705, 1447, 1376, 1337, 1292, 1191, 1163, 1140, 1118, 1098, 1072, 1044, 1000, 980, 963, 940, 929, 902, 890, 880, 868, 825 cm^{-1} (sh not listed); $[\alpha]^{27}_D$ +48.8 ± 2° (*c* 1.64, $CHCl_3$).

(7) A thorough spectroscopic and chemical investigation of this question was required at the time because of misleading results of a mass spectrometric investigation which will be reported elsewhere.

(8) D. Gagnaire and M. Vincendon, *Chem. Commun.*, 509–510 (1977).

(9) K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Lett.*, 179–182 (1977). R. Kasai, M. Suzuo, T. Asakawa, and O. Tanaka, *ibid.*, 175–178 (1977).

(10) Detailed characterizations of these products are included in the Experimental Section.

(11) W. D. Celmer and D. C. Hobbs, *Carbohydr. Res.*, **1**, 137 (1965); H. Els, W. D. Celmer, and K. Murai, *J. Am. Chem. Soc.*, **80**, 3777 (1958); T. Reichstein and E. Weiss, *Adv. Carbohydr. Chem.*, **17**, 65–120 (1962).

Table II. ^{13}C NMR Data for the Four Major Avermectin Components and Some of Their Deglycosylation Products[a,b]

	B_1	B_1 (ms)[b]	B_2	B_2 (ms)[b]	A_1	A_2	A_2 (ms)[b]	A_2 (agl)[b]
28	12.0	12.1 (q*)	11.8	11.8 (q*)	12.0 (q*)	11.8 (q*)	11.8	11.5 (q*)
26a	12.9	13.0 (q*)	12.4	12.4 (q*)	13.0 (q*)	12.4 (q*)	12.4	12.5 (q*)
24a			13.8	13.8 (q*)		13.8 (q*)	13.8	13.8 (q*)
14a	15.1	15.1 (q)	15.1	15.1 (q)	15.1 (q)	15.1 (q)	15.1	14.6 (q)
24a	16.4	16.4 (q*)			16.4 (q*)			
6'	17.7	17.8 (q)	17.7	17.8 (q)	17.7 (q)	17.7 (q)	17.7	
6"	18.4		18.4		18.4 (q)	18.4 (q)		
4a	19.9	19.9 (q)	19.9	19.9 (q)	19.9 (q)	19.9 (q)	19.9	19.9 (q)
12a	20.2	20.2 (q*)	20.2	20.2 (q*)	20.3 (q)	20.3 (q*)	20.2	19.3 (q*)
ΣCH_3	8	7	8	7	8	8	7	6
27	27.5	27.5 (t)	27.3	27.3	27.5 (t)	27.3 (t)	27.2	27.5 (t)
24	30.6	30.6 (d)			30.6 (d)			
16	~34.3	34.2 (t)	~34.3	~34.2	34.3 (t)	34.3 (t)	~34.2	34.3 (t)
2'	~34.3	34.0 (t)	~34.3	~34.2	34.3 (t)	34.3 (t)	~34.2	
2"	~34.3		~34.3		34.3 (t)	34.3 (t)		
26	35.2	35.2	35.2	35.2	35.2 (d)	35.2 (d)	35.1	35.2 (d)
24			35.8	35.8		35.8 (d)	35.7	35.7 (d)
18	36.6	36.6 (t)	36.5	36.5	36.6 (t)	36.5 (t)	~36.3	36.2 (t)
12	39.8	39.8 (d)	39.8	39.8 (d)	39.7 (d)	39.8 (d)	39.7	40.1 (d)
20	40.5	40.6 (t)	40.8	40.9	40.5 (t)	40.8	~41.0	40.9 (t)
22			41.2	41.2		41.2 (t)	~41.0	40.9 (t)
2	45.7	45.7 (d)	45.8	45.8	45.7 (d)	45.7 (d)	45.6	45.7 (d)
$-CH_2-$	6	5	7	6	6	7	6	5
>CH-	4	4	4	4	4	4	4	4
$\Sigma(CH_2/CH)$	10	9	11	10	10	11	10	9
3'a	56.4	56.6	56.4	56.6	56.4 (q)	56.4 (q)	56.6	
3"a	56.4		56.4		56.4 (q)	56.4 (q)		
5a					57.7 (q)	57.7 (q)	57.6	57.7 (q)
$\Sigma(OCH_3)$	2	1	2	1	3	3	2	1
5'	67.3		67.3		67.3 (d)	67.3 (d)		
5	67.8	67.7	67.7	67.7 (d)				
19	~68.2	~68.4	67.7	67.7 (d)	68.2 (d)	67.7 (d)	67.6	67.6 (d)
8a	~68.2	~68.4	~68.3	68.3	68.2 (t)	68.2 (t)	68.2	68.2 (t)
17	~68.4	~68.4	~68.4	68.3	68.4 (d)	68.2 (d)	68.2	68.4 (d)
5'		~68.4		68.3			68.2	
5"	~68.4		~68.3		68.4 (d)	68.3 (d)		
23			69.9	69.9		69.9 (d)	69.8	70.0
25			70.9	70.9		70.8 (d)	70.7	71.3
25	75.0	74.9			74.9 (d)			
4'		76.1		76.1			75.9	
4"	76.1		76.1		76.1 (d)	76.1 (d)		
5 or 6					77.0 (d)	77.0 (d)	76.9	77.0 (d)
6 or 5					77.5 (d)	77.6 (d)	77.7	77.6 (d)
13								77.4 (d)
3"	78.3		78.3		78.3 (d)	78.3 (d)		
3'		78.4		78.4 (d)			78.3	
3'	79.4		79.4		79.4 (d)	79.4 (d)		
6	79.4	79.3	79.4	79.5				
4'	80.5		80.5		80.6 (d)	80.6 (d)		
7	80.5 (s)	80.4 (s)	80.5 (s)	80.5 (s)	80.6 (s)	80.6 (s)	80.6 (s)	80.5 (s)
13	82.0	81.8 (d)	81.8	81.7 (d)	82.0 (d)	81.8 (d)	81.6	
CH_2O	1	1	1	1	1	1	1	1
CH-O	12	9	13	10	12	13	10	7
C-O	1	1	1	1	1	1	1	1
$\Sigma(CH_xO)$	14	11	15	12	14	15	12	9
1'	95.0	95.1 (d)	94.9	95.0 (d)	95.0 (d)	94.9 (d)	95.0	
21	95.8	95.8 (s)			95.8 (s)			
1"	98.5		98.6		98.5 (d)	98.6 (d)		
21			99.7	99.7 (s)		99.7 (s)	99.6 (s)	99.6 (s)
	2	1	2	1	2	2	1	0
	1	1	1	1	1	1	1	1
$\Sigma(CH_xO_2)$	3	2	3	2	3	3	2	1
15	118.1	118.1	117.7	117.7	118.4 (d)	117.7 (d)	117.6	116.5 (d)
3	118.4	118.4	118.0	118.1	118.4 (d)	118.4 (d)	118.7	118.5
9	120.4	120.4	120.4	120.5	119.7 (d*)	119.7 (d*)	119.8	119.8 (d*)
10	124.8	124.8	124.8	124.8	124.9 (d**)	124.9 (d**)	124.9	124.8 (d**)
23	127.9	127.8			127.8 (d*)			

Table II (*Continued*)

	B_1	B_1 (ms)[b]	B_2	B_2 (ms)[b]	A_1	A_2	A_2 (ms)[b]	A_2 (agl)[b]
14	135.2	135.1 (s)	135.7	135.7	135.2 (s)	135.7 (s)	135.6	139.3
4					136.0 (s)	136.1 (s)	135.7	135.8
22	136.2	136.2			136.1 (d*)			
4	137.9	137.9 (s)	138.0	137.9				
11	~138.0	~138.0	138.0	137.9	137.6 (d**)	137.6 (d**)	137.5	136.9 (d**)
8	139.7	139.6 (s)	139.8	139.7	139.9 (s)	140.0 (s)	139.8 (s)	139.9 (s)
-*C*H=C	7	7	5	5	7	5	5	5
>*C*=C	3	3	3	3	3	3	3	3
$\Sigma(CH_x{=}C)$	10	10	8	8	10	8	8	8
$-C\overset{O^-}{\underset{O^-}{=}}$	173.6	173.6 (s)	173.5	173.4 (s)	173.9 (s)	173.7	173.2	173.4
$\Sigma(CO_2)$	1	1	1	1	1	1	1	1
overall Σ	48	41	48	41	49	49	42	35

[a] Multiplicities in proton single-frequency off-resonance decoupled (SFORD) ^{13}C spectra are denoted as s, d, t, or q, respectively, for singlet, doublet, triplet, or quartet patterns. An asterisk followed by the letter designation signifies a pattern containing recognizable "second order" structure, due to strong coupling conditions involving the proton(s) directly bonded to the carbon nucleus in question under SFORD conditions. The double asterisk appearing for certain olefinic carbons indicates an extraordinarily complex SFORD pattern which is characteristic of a trans-disubstituted olefinic bond. SFORD observations were not made for all eight compounds in the table, particularly where assignments by analogy were obvious. The spectra were obtained in $CDCl_3$ solutions at 10–20% w/v concentrations and with Me_4Si as internal reference. [b] ms = monosaccharide degradation product; agl = aglycone.

$B_{1a/b}$: UV (CH_3OH) 237 nm (ε 29 120), 243 (31 850), 252 (20 510); IR ($CHCl_3$) 3562, 3460, 3004, 2968, 2936, 2876, 2825, 1706, 1447, 1375, 1337, 1290, 1265, 1190, 1156, 1139, 1115, 1101, 1064, 1043, 1004, 980, 961, 942, 930, 912, 903, 890, 862, 820 cm^{-1} (sh not listed); $[\alpha]^{27}_D$ +55.7 ± 2° (*c* 1.06, $CHCl_3$).

$B_{2a/b}$: UV (CH_3OH) 237 nm (ε 27 580), 243 (30 590), 252 (20 060); IR ($CHCl_3$) 3564, 3500, 3000, 2964, 2937, 2878, 2825, 1707, 1445, 1375, 1336, 1291, 1191, 1161, 1139, 1116, 1101, 1068, 1042, 1001, 980, 963, 940, 929, 908, 890, 879, 861, 825 cm^{-1} (sh not listed); $[\alpha]^{27}_D$ +38.3 ± 2° (*c* 0.87, $CHCl_3$).

Methanolysis of Avermectin A_{2a}. Avermectin A_{2a} (64.5 mg; containing some A_{2b}) was left overnight at room temperature in 0.05 N methanolic HCl. After this time, TLC showed no starting material but two new UV-fluorescence quenching products of higher R_f (1.31 and 1.75 × R_f of A_2). The solution was diluted with dioxane and lyophilized after evaporation of most of the methanol. The residue was chromatographed in hexane containing 10% isopropyl alcohol and rechromatographed in methylene chloride containing 3% isopropyl alcohol on 250 μm SiO_2 HF 254 plates. Areas of the chromatograms containing the methyl oleandrosides were separately collected and eluted. The more polar fluorescence quenching product was identified as the monosaccharide degradation product [37.3 mg; M^+ 760; UV (CH_3OH) 243 nm (ε 31 312, calcd 31 730)] and the faster moving one as the aglycone [16.3 mg; M^+ 616; UV (CH_3OH) 243 nm (ε 27 166)].

Avermectin $A_{2a/b}$ (1.0 g) was added at room temperature to a solution of 0.2 mL of concentrated H_2SO_4 in 20 mL of CH_3OH and kept overnight. The mixture was diluted with 175 mL of $CHCl_3$ and the solution washed with saturated $NaHCO_3$ solution and water and evaporated. The residue was chromatographed on 140 g of SiO_2 (silica gel 60, 70–230 mesh, E. Merck, Darmstadt) in $CHCl_3$ containing 2% THF. Fractions of 20 mL were collected. Fractions 60 to 80 were combined and yielded 532 mg (78.1%) of chromatographically pure avermectin $A_{2a/b}$ aglycone. An aliquot of 100 mg was recrystallized from 1 mL of CH_3OH, yielding 60 mg; mp 175–177 °C; M^+ 616. Anal. Calcd for $C_{35}H_{52}O_9$: C, 68.16; H, 8.58. Found: C, 68.30; H, 8.58. Treatment of the avermectin B_2 components in this fashion led to the corresponding monosaccharide and aglycone products. In the case of the A_1 and B_1 components, however, treatment with methanolic HCl resulted in substantial addition of solvent or acid to the 22,23 double bond. Good results were obtained with sulfuric acid in aqueous dioxane.

Spraying of TLC chromatograms of the crude methanolysis product of avermectin A_{2a} with anthrone reagent[12] revealed the presence of two carbohydrate components. With increasing reaction time the less polar component, migrating even faster than A_{2a} aglycone on SiO_2 GF 254 plates in hexane containing 14% isopropyl alcohol, accumulated at the expense of the more polar one whose R_f is intermediate between those of the A_{2a} monosaccharide and aglycone. The less polar sugar product could be recovered and gave the following mass spectral and 100 MHz ^1H NMR data: M^+ 176, *m/e*, 145, 127, 113, 95, 87, *m*/e* 88; ^1H NMR δ 1.31 (d, 3, *J* = 6.5 Hz, $CHCH_3$), ~2.28 (m, 2, CH_2), 3.13 (br t, 1, *J* = 9 Hz, C*H*–OH), 3.33 (s, 3, Cl–OCH_3), 3.38 (s, 3, C3–OCH_3), 3.4–3.8 (m, 2, C3*H*–OCH_3 and C5*H*–CH_3), 4.76 (dd, 1, *J* = 3.7 and 1.6 Hz, Cl–H).

Avermectin A_{2a} (20 g) was subjected to methanolysis as described above. After 18 h the reaction was diluted sixfold with dichloromethane and successively washed with sodium bicarbonate solution (2 × 50 mL) and water (4 × 50 mL). The aqueous extracts were combined, concentrated in vacuo below 30 °C to about 100 mL, saturated with NaCl, and extracted with diethyl ether (4 × 50 mL). The ether extracts were combined, dried over Na_2SO_4, and evaporated. The residues could be further purified by distillation, giving 450 mg (133 mol %) of a mixture of α- and β-methyl oleandroside. Column chromatography on silica gel in CH_2Cl_2 containing 2% methanol resulted in a α:β = 6:1 enriched preparation by NMR integration [α isomer, see above; β isomer, δ 1.35 (d, 3, *J* = 5.5 Hz; CH–CH_3), 3.36 (s, 3, C3–OCH_3), 3.50 (s, 3, Cl–OCH_3), 4.40 (dd, 1, *J* = 9.5 and 2.0 Hz, Cl–H); $[\alpha]^{27}_D$ –97.1 ± 1.3° (*c* 0.375, CH_3OH)].

Ozonolysis. General Procedure. A solution of 100 mg of avermectin in 20 mL of 10:7:3 CH_2Cl_2–CH_3OH–H_2O containing 20 mg of K_2HPO_4 was chilled to –75 °C in a dry ice–isopropyl alcohol bath and ozonized, using a Wellsbach T19 Ozonator until the blue color of excess dissolved ozone persisted. While at –75 °C, N_2 was bubbled through until the solution was colorless, 1.5 mL of $(CH_3)_2S$ was added, and the stirring solution was allowed to warm to 0 °C. The mixture was then placed in an ice bath, treated with 20 mg of $NaBH_4$, and allowed to warm to room temperature within 1 h (negative starch–iodide test). The mixture was concentrated to about 6 mL on the rotary evaporator and extracted 5 times with equal volumes of diethyl ether and the combined ether layers were dried over anhydrous $MgSO_4$ and evaporated. The residual oil was chromatographed by multiple development in $CHCl_3$–CH_3OH 19:1 on preparative silica gel plates (Analtech) and the products eluted with ethyl acetate.

(12) G. Zweig and J. Sherma, Eds., "CRC Handbook of Chromatography", CRC Press, Cleveland, Ohio, 1972, p 125.

Scheme I

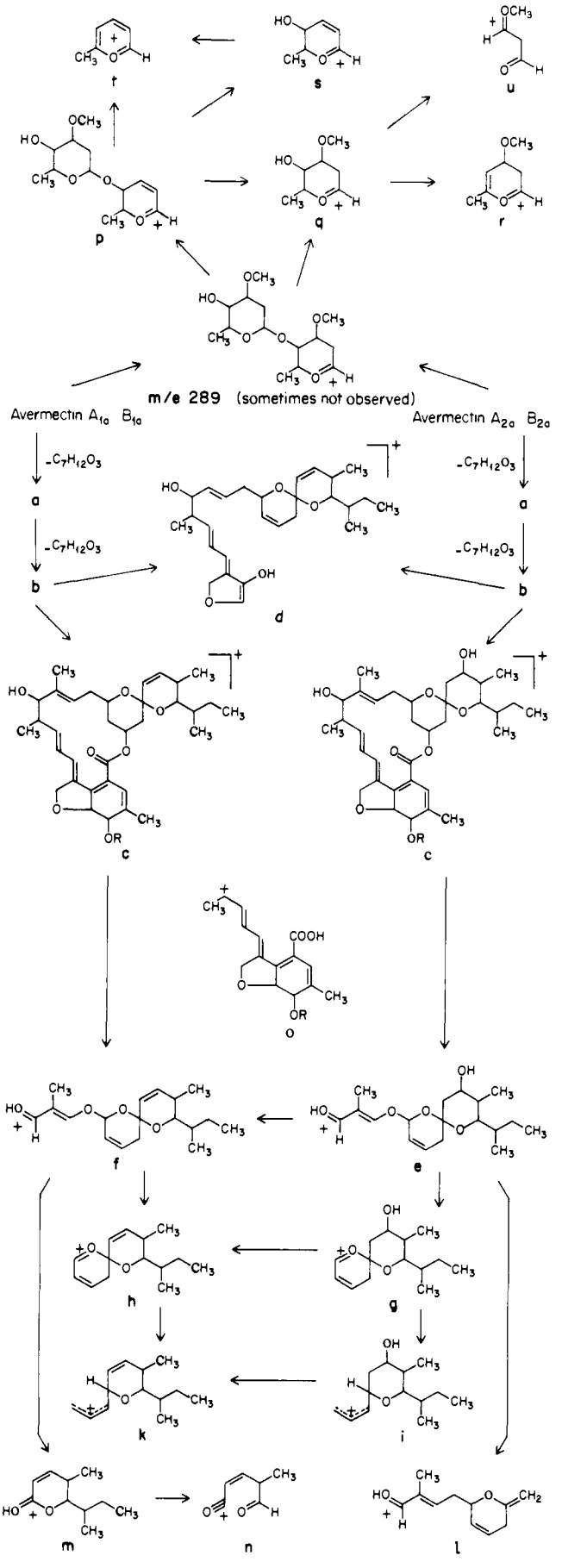

Table III. ^{1}H NMR Data for Avermectin A_{2a} and A_{2a} Aglycon[a]

	A_{2a}	A_{2a} aglycon
C2-H	3.35 (q, J = 2.0)	3.32 (q, J = 2.2)
C3-H	5.42 (q, $J \simeq 1.0$)	5.42 (q, $J \simeq 1.0$)
C4-CH_3	1.83 (s)	1.82 (s)
C5-H	3.99 (d, J = 5.5)	3.99 (d, J = 5.5)
C5-OCH_3	3.51 (s)	3.51 (s)
C6-H	4.05 (d, J = 5.5)	4.04 (d, J = 5.5)
C7-OH	4.07 (s)	3.98 (s)
C8-CH_2	4.65 (dd, J = 14.2, 2.0)	4.65 (dd, J = 14.0, 2.0)
	4.71 (dd, J = 14.0, 2.0)	4.70 (dd, J = 14.0, 2.0)
C9-H	5.85 (m)	5.80 (dt, J = 10.0, 2.0)
C10-H	5.94 (m)	5.77 (dd, J = 15.3, 10.0)
C11-H	5.94 (m)	5.72 (dd, J = 14.3, 9)
C12-H	2.54 (m)	2.54 (qn, J = 7.0)
C12-CH_3	1.18 (d, J = 7.0)	1.17 (d, J = 7.0)
C13-H	3.97 (br s)	4.02 (br s)
C13-OH		
C14-CH_3	1.51 (s)	1.52 (s)
C15-H	5.34 (t, J = 7.5)	5.32 (t)
C16-Ha	*[b]	2.36 (dd, J = 13.5, 11.0)
C16-He	*	2.29 (broadened)
C17-H	*	3.78 (m, $J_{17/18}$ = 11.0)
C18-Ha	*	0.86 (q, J = 12.0)
C18-He	1.79 (br d, J = 14.0)	1.78 (dddd, J = 12.0, 5.0, 2, 1.5)
C19-H	5.34 (tt, J = 11.5, 5.0)	5.28 (tt, J = 11.5, 5.0)
C20-Ha	1.46 (t, J = 11.5)	1.42 (t, J = 12.0)
C20-He	2.01 (dd, J = 11.0, 4.5)	2.02 (ddd, J = 12.0, 4.5, 1.5)
C22-Ha	1.69 (dd, J = 14.0, 3.5)	1.68 (dd, J = 14.0, 3.0)
C22-He	2.00 (dd, J = 14.0, 3.0)	1.99 (dd, J = 14.0, 2.5)
C23-H	3.8 (m)	3.78 (m)
C23-OH	3.54 (d, J = 10)	
C24-H	~1.58	1.58 (qdd, J = 11.0, 6.5, 1.0)
C24-CH_3	0.89 or 0.92 (d, J = 6.5)	0.91 (d, J = 6.5)
C25-H	3.59 (d, J = 11.0)	3.56 (dd, J = 11.0, 1.0)
C26-H	~1.58	1.54 (m)
C26-CH_3	0.92 or 0.89 (d, J = 6.5)	0.87 (d, J = 6.2)
C27-H2	*	1.48 (qn, J = 7.0)
C28-H3	0.97 (t, J = 7.0)	0.98 (t, J = 7.2)
C1'-H	4.78 (d, J = 3.2)	
C2'-H2	*	
C3'-H	3.8 or 3.6	
C3'-OCH_3	3.43 or 3.44 (s)	
C4'-H	3.26 (t, J = 9.0)	
C5'-H	3.85 (dq, J = 9.2, 6.5)	
C5'-CH_3	1.29 or 1.26 (d)	
C1"-H	5.42 (d, J = 3.0)	
C2"-H2	*	
C3"-H	3.6 or 3.8	
C3"-OCH_3	3.44 or 3.43	
C4"-H	3.18 (dt, J = 1.4, 9.0)	
C4"-OH	2.50 (d, J = 1.9)	
C5"-H	3.78 (dq, J = 9.5, 7.0)	
C5"-CH_3	1.26 or 1.29 (d, J = 7.0)	

[a] Spectra were recorded in $CDCl_3$ solution; chemical shifts are given in ppm relative to internal tetramethylsilane; coupling constants are given in Hz. [b] Unresolved in spectra of the natural disaccharide derivatives. Abbreviations: qn = quintet, unres = unresolved, a = axial, e = equatorial.

Epimeric disaccharide derivative **2** (R_f 0.45 on SiO_2 in $CHCl_3$–CH_3OH 9:1; 65%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 and 1.08 [d, 3, J = 7 Hz, 12-CH_3 in 1:3 ratio], 1.24 and 1.26 [d, 3, J = 6.8 Hz, 14-CH_3 in 1:3 ratio], 1.29 and 1.32 [d, 3, J = 7 Hz, 6'-CH_3 and 6"-CH_3], 1.52 and 1.53 [ddd, 1, J = 3.8, 11.5 and 13.3 Hz, C2'-H(a) in 1:3 ratio], 1.72 [ddd, 1, J = 4.2, 10.3 and 13.4 Hz, C2"-H(a)], 1.97 [br m, C12-H], 2.28 [m, C2'-H(e)], 2.33 [m, C2"-H(e)], 3.18 [t, 1, J = 9 Hz, C4"-H], 3.31 [dd, 1, J = 7.7 and 9.6 Hz, C4'-H], 3.37 and 3.38 [s, 3, C3'-OCH_3 in 1:3 ratio], 3.43 [s, 3, C3"-OCH_3], 3.45–3.95 [m, H on C3', C3", C5', C5", C11, C13, and C14]; 5.04 [dd, 1, J = 2.8 and 4.0 Hz, C1'-H, minor isomer], 5.12 [dd, 1, J = 1.7 and 3.9 Hz, C1'-H, major isomer], 5.37 [dd, 1, J = 1.1 and 4.0 Hz, C1"-H]. Spi-

roketal **3** (R_f 0.39, 62%): δ0.85 [d, 3, J = 6.8 Hz, 26a-CH_3], 0.92 [d, 3, J = 6.6 Hz, 24-CH_3], 0.96 [t, 3, J = 6.9 Hz, 28-CH_3], 1.27 [q, 1, J = 12 Hz, C18–$H_{(a)}$], 1.34 [t, 1, J = 12 Hz, C20–$H_{(a)}$], 1.40–2.15 [m, H on C16, C18(e), C20(e), C22, C24, C26, C27], 3.65 [dd, 1, J = 1.4 and 10.4 Hz, C25–H], 3.88 [m, H on C15, C17, and C23], 3.96 [tt, 1, J = 11 Hz; C19–H]; M^+ 302, *m/e* 284, 266, 257, 245, 239, 227, 221, 209, 187, 169, 161, and 111.

2 (20 mg) was stirred in 0.5 mL of 1% methanolic H_2SO_4. After 18 h, TLC (SiO_2, $CHCl_3$–CH_3OH 9:1) revealed three products of R_f 0.47, 0.46 (α- and β-methyl oleandroside), and 0.16. The solution was neutralized with solid $NaHCO_3$, filtered, and evaporated and the products were isolated by preparative TLC. By repeated chromatography, the epimeric 2-methyl pentanetriols could be separated. Tentative assignments of the 14*S* configuration to the minor and of the 14*R* configuration to the major isomer were based on Felkin's rules.[13] (14*S*)-Triol **4**: ^1H NMR [$CDCl_3$] δ 0.88 [d, 3, J = 7.0 Hz, 12a-CH_3], 1.22 [d, 3, J = 7.0 Hz, 14a-CH_3], 1.57 [OH], ~1.92 [m, C12–H], 2.53 [OH], 3.08 [OH], 3.61 [dd, 1, J = 3.7 and 7.3 Hz, C13–H], 3.69 [t, 1, J = 7.4 Hz, C11–H], ~3.95 [dq, 1, J = 4.1 and 6.2 Hz, C14–H]; M^+ 134.0936 (measured on tri-TMSi derivative), *m/e* 133, 116, 103, 89, 75, 71, 59, 45, and 32. (14*R*)-Triol **4**: ^1H NMR δ 0.99 [d, 3, J = 7.0 Hz, 12a-CH_3], 1.26 [d, 3, J = 7.0 Hz, 14a-CH_3], 1.57 [OH], 1.90 [m, C12–H], 2.53 [OH], 3.08 [OH], 3.35 [dd, 1, J = 3.0 and 5.6 Hz, C13–H], 3.70 [dd, 1, J = 3.7 and 10.7 Hz, C11–H], 3.79 [dd, 1, J = 3.7 and 10.7 Hz, C11–H], 3.91 [dq, 1, J = 3.1 and 6.2 Hz, C14–H].

(13) M. Cherest, H. Felkin, and N. Prudent, *Tetrahedron Lett.*, 2199–2204 (1968).

The Absolute Stereochemistry and Conformation of Avermectin B_{2a} Aglycon and Avermectin B_{1a}

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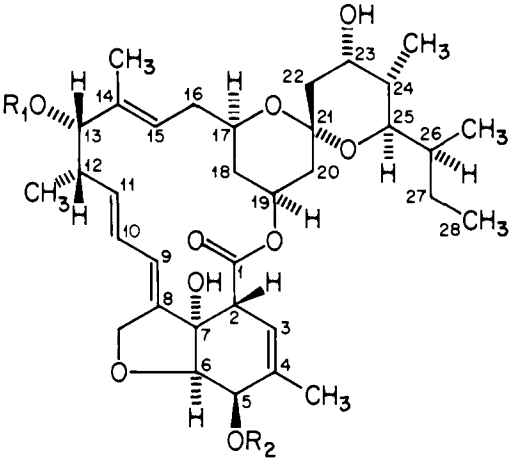
Abstract: The crystal structures of the potent antiparasitic agents avermectin B_{2a} aglycon (**1b**) [a = 15.061 (8), b = 9.005 (2), c = 14.624 (7) Å, β = 96.37 (4)°, $P2_1$, Z = 2, $C_{34}H_{50}O_9$] and avermectin B_{1a} (**3**) [a = 39.362 (13), b = 9.500 (3), c = 14.694 (2) Å, β = 106.43(2)°, $C2$, Z = 4, $C_{48}H_{72}O_{14}$] were solved to establish both the relative and absolute stereochemistry of these *Streptomyces* metabolites. Detailed ^1H NMR analyses showed that the solution conformation of the basic avermectin skeleton is virtually identical with the solid state conformation.

The avermectins are a previously undescribed series of compounds isolated from *Streptomyces avermitilis* with potent anthelmintic as well as ectoparasitic activity.[1] Initial isolation as well as structural and biological characterization indicated that at least eight related compounds possessed this remarkable activity. To unambiguously define the absolute stereochemistry as well as the conformation of the avermectins, single-crystal X-ray diffraction experiments of avermectin B_{2a} aglycon (**1b**) and avermectin B_{1a} (**3**) were undertaken.

Experimental and Methods

Avermectin B_{2a} Aglycon (1b). One of the major components, avermectin B_{2a} (**1a**), was subjected to acidic methanolic hydrolysis conditions to yield avermectin B_{2a} aglycon (**1b**).[1a] The aglycon ($C_{34}H_{50}O_9$) was recrystallized from methanol to give colorless crystals of symmetry $P2_1$ with cell unit dimensions of a = 15.061 (8), b = 9.005(2), and c = 14.624 (7) Å and β = 96.37 (4)°. The calculated density was 1.18 g/cm^3 for



1a : R_1 = $C_{14}H_{25}O_6$ (disaccharide 2)
R_2 = H
b : R_1 = H; R_2 = H
c : R_1 = H; R_2 = CH_3

Z = 2 with six molecules of methanol in the unit cell (vide infra). A suitable crystal was mounted in a Lindemann glass capillary with mother liquor. Of the 2902 unique reflections measured ($2\theta \leq 115°$) with graphite monochromated Cu Kα radiation (λ = 1.5418 Å), 2510 (86%) were significant ($I \geq 3\sigma I$). These observed reflections were corrected for

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