L-155,175: A NEW ANTIPARASITIC MACROLIDE

FERMENTATION, ISOLATION AND STRUCTURE

MICHAEL A. GOETZ*, PAMELA A. MCCORMICK, RICHARD L. MONAGHAN, DAN A. OSTLIND

Merck Sharp and Dohme Research Laboratories

OTTO D. HENSENS*, JERROLD M. LIESCH and GEORG ALBERS-SCHONBERG

Merck Institute for Therapeutic Research
Rahway, New Jersey 07065, U.S.A.

(Received for publication September 10, 1984)

A new antiparasitic macrolide, L-155,175, produced by a strain of *Streptomyces hygroscopicus*, has been isolated; its structure was determined by physico-chemical means. It is active against the tapeworm *Hymenolepis diminuta* in rats.

In the course of screening of fermentation broths for antiparasitic compounds, we have discovered a new macrolide designated as L-155,175 produced by a strain of *Streptomyces hygroscopicus*. The compound is effective against the tapeworm *Hymenolepis diminuta* in rats.

This paper describes the production of L-155,175 by fermentation, a scheme for its isolation and purification and its structural elucidation by physico-chemical means.

Microorganism

The producing microorganism was a new soil isolate identified as a strain of *Streptomyces hygroscopicus* (MA5285; deposited as ATCC 31955).

Fermentation

The organism was preserved in lyophiles as MA5285. A lyophile was used to inoculate seed medium A (54 ml of A per 250-ml baffled flask; see Table 1). After 2 days incubation at 28°C and 220

Table 1. Compositions of fermentation media.

Seed Medium A					
Dextrose	1.0 g	$MgSO_4 \cdot 7H_2O$	0.05 g		
Soluble starch	10.0 g	0.5 M KH_2PO_4/Na_2HPO_4 buffer (pH 7)	2.0 ml		
Beef extract	3.0 g	$CaCO_3$	0.5 g		
Yeast auto lysate	5.0 g	Distilled H_2O	1,000 ml		
N-Z-Amine Type E	5.0 g	pH adjusted to 7.0~7.2			
Medium B		Medium C		Basal Medium D	
Tomato paste	40.0 g	Oat flour	10.0 g	K_2HPO_4	0.1 g
Oat flour	10.0 g	Dextrose	10.0 g	$MgSO_4 \cdot 7H_2O$	0.5 g
Distilled H_2O	1,000 ml	Asparagine	1.0 g	Trace element mixture	10 ml
pH adjusted to 7.0		K_2HPO_4	0.1 g	$CaCO_3$	3.0 g
		$MgSO_4 \cdot 7H_2O$	0.5 g	Distilled H_2O	1,000 ml
		Yeast extract	0.5 g		
		Trace element mixture	10 ml		
		$CaCO_3$	3.0 g		
		Distilled H_2O	1,000 ml		
		pH adjusted to 7.2			

rpm, approximately 40 ml of the contents of one flask were used to inoculate each flask of medium B or C (300~500 ml per 2-liter unbaffled flask). The broth was harvested after 4 days incubation at 28°C and 150 rpm.

Studies on the nutrient requirements of the fermentation were carried out in modifications of a basal medium D. Nitrogen sources were added at 0.1~0.5%, carbon sources at 1.0~4.0%, and the pH of each specific medium was adjusted to 7.0~7.2, unless otherwise indicated. In these studies, 1~2 ml of seed flask A growth were used to inoculate 50 ml of the modified medium D (per 250-ml flask) and all incubations were carried out at 28°C and 220 rpm.

Fermentation Results

In media B and C, production titers (determined by HPLC) increased over the fermentation period of 4 days, although no increase in biomass was noted after day 2. Maximum titers of L-155,175 obtained in B and C were comparable, ranging from 15~20 μg/ml. The studies in medium D suggested that some pH control was necessary. The presence of $CaCO_3$ stabilized the pH at values of 6.0~8.0; however, replacement of the $CaCO_3$ with buffers such as 0.1 M 4-morpholinopropanesulfonic acid or 2-(*N*-morpholino)ethanesulfonic acid, pH 7.0, was less favorable to production.

A variety of carbon sources were tested for ability to support growth and production of L-155,175. With $(NH_4)_2SO_4$ as the nitrogen source, citrate and propionate did not support growth; glucose and acetate gave minimal growth, but less than 1 μg/ml production; sucrose, glycerol, ribose, maltose and lactose supported moderate growth while L-155,175 titers ranged from 2.5~4.0 μg/ml.

Nitrogen sources such as yeast extract and yeast ribonucleic acid offered better growth and higher production (4~10 μg/ml). The addition of glycerol and a second, complex carbon source such as oat flour improved production to 10~20 μg/ml.

Assay of L-155,175 in Fermentation Broths

All assays were performed by reverse-phase HPLC. Whole broth samples (40 ml) were extracted twice with 20 ml of ethyl acetate; the combined extracts were evaporated to dryness under reduced pressure. The residue was dissolved in 2.0 ml of methanol - methylene chloride (4: 1); 10 μl of this solution was used for assay on a Whatman ODS-3 column (4.6×25 mm, packed with 10 μm particles and kept at 40°C with a constant temperature water-bath). The eluent, delivered at 2.0 ml/minute, consisted of acetonitrile - 0.01 M ammonium phosphate at pH 3 (7: 3).

Isolation and Purification

The fermentation broth (10 liters) was extracted three times with 3 liters of ethyl acetate. The combined extracts were concentrated under reduced pressure to an oil, diluted to 60 ml with methylene chloride - methanol (9: 1) and applied onto a column packed with 1 kg of silica gel (E. Merck, Silica Gel 60). After washing with 4 liters of methylene chloride, the column was eluted with methylene chloride - methanol, 95: 5. The fractions containing the crude L-155,175 were pooled and evaporated to dryness under reduced pressure. The residue was dissolved in 10 ml of methanol - methylene chloride (2: 1) and further fractionated on a column of Sephadex LH-20 (Pharmacia Fine Chemicals: column dimensions 2.5×200 cm). Final purification of the active compound was achieved by reverse-phase HPLC (1.4×183 cm column packed with E. Merck LiChroprep RP-18, 25~40 μm particles) using a stepwise gradient of acetonitrile in 0.01 M ammonium phosphate at pH 3. Pure L-155,175 eluted with 70% acetonitrile; it was extracted from the eluate with methylene chloride and its homogeneity ascertained in

several TLC systems (E. Merck Silica Gel 60 F_{254}: methylene chloride - methanol, 9: 1; hexane - acetone, 3: 2; toluene - ethyl acetate, 1: 1: Whatman $KC_{18}F$: methanol-water, 85: 15; acetonitrile - water -acetic acid, 75: 24: 1) and by HPLC (Whatman ODS-3, acetonitrile - 0.01 M ammo - nium phosphate at pH 3, 7: 3; Whatman PXS silica gel, chloroform - acetonitrile - acetic acid, 90: 9: 1). Yield 160 mg.

Table 2. Efficacy of L-155,175 vs. *H. diminuta* in rats.

Treatment[a)]	Dosage (mg/kg)	Number of rats	Average number of worms at necropsy
Placebo	—	6	5.5
L-155,175	25	1	0
L-155,175	12.5	2	3
L-155,175	6.25	2	6
L-155,175	3.125	1	5

[a)] Dissolved in PEG 400-DMSO (1: 2).

Physico-chemical Properties

L-155,175 was obtained as a yellow amorphous powder, soluble in methylene chloride, acetone, ethyl acetate and the lower alcohols but virtually insoluble in water. It is fairly stable in acid and in base. Its UV spectrum exhibited the following maxima: λ_{max}^{MeOH} nm (ε) 248 (30,900), 275 (sh, 16,800), 324 (sh, 3,000). Specific rotation: $[\alpha]_D^{22}$ +16.6° (*c* 0.29, MeOH); mp >106°C (dec).

Biological Properties

Table 2 gives data on the efficacy of L-155,175 against *H. diminuta* in rats. In these studies, each rat was infected with 8 cysticercoids 12 weeks prior to treatment. The placebo or L-155,175 was administered as a single oral dose. Necropsy was performed six days after treatment.

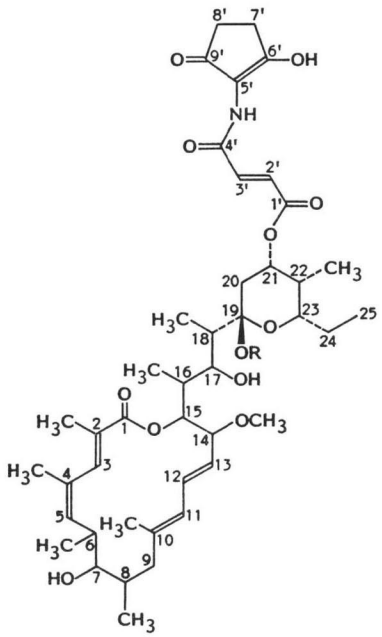
Characterization and Structural Elucidation

On the basis of spectroscopic comparisons with the Na^+-K^+ ATPase inhibitor components of L-681,110 (Fig. 1) previously discovered in these laboratories[1)], structure **1** is proposed for L-155,175.

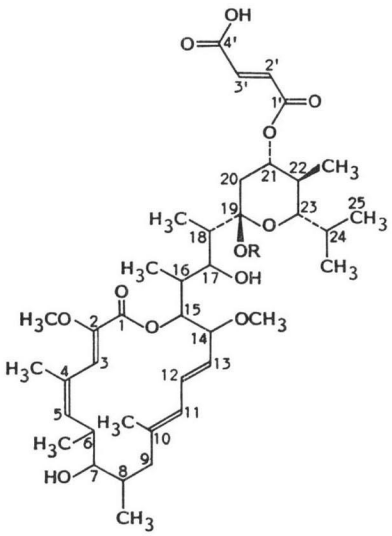
Electron-impact (EI) spectra afforded a highest mass ion at *m/z* 574.3879 corresponding to $C_{34}H_{54}O_7$ (M^+_{app}) (calcd 574.3870) and a base peak at *m/z* 211.0475 corresponding to $C_9H_9NO_5$ (calcd 211.0480). The ^{13}C NMR spectrum in $CDCl_3$ (Table 3) indicated a total of 43 carbons and a non-active proton count of 58 from 'coupled' spectra. Five active protons were evident from ^1H NMR spectra in $CDCl_3$ or benzene-d_6 (Table 4) thereby strongly suggesting the sum of the two moieties, $C_{43}H_{63}NO_{12}$ (MW 785) as the molecular formula for L-155,175. This is in agreement with the weak $M+Na^+$ peak at *m/z* 808 by field-desorption (FD) MS, the most prominent ions appearing at *m/z* 597 ($M^+_{app}+Na$) and *m/z* 574 (M^+_{app}). Apparently, a very facile decomposition occurs under all MS conditions.

The nature of the $C_9H_9NO_5$ fragment became clear from an analysis of the ^1H NMR spectrum in $CDCl_3$. The spectrum contains two active protons at δ 13.27 (hydrogen-bonded OH) and δ 8.40 (slow-exchanging amide proton) and a $-CH_2CH_2-$ multiplet centered at δ 2.61 suggestive of a 2-acylamino-cyclopentan-1,3-dione moiety previously observed in manumycin[2)], asukamycin[3)] and reductiomycin[4,5)]. The spectrum of the ATPase inhibitor **3** in CD_3OD contains two broad doublets at δ 6.65 and 6.91 (J= 16 Hz)[1)] which are also present in the spectrum of L-155,175 but to lower field at δ 6.91 and 7.18 and which are no longer broadened. The data for the $C_9H_9NO_5$ moiety are therefore consistent with the fumarate amide of 2-aminocyclopentan-1,3-dione which is identical to flavensomycinic acid (**6**) (Fig. 2) previously isolated from the antifungal and insecticidal natural product flavensomycin by acid hydrolysis[6,7,8)]. The presence of this moiety in L-155,175 accounts for its yellow color and strong UV maximum at 248 nm (ε 30,900) and weak absorption near 324 nm (ε 3,000) in methanol. The moiety is also well characterized by ^{13}C NMR (Table 3). Assignment of the 2-acylaminocyclopentan-1,3-dione carbons follows readily from those reported for reductiomycin[4)]. The two methylene triplets at 25.9 and 32.3

Fig. 1. Structures of L-155,175 and L-681,110 components.

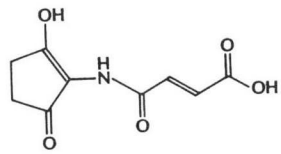


L-155,175 (**1**) R = H
2 R = CH_3



L-681,110 A_1 (**3**) R = H
L-681,110 A_2 (**4**) R = CH_3
L-681,110 B_1 (**5**) R = H and substitution of OCH_3 for fumarate moiety.

Fig. 2. Structure of flavensomycinic acid.



6

ppm are clearly distinguishable from those of the aglycone on the basis of their large $^1J_{^{13}C-^1H}$ values. Similarly, the olefinic methynes of the fumarate portion assigned by comparison with ethyl fumarate[1], are also characterized by larger $^1J_{^{13}C-^1H}$ values than those of the aglycone.

Both **1** and **3** behave chromatographically as carboxylic acids as their mobilities on TLC are retarded when ammonia is present in the solvent system. The enol is sufficiently acidic to readily catalyze formation of the methylketal **2** (Fig. 1) in refluxing methanol as observed for L-681,110A_1[1], but is sufficiently intramolecularly hydrogen-bonded (as inferred from the downfield shifted OH proton near δ 13.3) not to cause streaking on TLC.

Besides the difference in the nature of the ester side chain, comparison of the ^1H NMR spectral data with those of L-681,110B_1 lacking the fumarate moiety, reveals two other regions of difference in the structure which will now be discussed. The results are summarized for benzene-d_6 in Table 4 but confirmatory decoupling studies were also made in $CDCl_3$ and benzene-d_6 - $CDCl_3$ mixtures.

Dienoic Acid Chromophore

The UV maximum at 284 nm of L-681,110B_1, which is attributed to the α-methoxy-γ-methylhexadienoic acid chromophore[1], appears as a shoulder near 275 nm (ε 16,800) in L-155,175. Modification of

Table 3. ^{13}C NMR assignments of L-155,175 and L-681,110A$_1$[1] in $CDCl_3$ at 25°C[a].

Assignment	L-155,175	L-681,110A$_1$	Assignment	L-155,175	L-681,110A$_1$
C2-CH_3	5.0 q (126)	—	C15	76.3 d (~146)	77.1 d (145)
C4-CH_3[b]	7.0 q (127)	7.1 q (127)	C7[e]	81.5 d (142)	81.3 d (141)
C10-CH_3[b]	9.8 q (127)	9.9 q (127)	C17[e]	82.8 d (142)	82.5 d (143)
C24-CH_3[c]	10.8 q (126)	12.3 q (126)	C19	99.6 s	99.2 s
C6-CH_3[c]	13.8 q (128)	14.0 q	C12[f]	125.6 d (~150)	125.6 d (~146)
C22-CH_3	15.3 q (128)	14.4 q (126)	C13[f]	127.7 d (~157)	127.4 d (151±2)
C8-CH_3[c]	17.6 q (~129)	17.3 q (126)	C4	134.9 s	133.3 s
C16-CH_3[c]	20.2 q (126)	20.2 q	C11	132.9 d (149)	133.5 d (149)
C24-CH_3	—	21.2 q (126)	C3	145.2 d (~148)	134.0 d (151)
C18-CH_3[c]	21.6 q (127)	21.8 q (126)	C2	122.6 s	~141.4 s
C24	25.4 t (126)	28.0 d (124)	C5	147.1 d (~150)	143.4 d (~153)
C6[d]	35.5 d (130)	36.9 d (124)	C10	143.1 s	143.6 s
C8[d]	36.8 d (~126)	37.3 d	C1	172.6 s	167.8 s
C16[d]	38.0 d (129)	38.2 d			
C22	39.9 d (~133)	40.2 d	C7′	25.9 t (136)	—
C20	34.4 t (128)	40.0 t	C8′	32.3 t (135)	—
C9	41.3 t (128)	41.4 t	C5′	115.4 s	—
C18	41.7 d (~131)	42.1 d	C2′	133.7 d (168)	~136.4 v br
C2-OCH_3	—	60.1 q (145)	C3′	133.8 d (165)	~130.5 v br
C14-OCH_3	55.7 q (142)	55.7 q (142)	C4′	164.3 s	~170.4 v br
C14	70.5 d (146)	70.9 d (146)	C1′	164.5 s	~165.4 v br
C23	71.3 d (~146)	75.4 d (~144)	C6′	176.1 s	—
C21	73.3 d (~154)	76.1 d (~146)	C9′	198.5 s	—

[a] ^{13}C NMR data were determined at 75 MHz on a Varian SC-300 instrument. Chemical shifts are in ppm downfield of TMS. $^1J_{^{13}C-^1H}$ values in Hz±1.0 Hz are given in parentheses; where not given, ^1J values could not be determined because of overlap of signals.
Abbreviations: s=singlet, d=doublet, t=triplet, q=quartet, v=very, br=broad.
[b,c,d,e,f]: Interchangeable assignments.

the chromophore is supported by the absence of the C2-OCH_3 singlet at δ 3.66 in the ^1H NMR spectrum in benzene-d_6 (Table 4). Moreover, the sharp singlet for H3 at δ 6.84 is found at lower field (δ 7.40) and is broadened because of allylic coupling to one of three vinylic methyl groups at δ 2.11 not present in L-681,110B$_1$. The resulting α,γ-dimethylhexadienoic acid thus satisfactorily accounts for the UV adsorption near 274 nm and appreciable downfield shift of the H3 proton[10].

Tetrahydropyran Ring

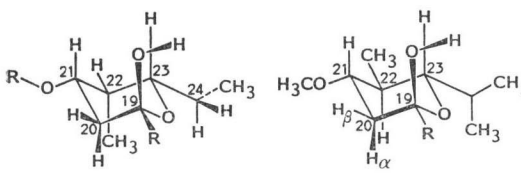
The nature of the tetrahydropyran ring was determined by a series of decoupling experiments in benzene-d_6 (Fig. 3 and Table 4). Irradiation of the doublet of triplets at δ 5.89 (H21) resulted in collapse of H20β from a doublet of doublets to a doublet (J=12 Hz) and H20α from a broad triplet to a broad doublet (J=12 Hz). The latter was shown to be long-range coupled (J=1.5 Hz) to the active proton at δ 6.19 by irradiation at either position (or on spiking with D_2O or CD_3OD) similar to that observed in L-681,110B$_1$[1]. Furthermore, irradiation of H21 results in collapse of H22 at δ 2.15 from a multiplet to a broad quartet (J=6.5 Hz) whereas irradiation of the methyl doublet at C22 collapses H22 to a broad doublet ($J_{H21,H22}$=4.5, $J_{H22,H23}$=1.5 Hz). Based on the coupling constants, the stereochemistry at C19 and C21 is therefore the same as in L-681,110B$_1$ but inverted at C22, *i.e.* the methyl group has the axial configuration. Irradiation of H23 at δ 4.22 slightly sharpens the multiplets at δ 2.15 (H22) and δ 1.20 (H24β) the latter being partly obscured by H20α. Irradiation at δ 1.20 and 2.15 in turn collapses the methyl triplet at δ 1.01 to a doublet (J=6.5 Hz) and establishes $J_{H23,H24\alpha}$=9 and $J_{H23,H24\beta}$=3.0 Hz.

Table 4. ^{1}H NMR assignments of L-155,175 and L-681,110B$_1$[1] in benzene-d_6 at 25°C[a].

Assignment	L-155,175	L-681,110B$_1$	Assignment	L-155,175	L-681,110B$_1$
C2-OCH$_3$	—	3.66 s	C17-OH	5.44 d (~3)	5.13 d (4)
C2-CH$_3$	2.11 br s	—	H18	~1.90 m	1.92 dq (1.5, 7)
H3	7.40 br s	6.84 s	C18-CH$_3$	1.13 d (7)	0.92 d (7)
C4-CH$_3$	1.68 br s	2.02 br s	C19-OH	6.19 br s	5.78 d (2.0)
H5	5.91 d (8.5)	5.84 br d (9)	H20α	1.53 br t (12)	1.22 dt (2, 12, 12)
H6	2.26 obsc	2.33 ddq (1.5, 7, 9)	H20β	2.30 dd (4.5, 12)	2.68 dd (4.5, 12)
C6-CH$_3$	0.85 d (7)	0.89 d (7)	H21	5.89 dt (4.5, 4.5, 12)	3.54 dt (4.5, ~11.5)
H7	2.87 br d (5.5)	2.91 br m	C21-OCH$_3$	—	3.10 s
C7-OH	~1.96 br s	~0.90 br s	H22	2.15 m	1.71 tq (7, ~10, ~10)
H8	1.76 m	1.77 m	C22-CH$_3$	0.90 d (7)	1.04 d (7)
C8-CH$_3$	0.72 d (~6)	0.75 d (7)	H23	4.22 br d (9)	3.84 dd (2, 10)
C9$\langle^{H}_{H}$	~2.02 m	~1.97 m	C24$\langle^{H}_{H}$	1.20 m 1.61 m	— 1.92 m
C10-CH$_3$	1.87 br s	1.88 br s	C24-CH$_3$	1.01 t (7)	1.20 d (7) 1.10 d (7)
H11	5.74 br d (11)	5.67 br d (11)	H2′	7.13 obsc	
H12	6.54 dd (11, 15)	6.51 dd (11, 15.5)	H3′	7.25 d (16)	
H13	5.22 dd (9, 15)	5.13 dd (9, 15.5)	CONH	8.89 br s	
H14	3.93 t (9)	3.92 t (9)	C6′-OH	~13.86 br s	
C14-OCH$_3$	3.11 s	3.27 s	C7′$\langle^{H}_{H}$	~2.02 m	
H15	5.43 br d (~8)	5.38 dd (1.5, 9)	C8′$\langle^{H}_{H}$	~1.90 m	
H16	2.42 ddq (~1.5, 7, 10.5)	2.45 ddq (1.5, 7, 10.5)			
C16-CH$_3$	0.80 d (6.5)	0.86 d (7)			
H17	4.56 br d (10.5)	4.54 ddd (1.5, 4, 10.5)			

[a] Chemical shifts are given in ppm downfield of internal TMS at 300 MHz. Coupling constants in Hz (±0.5) are given in parentheses.
Abbreviations: m=multiplet, obsc=obscured (overlapping signals), see footnote of Table 3.

Fig. 3. Tetrahydropyran partial structures in L-155,175 (**1**) and L-681,110B$_1$ (**5**) showing relative stereochemistry.



The isopropyl group at C23 in L-681,110B$_1$ is thus replaced by an ethyl group, which probably has the stable equatorial orientation although the small coupling of ~1.5 Hz between H22 and H23 does not rule out an axial configuration.

Substitution of the fumarate moiety ($C_4H_4O_4$) at C21 of L-681,110A$_1$ ($C_{39}H_{60}O_{12}$) **3** for flavensomycinic acid ($C_9H_9NO_5$), an ethyl for an isopropyl group at C23 and a methyl for a methoxy group at C2, are consistent with the molecular formula $C_{43}H_{63}NO_{12}$ for L-155,175 as well as the ^{13}C NMR data. By comparison with L-681,110A$_1$ (Table 3), there should be no net change in the number of upfield methyl carbons and one instead of two methoxy carbons as observed. Substitution of the C2-OCH$_3$ group by a methyl group has the same expected effect on the chemical shift of C3 as H3[10,11] *i.e.*, a downfield shift from 134.0 to 145.2 ppm.

HRMS data on L-155,175 (Table 5) lend further support to the structure. The apparent molecular ion M^+_{app} 574 readily loses two molecules of water and one of methanol as depicted in Scheme 1. Loss

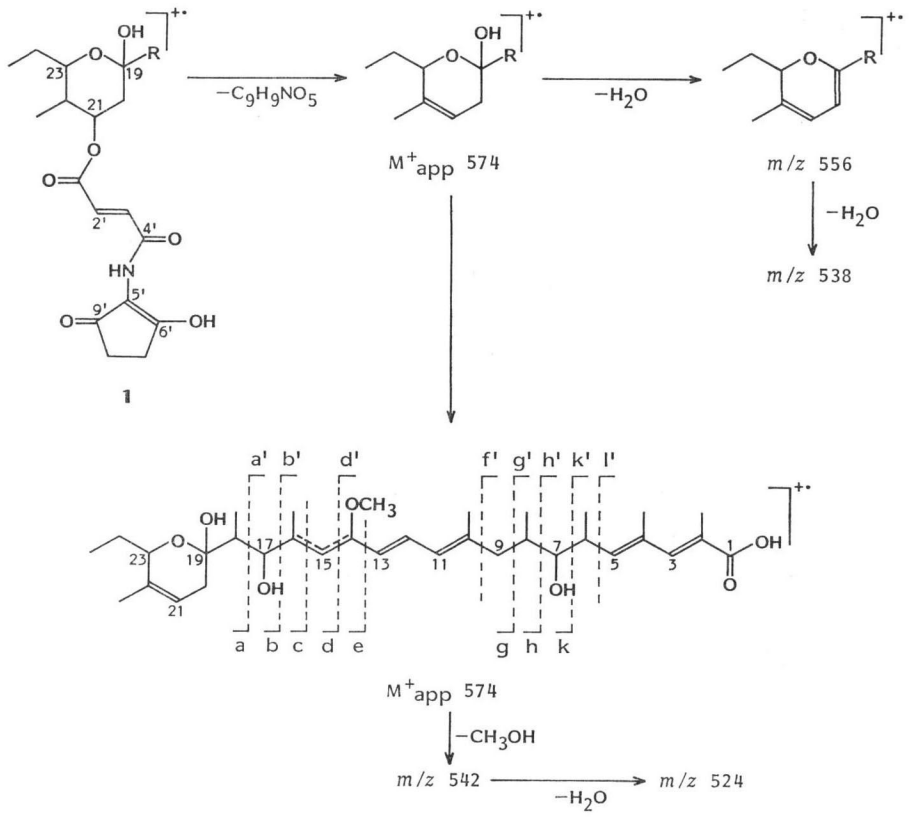
Table 5. High resolution mass spectral data for L-155,175.

Fragment ion[a]	Formula	Mass		Fragment ion[a]	Formula	Mass	
		Calcd	Found			Calcd	Found
M^+_{app}	$C_{34}H_{54}O_7$	574.3870	574.3879	e+H−$2H_2O$	$C_{14}H_{19}O_2$	219.1385	219.1354
$M^+_{app}-H_2O$	$C_{34}H_{52}O_6$	556.3764	556.3760	$-CH_3CH_2\cdot$	$C_9H_9NO_5$	211.0480	211.0475
$M^+_{app}-2H_2O$	$C_{34}H_{50}O_5$	538.3658	538.3654	f′−H_2O	$C_{13}H_{19}O_2$	207.1385	207.1380
$M^+_{app}-CH_3OH$	$C_{33}H_{50}O_6$	542.3607	542.3563	g−H_2O	$C_{12}H_{17}O_2$	193.1228	193.1234
$M^+_{app}-CH_3OH$				c+H−$2H_2O$	$C_{13}H_{19}O$	191.1436	191.1402
$-H_2O$	$C_{33}H_{48}O_5$	524	524[b]	h′	$C_{10}H_{15}O_3$	183.1021	183.1035
a′−H	$C_{24}H_{36}O_5$	404	404[b]	b−H_2O	$C_{11}H_{17}O_2$	181.1228	181.1207
a′−H−H_2O	$C_{24}H_{34}O_4$	386.2457	386.2410	h′−H_2O	$C_{10}H_{13}O_2$	165.0916	165.0910
b′	$C_{23}H_{35}O_4$	375.2535	375.2575	b−$2H_2O$	$C_{11}H_{15}O$	163.1123	163.1166
g−H−H_2O or	$C_{22}H_{32}O_3$	344.2352	344.2347	c+H−$2H_2O$			
h−H_2O−$CH_3CH_2\cdot$				$-CH_3CH_2\cdot$	$C_{11}H_{14}O$	162.1045	162.1083
d′	$C_{20}H_{31}O_4$	335.2222	335.2233	k′	$C_9H_{13}O_2$	153.0916	153.0899
d′+H−H_2O	$C_{20}H_{30}O_3$	318.2195	318.2153	a−H_2O	$C_{10}H_{15}O$	151.1123	151.1132
d′−H−CO_2	$C_{19}H_{30}O_2$	290.2246	290.2273	h′−CO_2	$C_9H_{15}O$	139.1123	139.1143
d	$C_{14}H_{23}O_3$	239.1647	239.1655	b−$2H_2O$			
e+H−CH_3OH				$-CH_3CH_2\cdot$	$C_9H_{11}O$	135.0810	135.0818
$-H_2O$	$C_{15}H_{22}O_2$	234.1619	234.1617	1′	$C_7H_9O_2$	125.0603	125.0615

[a] See Scheme 1. HRMS measurements were performed on a Varian MAT-731 mass spectrometer. M^+_{app}=apparent molecular ion.

[b] Only low-resolution values.

Scheme 1. Mass spectral fragmentation of L-155,175 (**1**).



of the ethyl side chain is less prominent than for the isopropyl equivalent in L-681,110A_1 and can only be observed from the smaller fragments b, c, e and g. The main fragmentations are reminiscent of those in L-681,110A_1[1] and confirm the $CH_3O \rightarrow CH_3$ and isopropyl→ethyl substitutions. In particular, the abundant fragments a′ at *m/z* 420 ($C_{24}H_{36}O_6$) and d′ − H_2O at *m/z* 334 ($C_{20}H_{30}O_4$) in L-681,110A_1, occur at 16 mass units lower at *m/z* 404 ($C_{24}H_{36}O_5$) and *m/z* 318 ($C_{20}H_{30}O_3$) respectively in L-155,175.

Besides flavensomycin, two other insecticidal metabolites of *Streptomyces* species, prasinons A and B[12,13], have been found to liberate flavensomycinic acid (**6**) on acid hydrolysis. From the limited physico-chemical properties which have been reported for these compounds it appears that prasinon B is closely related to, or identical with, L-155,175 (**1**). Two further macrolides containing a flavensomycinic acid moiety have recently been reported: bafilomycin B[14] and virustomycin A[15].

References

1) Hensens, O. D.; R. L. Monaghan, L. Huang & G. Albers-Schönberg: Structure of the sodium and potassium ion activated adenosinetriphosphatase inhibitor L-681,110. J. Am. Chem. Soc. 105: 3672～3679, 1983
2) Schroder, K. & A. Zeeck: Manumycin. Tetrahedon Lett. 1973: 4995～4998, 1973
3) Kakinuma, K.; N. Ikekawa, A. Nakagawa & S. Ōmura: The structure of asukamycin, a possible shunt metabolite from 3-dehydroquinic acid in the shikimate pathway. J. Am. Chem. Soc. 101: 3402～3404, 1979
4) Shizuri, Y.; M. Ojika & K. Yamada: Structure of an antitumor antibiotic, reductiomycin. Tetrahedron Lett. 1981: 4291～4294, 1981
5) Shimizu, K. & G. Tamura: Reductiomycin, a new antibiotic. II. Structural elucidation by spectroscopic studies. J. Antibiotics 34: 654～657, 1981
6) Canonica, L.; A. Corbella, G. Jommi, F. Pelizzoni & C. Scolastico: The synthesis of flavensomycinic acid. Tetrahedron Lett. 1966: 3031～3034, 1966
7) Gottlieb, D.: Flavensomycin. *In* Antibiotics. I. Mechanism of Action. *Ed.*, Gottlieb, D. & P. D. Shaw, pp. 617～620, Springer, Berlin, 1967
8) Canonica, L.; G. Jommi & F. Pelizzoni: Structure of flavensomycinic acid. Tetrahedron Lett. 1961: 537～543, 1961
9) Korte, F. & D. Scharf: Zur Darstellung und Stereochemie der 3,5-Dimethyl- und 2,3,5-Trimethyl-sorbinsäure und deren Ester. Chem. Ber. 95: 443～454, 1962
10) Brouwer, H. & J. B. Stothers: ^{13}C Nuclear magnetic resonance studies. XVI. ^{13}C Spectra of some substituted acrylic acids and their methyl esters. A correlation of olefinic shieldings in α,β-unsaturated carbonyl systems. Canad. J. Chem. 50: 601～611, 1972
11) Rojas, A. C. & J. K. Crandall: Carbon-13 nuclear magnetic resonance spectroscopy. Substituted vinyl ethers and acetates. J. Org. Chem. 40: 2225～2229, 1975
12) Box, S. J.; M. Cole & G. H. Yeoman: Prasinons A and B: Potent insecticides from *Streptomyces prasinus*. Appl. Microbiol. 26: 699～704, 1973
13) Grove, J. F.; M. Fukuoka, A. W. Johnston & J. N. C. Lopes: The chemistry of prasinons A and B. Identification of the nitrogen-containing moiety. J. Chem. Soc., Perkin I 1977: 2441～2443, 1977
14) Werner, G.; H. Hagenmaier, H. Drautz, A. Baumgartner & H. Zähner: Metabolic products of microorganisms. 224. Bafilomycins, a new group of macrolide antibiotics. Production, isolation, chemical structure and biological activity. J. Antibiotics 37: 110～117, 1984
15) Ōmura, S.; N. Imamura, K. Hinotozawa, K. Otoguro, G. Lukacs, R. Faghih, R. Tolmann, B. H. Arison & J. L. Smith: The structure of virustomycin A. J. Antibiotics 36: 1783～1786, 1983