Structure Determination of LL-F28249α, β, γ, and λ, Potent Antiparasitic Macrolides from *Streptomyces cyaneogriseus* ssp. *noncyanogenus*

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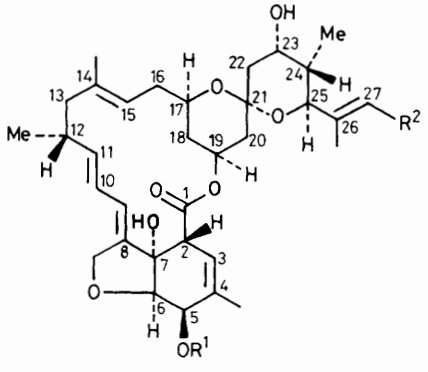
The structures of four antiparasitic macrocyclic lactones, LL-F28249α, λ, β, and γ (**1**)—(**4**) derived from the actinomycete *Streptomyces cyaneogriseus* ssp. *noncyanogenus*, were determined by spectroscopic methods and by *X*-ray crystallography of the γ-component (**4**).

Structures have been determined for a new family of potent antiparasitic antibiotics derived from *Streptomyces cyaneogriseus* ssp. *noncyanogenus*. These compounds designated LL-F28249α, λ, β, and γ (**1**)—(**4**) possess potent activity against commercially important endo- and ecto-parasites of cattle, sheep, swine, dogs, and horses.† The LL-F28249 compounds are related to the milbemycins[1] and avermectins[2] but are unique in that they each contain a trisubstituted double bond (Δ^{26}) in their side chains. This feature, combined with the rare substitution pattern for C-22 and -23 (found only in the avermectin '2' series)[3] may be responsible for their highly potent parasiticidal activity.

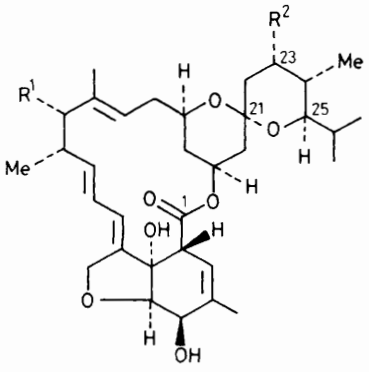


		R^1	R^2
(**1**)	LL-F28249 α	H	Pr^i
(**2**)	LL-F28249 λ	Me	Pr^i
(**3**)	LL-F28249 β	H	Me
(**4**)	LL-F28249 γ	Me	Me

† The spectrum of antiparasitic activity demonstrated by these compounds is similar to that obtained with the avermectin derivative ivermectin. Details concerning antiparasitic efficacy will be published separately.

LL-F28249α (**1**) was determined by high-resolution electron-impact mass spectrometry (h.r.e.i.m.s.) to have the formula $C_{36}H_{52}O_8$.‡ Its characteristic u.v. chromophore and e.i.m.s. fragment ions at *m/z* 151, 248, and 314 were clear indicators of the basic milbemycin structure.[1] Comparison of ^1H and ^{13}C n.m.r. data with those of the known milbemycins indicated identity between (**1**) and milbemycin D (**5**) for C-1—21, comprising the macrocycle and left-hand portion of

‡ F28249α (**1**): $C_{36}H_{52}O_8$ (M^+ *m/z* 612.3705, calc. 612.3662); $[\alpha]_D^{26}$ +133° (*c* 0.3, acetone); $\lambda_{max.}$ (MeOH) (log ε) 244 (4.47) nm; $\nu_{max.}$ (KBr) 3439, 2960, 2925, 1714, 1454, 1374, 1338, 1171, 1120, 996, and 967 cm^{-1}; *m/z* (electron impact, 70 eV) 612 (M^+; 16%), 594 (17), 466 (55), 448 (17), 425 (15), 354 (30), 314 (27), 248 (13), 247 (12), 237 (11), 219 (13), 204 (17), 152 (88), 151 (93), and 95 (100); ^1H n.m.r. ($CDCl_3$, 300 MHz), δ(Me_4Si) (numbering corresponds to that in Figure 2) 0.802 (3H, d, *J* 5.9 Hz, Me-24a), 0.883 (1H, m, 18-H_{ax}), 0.953 (3H, d, *J* 6.6 Hz, Me-29 or -28a), 1.00 (3H, d, *J* 6.6 Hz, Me-28a or -29), 1.05 (3H, d, *J* 6.6 Hz, Me-12a), 1.40 (1H, t, *J* 12 Hz, 20-H_{ax}), 1.53 (3H, s, Me-14a), 1.61 (3H, s, Me-26a), 1.70 (1H, dd, *J* 14, 3.2 Hz, 22-H_{ax}), 1.87 (3H, br.s, Me-4a), 1.99 (1H, dd, *J* 14, 2.4 Hz, 22-H_{eq}), 2.58 (1H, m, 28-H), 3.27 (1H, q, *J* 2.0 Hz, 2-H), 3.62 (1H, m, 17-H), 3.74 (1H, d, *J* 11 Hz, 25-H), 3.80 (1H, m, 23-H), 3.95 (1H, d, *J* 6.2 Hz, 6-H), 4.29 (1H, br.d, *J* 6.0 Hz, 5-H), 4.68 (2H, AB, CH_2-8a), 4.95 (1H, dd, *J* 11, 5 Hz, 15-H), 5.20 (1H, d, *J* 9.1 Hz, 27-H), and 5.41 (1H, br.s, 3-H); ^{13}C n.m.r. ($CDCl_3$, 75 MHz), δ(Me_4Si), 11.0 (26a), 13.9 (24a), 15.5 (14a), 19.9 (4a), 22.2 (12a), 22.8 (28a, 29 unresolved), 26.8 (28), 34.7 (16), 35.9 (24), 36.0 (12), 36.1 (18), 40.7 (20), 41.1 (22), 45.7 (2), 48.4 (13), 67.7 (17), 67.8 (5), 68.4 (8a), 68.5 (19), 69.3 (23), 76.7 (25), 79.3 (6), 80.2 (7), 99.7 (21), 118.0 (3), 120.3 (9, 15 unresolved), 123.3 (10), 130.6 (26), 137.2 (27), 137.3 (14), 137.7 (4), 139.4 (8), 142.8 (11), and 173.4 (1).



	R^1	R^2
(5)	H	H
(6)	O(L-oleandrose)$_2$	OH

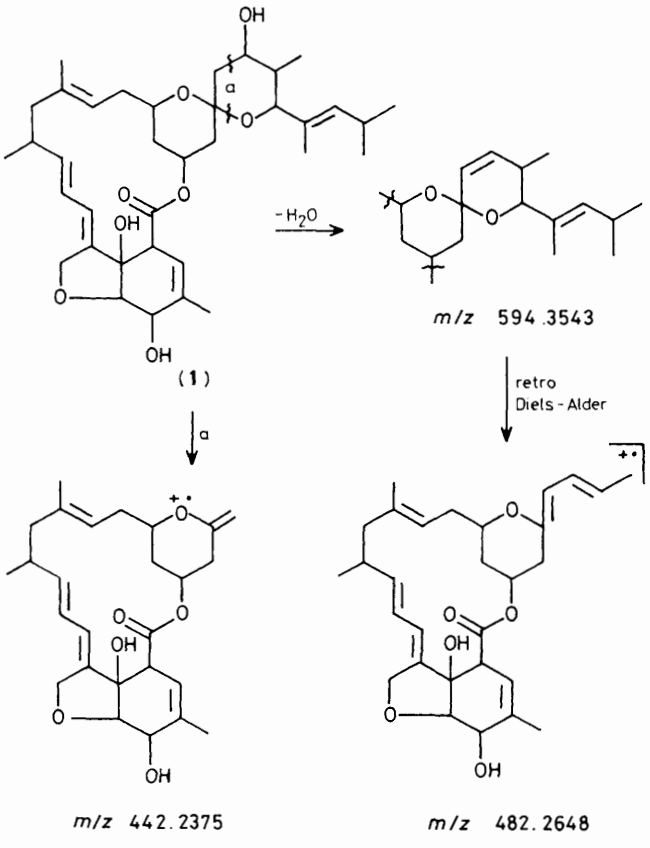


Figure 1. E.i.m.s. fragmentation of LL-F28249α (**1**).

the spiroacetal system. The remaining portion of the molecule, including the right side of the spiro system, consists of the fragment $C_{11}H_{20}O_2$. Hydroxy substitution of this ring was suggested by observation of several ions in the e.i. mass spectrum of (**1**) which indicated an additional unsaturation not present in the parent molecule (Figure 1). One of these, *m/z* 482.2648 ($C_{29}H_{38}O_6$), corresponds to loss of the side chain plus C-25 and the attached oxygen. This ion presumably arises through dehydration to the Δ^{22} compound followed by retro-Diels–Alder fragmentation of the ring, demanding

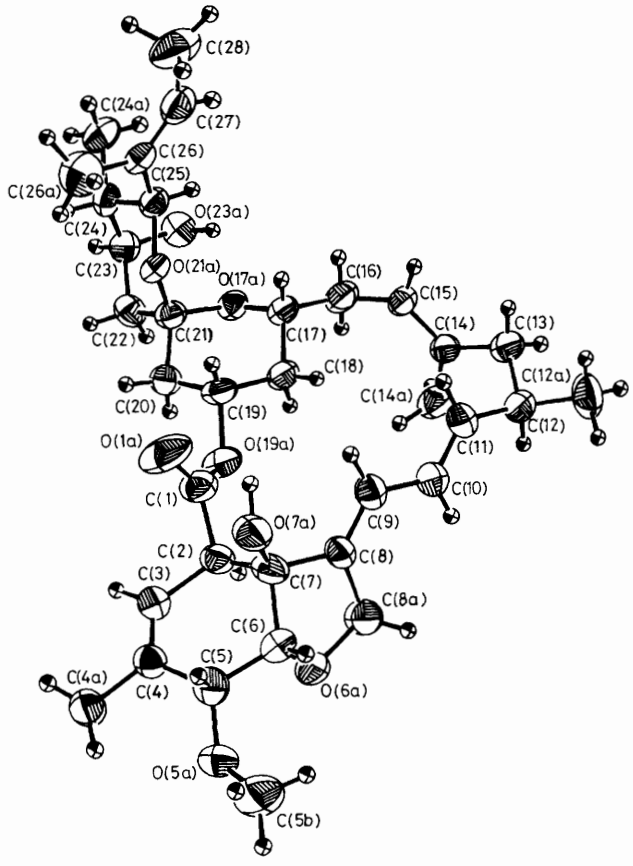


Figure 2. ORTEP drawing of LL-F28249γ (**4**).

hydroxy substitution at either the 22 or the 23 position. Evidence for 23-hydroxy substitution comes from the formation of the *m/z* 442.2375 ($C_{26}H_{34}O_6$) ion resulting from loss of C-23—C-29 including 2 oxygens. The 23-hydroxy promotes the cleavage of the C-22–C-23 bond and the fragmentation is completed by fission of the C-21–O bond.

The structure of the novel six-carbon side chain of (**1**) was determined by analysis of ^{1}H and ^{13}C n.m.r. data. The trisubstituted double bond was readily identified from ^{13}C n.m.r. signals at δ 130.6 (s) and 137.2 (d). The position of the isopropyl and methyl substituents about the double bond was assured by the observed 9 Hz doublet for 27-H at δ 5.20, indicating vicinal coupling to the isopropyl methine.

LL-F28249λ (**2**) was readily recognized as a methoxy derivative of (**1**) by characteristic ^{1}H (δ 3.50, 3H, s) and ^{13}C (δ 51.7, q) n.m.r. signals for the *O*-methyl group. Location of the methyl on the 5-oxygen was established from the appearance of appropriately shifted e.i.m.s. fragment ions. Similar arguments were applied for the structure assignments of the β (**3**) and γ components (**4**), which contain a terminal methyl substituent on the side chain in place of the isopropyl group of (**1**) and (**2**). In the ^{1}H n.m.r. spectra of (**3**) and (**4**) the proton on the double bond in the side chain gives rise to a doublet of quartets showing vicinal coupling (6.5—7.0 Hz) to the terminal methyl and allylic coupling (1.0—1.2 Hz) to the 25-H.

Slow evaporation of methanol–water solutions obtained from reversed-phase chromatography yielded crystals of

LL-F28249γ (**4**), which were used for *X*-ray crystallography.§ The resulting structure is given in Figure 2. As expected from n.m.r. data, the relative stereochemistry of all substituents is identical to that found for avermectin B_{2b} (**6**).[4] In addition, the Δ^{26} double bond was clearly shown to have the *trans*-configuration.

Preliminary results from biosynthetic experiments indicated identical labelling patterns with acetate and propionate in the macrocycle and spiroacetal rings as was found for milbemycin D[5] and avermectin.[6] The structure of the side chains in (**1**)—(**4**), however, reflects a fundamental difference in their biosynthesis. Whereas all previously described members of this general family incorporate a single acyl unit to form the C-25 side chain (the carboxy carbon of this unit becomes C-25), the LL-F28249 macrolides have side chains originating from two acyl units. For (**1**) and (**2**) the terminal unit is derived from isobutyrate which is linked *via* the double bond to the propionate-derived unit at C-26. Compounds (**3**) and (**4**) have incorporated acetate in place of isobutyrate as the terminal unit. Thus, in general, the polyketide precursors of the LL-F28249 macrolides are one unit longer than those which give rise to other known milbemycin derivatives.

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§ *Crystal data* for (**4**): $C_{35}H_{50}O_8$, M = 598.78, orthorhombic, space group $P2_12_12_1$, a = 13.316(4), b = 10.154(2), c = 25.021(5) Å, U = 3383.3 Å^3, Z = 4, D_c = 1.18 g/cm^3, λ(Cu-K_α) = 1.54184 Å, μ(Cu-K_α) = 6.7 cm^{-1}, F(000) = 1296. The structure was solved by direct methods and refined by full-matrix least-squares methods. 2209 reflections with $F_o^2 > 3.0\sigma(F_o^2)$ were collected on an Enraf–Nonius CAD4 diffractometer in the range $12 < \theta < 29°$. Refinement led to conventional values of R = 0.069 and R_w = 0.086. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

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