LL-E19020α AND β, NOVEL GROWTH PROMOTING AGENTS: ISOLATION, CHARACTERIZATION AND STRUCTURES

Sir:

We have recently discovered two new antibiotics, designated LL-E19020α (**1**) and β (**2**), which are highly modified versions of the aurodox family[1]. The LL-E19020 antibiotics have an exceptionally narrow antimicrobial spectrum against human pathogens, showing meaningful activity versus *Streptococcus* species and certain anaerobes (MIC 0.1~1 μg/ml). These compounds are highly effective as growth promoting agents in animals, producing superior performance in chicks in comparison trials with bacitracin and virginiamycin. The details of the biological activity of these compounds, the taxonomy and fermentation of the producing organism, and the structure elucidation will be the subjects of separate reports.

Flask fermentations of culture LL-E19020, *Streptomyces lydicus* sp. nov. *tanzanius*, were carried out in a medium consisting of (g/liter) dextrin (20), glucose (5), soy flour (15), corn steep liquor (5) and $CaCO_3$ (3). The flasks were incubated on a rotary shaker at 28°C for 5 days. The antibiotic titers averaged 825 μg/ml for LL-E19020α and 350 μg/ml for LL-E19020β under these conditions. The antibiotics were recovered by extraction of the whole harvest mash with one-half volume of MeOH. The resulting solution was concentrated under reduced pressure to remove the bulk of the MeOH. Extraction of the aqueous suspension with EtOAc partitioned the antibiotics into the organic phase. The residue obtained upon concentration of the EtOAc was purified by reversed-phase chromatography on a C_{18} column, employing mixtures of acetonitrile and 0.1 M NH_4OAc (pH 4.5) buffer as the mobile phase, to yield the pure compounds **1** and **2**. The compounds were freeze-dried from *tert*-butyl alcohol yielding off-white solids soluble in most organic solvents and sparingly soluble in hexane and water.

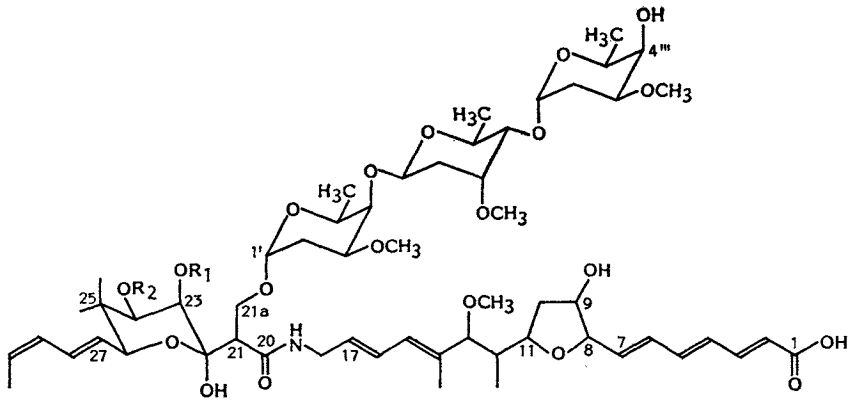
The compounds are isomers of molecular weight 1,225 with very similar physico-chemical profiles (Table 1). The only difference between the two is that LL-E19020α (**1**) has the phenylacetate ester linked at C-23, whereas LL-19020β (**2**) has this ester group at C-24 (see Fig. 1). In the following discussion only the characterization data for **1** will be presented, due to the nearly identical structures of the two compounds. The IR absorption spectrum for **1** (Fig. 2) contains two obvious features, a broad OH absorption band between 3200~3600 cm^{-1} and a carbonyl region containing overlapping bands (1600~1740 cm^{-1}). In the ^{13}C NMR spectrum (Table 2), three carbonyl signals were observed (δ 170.4, 171.4 and 173.3). One of these carbonyl signals is due to a carboxylic acid group as indicated by the formation of methyl ester derivative (fast atom bombardment mass spectrum (FAB-MS) $(M+Na)^+$ *m/z* 1,262; IR 1720 cm^{-1}; ^{13}C NMR δ 167.3) upon treatment of **1** with ethereal diazomethane. The ^{13}C NMR spectrum contains resonances attributed to twenty additional sp^2 carbons in the range δ 120~147. Six of these were accounted for by the identification of phenylacetic acid by GC-MS analysis of a basic degradation mixture. Catalytic hydrogenation of **1** (1 atm H_2, 10% Pd/C in MeOH) yielded a tetradecahydro derivative (FAB-MS $(M+K)^+$ *m/z* 1,278) corresponding to the hydrogenation of seven double bonds. On the basis of ^1H-^1H correlation spectroscopy (COSY) experiments

Table 1. Selected physico-chemical properties of LL-E19020α (**1**) and β (**2**).

	1	**2**
Molecular formula	$C_{65}H_{95}NO_{21}$	$C_{65}H_{95}NO_{21}$
MW	1,225	1,225
Molecular ion $(M+Na)^+$ (HRFAB-MS)	1248.6187 (calcd 1248.6270)	1248.6193 (calcd 1248.6270)
$[\alpha]_D^{26}$ (MeOH)	−8 (1.0%)	−17 (0.46%)
UV λ_{max}^{MeOH} nm (ε)	233 (49,800), 290 (36,600)	233 (47,000), 290 (34,100)
IR (KBr) cm^{-1}	3420, 2970, 2925, 1717, 1695, 1647, 1617, 1525, 1445, 1365, 1092, 1018	3430, 2970, 2930, 1712, 1648, 1620, 1543, 1454, 1367, 1265, 1098, 1020

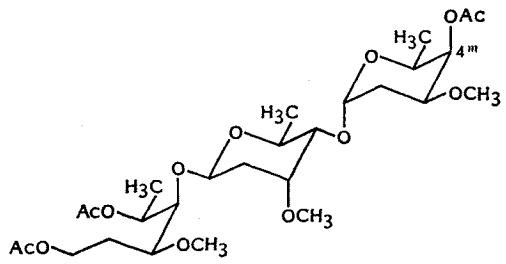
HRFAB-MS: High-resolution fast atom bonbardment mass spectrum.

Fig. 1. Structures of the LL-E19020 antibiotics and related compounds.



LL-E19020α (**1**) R_1 = $PhCH_2CO$ R_2 = H

LL-E19020β (**2**) R_1 = H R_2 = $PhCH_2CO$



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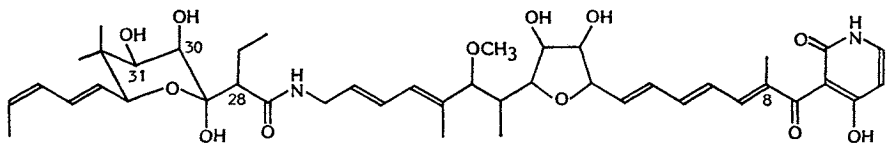

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and the UV absorbance data (Fig. 3 and Table 1) three of the double bonds are conjugated with the carboxylic acid (λ_{max} 290 nm, ε 36,600). The other four olefinic bonds are contained in two separate conjugated dienes, giving rise to overlapping UV absorption curves with a maximum at 233 nm (ε 49,800).

Further analysis of the ^{13}C NMR data for **1** reveals the presence of four acetal carbons (δ 97~101). Three of these resonances are due to the anomeric carbons of a trisaccharide which can be liberated under basic conditions (see below). Of the numerous oxygen-bearing sp^3 carbons, evidenced by ^{13}C NMR absorptions in the range of δ 55~90, three are alcohols which were readily acetylated upon treatment of **1** with acetic anhydride in pyridine at room temperature. The resulting triacetate was characterized by a FAB-MS containing $(M+K)^+$ *m/z* 1,390 and three additional ^{13}C NMR resonances in the carbonyl region. The shift of the characteristic *m/z* 145 ion in the electron impact mass spectrum (EI-MS) of **1** to *m/z* 189 in the acetyl derivative indicated one of the acetyl groups to be on the terminal unit of the trisaccharide (C-4‴). Therefore two remain on the aglycone.

Fig. 2. IR absorption spectrum of LL-E19020α (1).

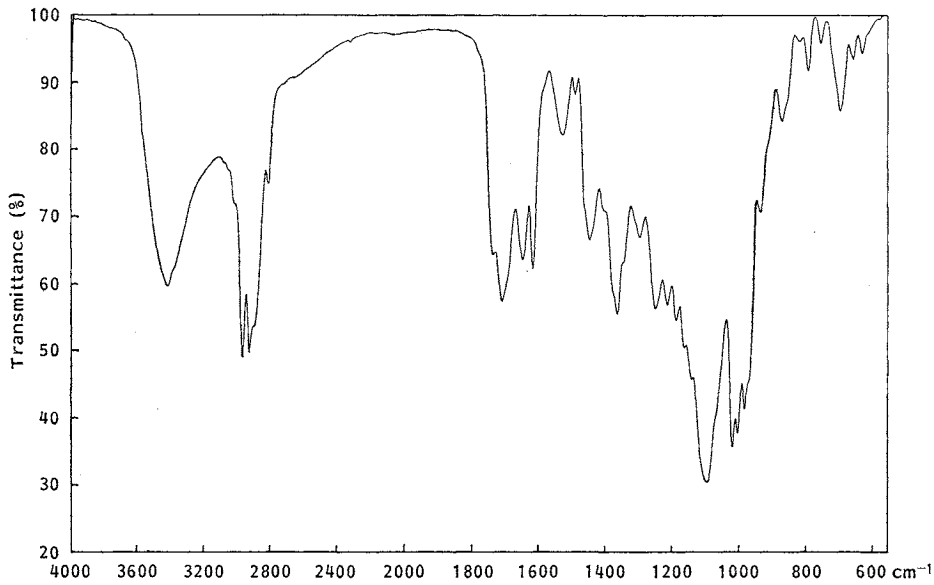


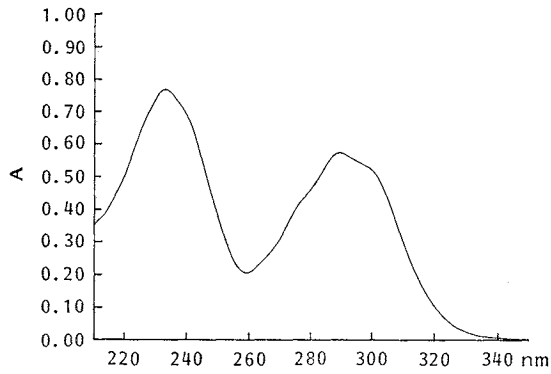
Table 2. ^{13}C NMR chemical shifts for LL-E19020α (1) ($CDCl_3$)[a].

173.3	100.6	56.53
171.4	98.99	56.08
170.4	97.30	55.63
145.9	97.06	55.45
140.3	89.26	49.87
137.2	83.17	41.79*
134.4	81.70	39.91
133.9	77.72	39.30
132.0	77.02	38.84
130.1	76.42	33.04
129.6*	74.66	31.08
129.0	74.45*	29.95
128.6*	74.28	23.75
128.5	72.29	18.13
128.3	72.01	17.22
128.2*	69.14	17.00
127.6	67.61	14.79
127.1	66.49	13.48
126.2	66.08	10.89
120.7	63.57	10.16

[a] Shifts reported in δ downfield from TMS.
* Two unresolved signals.

The ^{13}C NMR data show the presence of four *O*-methyl groups (δ 55.4~56.5), three on the trisaccharide, and eight *C*-methyl groups (δ 10.2~23.8) and again, three of these are located on the trisaccharide moiety. The complete assignments of the ^{13}C NMR signals will be presented in a paper covering the structure elucidation.

Fig. 3. UV absorption spectrum of LL-E19020α (1) (MeOH).



The trisaccharide was liberated from either **1** or **2** under basic conditions (*e.g.* NaOMe - MeOH or NH_3 - acetonitrile). The structure was determined by X-ray crystallographic analysis of the triacetate derivative **3** (^1H NMR δ 2.04 (3H, s), 2.08 (3H, s), 2.16 (3H, s); ^{13}C NMR δ 20.82, 20.95, 21.18, 170.1, 170.8, 171.0) obtained by reducing the anomeric center with $NaBH_4$ to form the open-chain diol followed by treatment with acetic anhydride in pyridine.

Methanolysis (HCl - MeOH) was used to obtain the aglycone and the compound which had two sugars cleaved off – the monosaccharide product.

Comparisons by ^{13}C NMR of the aglycone with the monosaccharide product provided evidence for the unprecedented glycosidic linkage at C-21a. A signal corresponding to an sp^3 methylene carbon bonded to oxygen which appears at δ 63.53 in the monosaccharide product is shifted to 59.71 in the aglycone. This result strongly suggests that this methylene carbon, assigned to C-21a, is the point of attachment for the trisaccharide. An additional experiment was performed to determine which ^{13}C NMR signals represented carbons bearing hydroxyl groups by observing small changes in chemical shifts induced by the addition of CD_3OD to the NMR samples[2]. The aglycone clearly shows a deuterium induced shift for a CH_2OH carbon which was not observed in the case of the monosaccharide product, again indicating this primary hydroxyl group is involved in the glycosidic bond.

The gross structural similarity of LL-E19020α (**1**) and β (**2**) to aurodox **4** extends up to the carbonyl group adjacent to the pyridone ring. In the case of **1** and **2**, the carbon chains terminate at what corresponds to the olefinic methyl group at C-8 of aurodox. This abbreviated carbon skeleton was first described for the related antibiotic L-681,217[3]. Another major difference is the replacement of the ethyl substituent (at C-28 of **4**) by the hydroxymethyl linked to the trisaccharide. No previously described member of this class of antibiotics has this particular modification. The presence of a phenylacetate group is also quite rare, and as previously indicated, the position of the ester constitutes the difference between LL-E19020α and β.

Subsequent to this structure assignment, a report appeared describing a related family of compounds, the phenelfamycins[4], two of which are isomeric with LL-E19020α and β. Phenelfamycins E and F each contain a phenylacetate ester at C-23 and the trisaccharide at C-24. Phenelfamycins E and F bear a diastereoisomeric relationship to one another, differing in configuration at C-21.

LL-E19020α and β are unique compounds bearing some relation to the aurodox class of antibiotics. The highly modified nature of these compounds, including the unique hydroxymethyl-linked trisaccharide and shortened carbon skeleton ending in a carboxylic acid, may explain their greatly enhanced growth promoting activity relative to aurodox.

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