9-*EPI*-LEUCOMYCIN A_5 SYNTHESIS AND ANTIMICROBIAL ACTIVITY

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(Received for publication August 24, 1981)

9-*epi*-Leucomycin A_5 has been obtained from leucomycin A_5 (**I**) by the following reaction sequence.

Leucomycin A_5 (**I**) was treated with Collins reagent (CrO_3-pyridine) in the presence of water (13%) to provide 9-dehydroleucomycin A_5 (**II**) in 95% yield. The formyl group was internally protected by the reaction of **II** with acetic anhydride-K_2CO_3 to afford 18,2′-di-*O*-acetyl-9-dehydroleucomycin A_5-3,18-hemiacetal (**III**). Sodium borohydride reaction of **II** provided a 1:1 mixture of natural **I** and its 9-epimer, 9-*epi*-leucomycin A_5 (**IV**), which were separated by silica gel chromatography. It was observed that the antimicrobial activities of both enantiomers were virtually identical with some test strains but that of **IV** is reduced in comparison with **I** in some bacteria such as *Staphylococcus epidermidis* sp-al-1 and *Streptococcus pyogenes* N. Y. 5.

Studies on the alteration of antimicrobial activity arising from the configurational change of a substituent in the lactone ring of a macrolide have been quite limited.[1~3]

This work describes the synthesis of 9-*epi*-leucomycin A_5 (**IV**) from leucomycin A_5 (**I**)[4,5] and some comparison of the physicochemical properties and antimicrobial activities of these macrolides. In an initial attempt, the C-9 hydroxyl group of leucomycin was oxidized into the corresponding ketone with manganese dioxide[6] or the CrO_3-pyridine complex[7] with only limited success; these methods provided the desired ketone in low yields. However, treatment of **I** with the CrO_3-pyridine complex in the presence of 13% water provided the desired 9-dehydroleucomycin A_5 (**II**) in a nearly quantitative yield (95%).

In order to protect the aldehyde group from sodium borohydride reduction in the subsequent step, **II** was heated with acetic anhydride in the presence of potassium carbonate at 60°C for 16 hours to give 18,2′-di-*O*-acetyl-9-dehydroleucomycin A_5-3,18-hemiacetal (**III**). Selective reduction of the C-9 carbonyl group in **III** was then effected by reduction with four equivalents of sodium borohydride in ethanol at ambient temperature for 25 minutes. Hydrolysis of the 18-*O*-acetyl-3,18-hemiacetal with sodium methoxide in methanol followed by removal of the 2′-*O*-acetyl group by refluxing in methanol provided a 1:1 mixture of 9-*epi*-leucomycin A_5 (**IV**) and natural **I**. Separation of **IV** was performed by silica gel column chromatography (benzene - ethyl acetate - methanol, 36:4:1 ~ 32:4:1 v/v) followed by aluminum oxide column chromatography (ethyl acetate - methanol, 50:1 v/v). The yields of **IV** and **I** were 10% and 25%, respectively. Stereochemical assignment of these epimers was made by the difference in the magnitude of the ^{1}H-coupling constant between H-9 and H-10, $J_{9,10}$; $J_{9,10}$'s were 3.0 Hz and 9.0 Hz for **IV** and

Fig. 1. The synthesis of 9-*epi*-leucomycin A_5 (IV) from leucomycin A_5 (I).

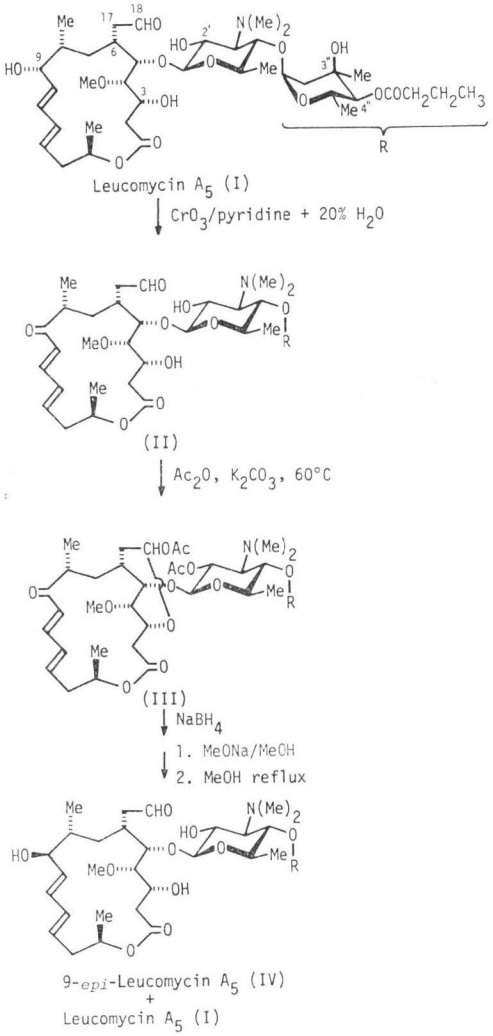

Table 1. Antibacterial spectra of leucomycin A_5 (I), 9-dehydroleucomycin A_5 (II) and 9-*epi*-leucomycin A_5 (IV).

Test organisms (10^6 cells/ml)	MIC (μg/ml)		
	I	II	IV
Staph. aureus ATCC 6538P	0.4	0.4	0.4
Staph. aureus MS353	0.8	0.8	0.8
Staph. aureus MS353 C36	0.4	0.4	0.4
Staph. aureus MS353 A0 (Mac^r A)	>100	>100	>100
Staph. epidermidis sp-al-1	0.8	0.8	1.6
Strept. pyogenes N. Y. 5	≤0.05	0.1	0.1
Strept. faecalis 1501	0.8	0.8	1.6
Strept. agalactiae 1020	0.2	0.2	0.2
Micrococcus luteus ATCC 9341	≤0.05	≤0.05	≤0.05
Micrococcus flavus ATCC 10240	0.1	0.1	0.1
Corynebact. diphtheriae P. W. 8	≤0.05	≤0.05	≤0.05
Bac. subtilis ATCC 6633	0.4	0.4	0.4
E. coli NIHJ-JC2	>100	>100	>100
Klebs. pneumoniae ATCC 10031	12.5	12.5	12.5
Salm. typhosa H 901	>100	>100	>100
Salm. enteritidis Gaertner	>100	>100	>100
Shigella flexineri type 3a	50	25	50
Shigella sonnei E33	>100	>100	>100
Proteus vulgaris OX19	100	50	100
Serratia marcescens	>100	>100	>100
Ps. aeruginosa IAM1095	>100	>100	>100

Media: HIA (Difco)
Mac^r: Macrolide-resistant.

I, respectively.[8~10]

The antimicrobial activities of leucomycin A_5 (I), 9-dehydroleucomycin A_5 (II) and 9-*epi*-leucomycin A_5 (IV) are compared in Table 1. Although there was virtually no difference in antimicrobial activity between I and II, a comparison between I and IV may be worth noting. The antimicrobial activities of I and IV were nearly identical against *Staphylococcus aureus*, but I was about two times more active than IV against *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Streptococcus faecalis*.

Experimental

Synthesis of 9-Dehydroleucomycin A_5

A solution of chromium trioxide (10 g) in water (10 ml) was added dropwise over 10 minutes to pyridine (40 ml) cooled in an ice bath. To the mixture was added a solution of leucomycin A_5 (I) (10 g) in pyridine (20 ml) and the reaction mixture was stirred for 2 hours at room temperature. It was then

poured into cold water (600 ml) and the pH adjusted to 9 by adding aqueous ammonia. The mixture was extracted with chloroform (200 ml) and the organic layer was washed sequentially with dilute HCl (pH 2, 200 ml), water (200 ml), and finally dilute aqueous ammonia (pH 9, 200 ml), dried over anhydrous magnesium sulfate, and concentrated *in vacuo* to provide 9.5 g powder of crude 9-dehydroleucomycin A_5 (**II**). [UV, λ_{max}^{EtOH} 279.5 nm (ε 21,600); Mass, *m/z* 769 (M^+), 682 (M^+-87), 555, 365, 215, 174, 173; NMR (100 MHz, in $CDCl_3$) δ 2.50 (s, $N(CH_3)_2$), 3.54 (s, 4-OCH_3), 6.29 (d, H-10, J=15.0 Hz), 7.27 (dd, H-11), 9.64 (s, CHO); Rf 0.48 (silica gel, Merck Art. 5721, chloroform - methanol - acetic acid - water, 80: 7: 7: 1 v/v)].

Synthesis of 18,2′-Di-*O*-acetyl-9-dehydroleucomycin A_5-3,18-hemiacetal

A mixture of 9-dehydroleucomycin A_5 (**II**) (6 g) and anhydrous potassium carbonate (2.1 g) in acetic anhydride (12 ml) was stirred at 60°C for 16 hours. The reaction mixture was poured into water (200 ml) and the pH adjusted to 9 with aqueous ammonia. It was then extracted with chloroform (150 ml). The organic layer was washed with water (150 ml), dried over anhydrous magnesium sulfate and concentrated *in vacuo* to obtain 5.5 g of a powder which was purified by silica gel column (silica gel 60, Merck Art. 7734, 2.4×100 cm, each fraction 18 ml) eluting with benzene - acetone, 9: 1 ~ 8: 1 (v/v). The fractions, which showed a spot at Rf 0.39 on TLC (silica gel, Merck, Art. 5721, benzene - acetone, 4: 1, visualized with H_2SO_4), were collected and concentrated to provide 18,2′-di-*O*-acetyl-9-dehydroleucomycin A_5-3, 18-hemiacetal (**III**) (3.2 g). **III** was further purified by column chromatography on silica gel using the same solvent system as described above. Characteristics of **III** are: UV, λ_{max}^{EtOH} 276 nm (ε 21,600); Mass, *m/z* 853 (M^+), 766 (M^+-87), 706 ($M^+-87-60$), 668, 638, 623, 608 (668−60), 578 (638−60), 563 (623 −60), 430, 407, 347, 342, 216, 215; NMR (100 MHz in $CDCl_3$) δ 2.03 (s, 18-OAc), 2.08 (s, 2′-OAc), 2.40 (s, $N(CH_3)_2$), 3.42 (s, 4-OCH_3); Rf 0.39 (silica gel, Merck Art. 5721, benzene - acetone, 4: 1).

Synthesis of 9-*epi*-Leucomycin A_5 (**IV**)

A solution of 18,2′-*O*-acetyl-9-dehydroleucomycin A_5-3,18-hemiacetal (**III**) (1.45 g) in ethanol (26 ml) was treated with sodium borohydride (257 mg) at room temperature for 25 minutes. The reaction mixture was poured into 50 ml water and extracted with chloroform (50 ml). The extract was dried over magnesium sulfate and concentration *in vacuo* to afford 1.42 g residue, which was dissolved in methanol (15 ml) and treated with methanolic sodium methoxide (2.8%, 0.6 ml) for 30 minutes at room temperature. The reaction mixture was poured into water (50 ml) and immediately extracted with chloroform (50 ml). The chloroform layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The crude product was dissolved in methanol (15 ml) and the solution was refluxed overnight. The cooled reaction mixture was then concentrated and dried *in vacuo* to provide a 1: 1 mixture of leucomycin A_5 (**I**) and 9-*epi*-leucomycin A_5 (**IV**) (1.2 g). Purification of **I** and **IV** required the following two steps; (1) the mixture was first purified by silica gel column chromatography (1×100 cm, each fraction 10 ml, eluting with benzene - acetone - methanol, 36: 4: 1 ~ 32: 4: 1, v/v) to provide three fractions (first fractions; 215 mg, Rf 0.44: second fractions; 404 mg, two spots on TLC, Rf 0.44 and Rf 0.33: third fractions; 523 mg, Rf 0.33: TLC performed on silica gel, Merck Art. 5721 using chloroform - methanol - acetic acid - water, 80: 7: 7: 1, v/v) and (2) the first fractions (Rf 0.44, 215 mg) collected were further purified by alumina column chromatography (aluminum oxide 90 Art. 1097, Merck, 1×20 cm, each fraction 3 ml) to provide pure 9-*epi*-leucomycin A_5 (**IV**) (148 mg) and impure 9-*epi*-leucomycin A_5 (**IV**) (55 mg). Finally the third fractions (Rf 0.33, 523 mg) were again purified by alumina column chromatography (1×20 cm, 3 ml fraction, eluting with ethyl acetate - methanol, 50: 1, v/v) to give pure leucomycin A_5 (**I**) (363 mg) and impure leucomycin A_5 (**I**) (28 mg). [9-*epi*-leucomycin A_5 (**IV**): UV, λ_{max}^{EtOH} 234 nm (ε 26,300); Mass, *m/z* 771 (M^+), 684 (M^+-87), 666, 586, 557, 430, 388, 367, 349, 300, 215, 190, 174, 173; NMR (100 MHz in $CDCl_3$) δ 2.49 (s, $N(CH_3)_2$), 3.50 (s, 4-OCH_3), 4.44 (d, 1′-H), 5.05 (d, 1″-H), 5.56 (m, 13-H), 5.72 (dd, 10-H), 6.04 (dd, 12-H), 6.35 (dd, 11-H), 9.74 (s, CHO), $J_{9,10}$=3 Hz, $J_{10,11}$=14 Hz, $J_{11,12}$=10 Hz, $J_{12,13}$=14 Hz. Rf 0.44 (silica gel, Merck Art. 5721, chloroform - methanol - acetic acid - water, 80: 7: 7: 1 v/v)]. [Leucomycin A_5 (**I**): UV, λ_{max}^{EtOH} 232 nm (ε 28,000); Mass, *m/z* 771 (M^+), 684 (M^+-87), 666, 586, 557, 430, 388, 367, 349, 300, 215, 190, 174, 173; NMR (100 MHz in $CDCl_3$) δ 2.50 (s, $N(CH_3)_2$), 3.49 (s, 4-OCH_3), 4.46 (d, 1′-H), 5.05 (d, 1″-H), 5.58 (m, 13-H), 5.65 (dd, 10-H), 5.99 (dd, 12-H), 6.26 (dd, 11-H), 9.77 (s, CHO), $J_{9,10}$=9 Hz, $J_{10,11}$=14 Hz, $J_{11,12}$=10 Hz, $J_{12,13}$=14 Hz, Rf 0.33

(silica gel, Merck Art. 5721, chloroform - methanol - acetic acid - water, 80: 7: 7: 1 v/v)].

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