Synthesis of Lactivicin and Its Derivatives

Hideaki Natsugari,* Yasuhiko Kawano, Akira Morimoto, Kouichi Yoshioka, and Michihiko Ochiai

Chemistry Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka 532, Japan

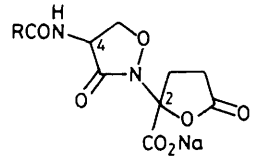
Lactivicin, a new type of antibiotic having β-lactam-like activity, and its derivatives were synthesized starting from 4-benzyloxycarbonylamino-3-isoxazolidinones and 2-oxoglutaric acid.

Lactivicin[1] (**1**) {2-[(4*S*)-4-acetamido-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylic acid sodium salt}[2]† is a novel antibiotic isolated from *Empedobacter lactamgenus* YK-258 and *Lysobacter albus* YK-422. Although lacking a β-lactam ring in its molecule, (**1**) has β-lactam-like biological activities: potent antibacterial activity, affinity to penicillin-binding proteins and susceptibility to β-lactamases.[1] These unique features stimulated our search for a new series of antibiotics. This communication describes a simple and efficient synthesis of (**1**) and its derivatives (**2a,b**) and (**3**) starting from 4-benzyloxycarbonylamino-3-isoxazolidinones (Cbz-cycloserines) (**7a,b**) and 2-oxoglutaric acid (**4**).

Our synthetic plan was initially designed to attempt alkylation of a cycloserine derivative with a halogeno-γ-lactone. The halogeno-γ-lactone (**6**) ($\nu_{max.}$ 1815, 1760 cm^{-1})‡ was easily prepared by chlorination of 2-oxoglutaric acid 1-*p*-nitrobenzyl (PNB) ester (**5**), which was synthesized selectively from (**4**) by esterification with PNB bromide (Scheme 1). Alkylation of (*S*)-Cbz-cycloserine (**7a**), prepared from L-serine *via* L-cycloserine,[3] with (**6**) in the presence of a base, *e.g.*, Et_3N, afforded the desired condensation product (**8a**)§ ($\nu_{max.}$ 1800, 1770—1700 cm^{-1}), a key intermediate for preparing lactivicin derivatives, in 60% yield. (*R*)-Cbz-cycloserine (**7b**),[4] obtained from a commercially available natural antibiotic, D-cycloserine, similarly reacted with (**6**) to give the (4*R*)-isomer (**8b**)§ (56%) (Scheme 2, method A).

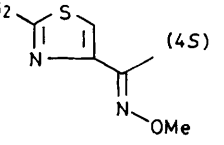
Taking into consideration the formation[5] of 2-acetamido-5-oxotetrahydrofuran-2-carboxylic acid from (**4**) and acetamide, and the mechanism[6] for the easy conversion of (**5**) into (**6**), we investigated the direct condensation of (**5**) with (**7a,b**). The reaction proceeded smoothly in the presence of a condensing agent, dicyclohexylcarbodiimide (DCC), to give the desired compounds in good yields [(**8a**) (66%); (**8b**) (67%)] (Scheme 2, method B); other condensing agents, such as 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, were also effective in this reaction, thus providing a simple and convenient one-step synthesis of a variety of lactivicin-like compounds.

Conversion of (**8a,b**) into (**1**) and its derivatives having various acylamino moieties at the C-4 position [*e.g.*, (**2a,b**)



(**1**): R = Me (4*S*) (Lactivicin)
(**2a**): R = $PhCH_2$ (4*S*)
(**2b**): R = $PhCH_2$ (4*R*)

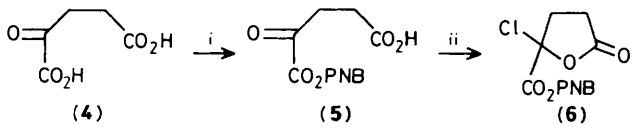
(**3**) R =



† (**1**) exists as an equilibrium mixture of epimers (*ca.* 1 : 1) at the C-2 of the 5-oxotetrahydrofuran (γ-lactone) moiety. The epimers of the *p*-nitrobenzyl (PNB) ester of (**1**) are separable by silica gel chromatography, each of which does not isomerize in 50% aq. MeOH, whereas the sodium salt (**1**) obtained from the separated epimer by removal of the ester group again reaches the equilibrium in aqueous solution.[2]

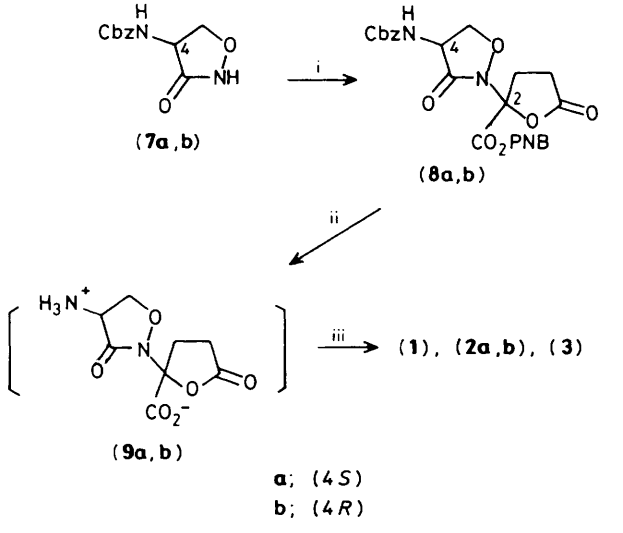
‡ Satisfactory spectral (^1H n.m.r., i.r.) data and elemental analyses and/or mass spectra were obtained for all new compounds.

§ As expected, (**8a,b**) were obtained as a mixture of diastereoisomers at the C-2 of the γ-lactone moiety, which showed overlapping spots on t.l.c. The ratio differed slightly depending on the reaction conditions (*ca.* 1 : 1 to 1 : 1.4 determined by h.p.l.c.).



PNB = *p*-nitrobenzyl

Scheme 1. *Reagents and conditions:* i, PNBBr (1.1 equiv.), dicyclohexylamine (1 equiv.), dimethylformamide, 50—70 °C, 15 min, 93%; ii, $SOCl_2$ (5.5 equiv.), $ClCH_2CH_2Cl$, reflux, 10 h, 85%.



Cbz = benzyloxycarbonyl

Scheme 2. *Reagents and conditions:* i, method A: (**6**) (1.3 equiv.), Et_3N (2 equiv.), CH_2Cl_2, 0 °C → room temp., 0.5 h, or method B: (**5**) (1.3 equiv.), DCC (1.3 equiv.), CH_2Cl_2, room temp., 14 h; ii, H_2, Pd–C, EtOAc–pH 7.0 buffer, 0 °C → room temp.; iii, for (**1**), Ac_2O (1.2 equiv.), $NaHCO_3$, tetrahydrofuran (THF)–H_2O, 0 °C, 28%; for (**2a,b**), $PhCH_2COCl$ (1.2 equiv.), $NaHCO_3$, THF–H_2O, 0 °C; and for (**3**), CATAM chloride (1.2 equiv.), $NaHCO_3$, THF–H_2O, 0 °C, then MeNHCSSNa (2 equiv.), room temp.

and (**3**)] was achieved by conventional methods *via* the betaine (**9a,b**). Thus, (**1**) was prepared by deprotection of (**8a**) by hydrogenolysis using Pd–C followed by acetylation. This material was identical (h.p.l.c., ^1H n.m.r., i.r., and u.v. spectroscopy, optical rotatory dispersion, circular dichroism, and antibacterial activity) with natural lactivicin. The (4*S*)- and (4*R*)-phenylacetyl derivatives, (**2a**) (63%) and (**2b**) (60%), were similarly prepared from (**8a,b**), respectively. The compound having a 2-aminothiazol-4-yl-(*Z*)-2-methoxyiminoacetyl side chain (**3**) (61%) was prepared from (**8a**) *via* hydrogenolysis, acylation with 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetyl (CATAM) chloride,[7] and removal of the chloroacetyl group with sodium *N*-methyldithiocarbamate.

Highly improved and enhanced antibacterial activities were observed in (**2a**) and (**3**) [MIC (minimum inhibitory concentration): μg/ml, 10^8 c.f.u. (colony forming units) ml^{-1}: *e.g.*, *Staphylococcus aureus* FDA 209P: (**1**), 3.13; (**2a**), 0.2; (**3**), 12.5: *Escherichia coli* 0–111: (**1**), 100; (**2a**), 6.25, (**3**), 0.39]. The (4*R*)-isomer (**2b**) showed significantly reduced activity compared with the (4*S*)-isomer (**2a**) [MIC: (**2b**); *S. aureus*, 6.25; *E. coli*, 100]. These structure–activity relationships together with the other biological features[1] suggest that lactivicin and its derivatives show biological activity *via* a mechanism similar to that of traditional β-lactams, in which the γ-lactone moiety may play an important role in activating the C–N bond of the cycloserine ring.¶

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¶ 2-(4-Phenylacetamido-3-oxo-2-isoxazolidinyl)propionic acids display no antibacterial activity.[8]