NOTES

GLYCOCINNAMOYLSPERMIDINES, A NEW CLASS OF ANTIBIOTICS IV. CHEMICAL MODIFICATION OF LL-BM123γ

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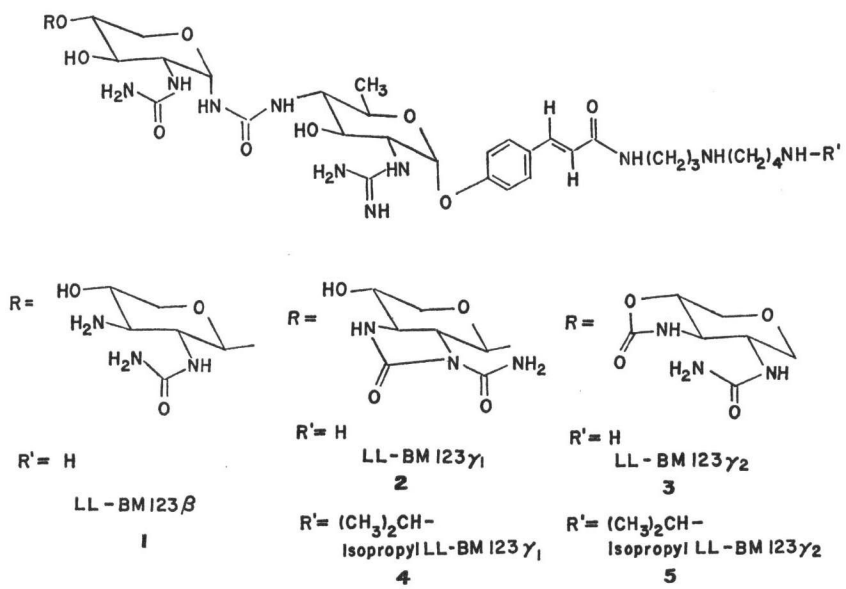
Recent communications from these laboratories have described the fermentation,[1] isolation[2] and structure[3] elucidation of a new class of potent antibiotics. These substances are unique in that they combine a *p*-hydroxycinnamoylspermidine unit with a trisaccharide and still possess the broad spectrum antibacterial activity and potency generally associated with the aminoglycoside antibiotics, *i.e.* gentamicin, kanamycin, *etc.*

The antibiotic complex as isolated from the fermentation broth was shown to be a mixture of three closely related compounds,* β, γ_1 and γ_2 (**1**, **2** and **3**). Since the γ_1 and γ_2 components (**2** and **3**) proved to be the more active (biologically) compounds, they were used in our structure-activity study. The two γ products differ only in the arrangement of the *trans* ring juncture in the terminal pentose; the γ_1 isomer (**2**) possessing a *trans* imidazolidone and the γ_2 isomer (**3**) has a *trans* oxazolidone ring. Since the separation of the isomers was somewhat time consuming, we carried out all the modification reactions described on a 50/50 mixture.

Although there are many reports in the literature describing successful chemical modifications of antibiotics to improve potency or broaden the antibacterial spectrum, there are few descriptions of successful attempts to decrease the toxicity of an antibiotic by varying its structure and at the same time retaining or improving its overall biological activity. We have been able to carry out a number of chemical modifications on these antibiotics which improved their antibacterial activity against experimental infections in mice and increased their safety margin (LD_{50}/ED_{50}) by as much as a factor of twelve.



* At the present time no generic or trade name has been assigned to these antibiotics and we normally refer to them by the following code numbers: LL-BM123γ_1, LL-BM123γ_2 and LL-BM123β. For the sake of brevity in this publication, we shall refer to them as γ_1, γ_2 and β.

Table 1. Activity-toxicity data

Antibiotic	Acute toxicity in mice LD_{50}[a] (single subcutaneous dose) (mg/kg)	*E. coli* infection No. 311 in mice, ED_{50}[b] (mg/kg) single subcutaneous dose	Safety margin LD_{50}/ED_{50}
Parent $\gamma_1+\gamma_2$ mixture (*trans*)	32~64	0.5	64~128
Parent $\gamma_1+\gamma_2$ mixture (*cis*)	32~64	1.0	32~64
N-Isopropyl $\gamma_1+\gamma_2$ mixture	128	0.08	1600
N-Cyclopentyl $\gamma_1+\gamma_2$ mixture	128	0.5	260
N-Tetrahydrothiopyran-4-yl $\gamma_1+\gamma_2$ mixture	256	1.0	256
N-Ethyl $\gamma_1+\gamma_2$ mixture	256	1.0	256
N-(β-Phenylethyl)-$\gamma_1+\gamma_2$ mixture	64	0.125	512

[a] Median lethal dose in noninfected mice (groups of 2~10 mice)
[b] Median effective dose (in 5 mice per group)

Selective mono-alkylation of the primary amino groups of spermidine using either an aldehyde or ketone as the alkylating agent and sodium cyanoborohydride[4] as the reducing agent afforded new, easily isolable products. We selected from a wide range of ketones and aldehydes for these alkylations so as to include many different types of alkyls in order to determine their effects on biological activity and acute toxicity. In general the ketone alkylated products were more active than the products obtained from aldehyde alkylations and the alkyl ketones yielded more active products than the aromatic or cycloalkyl ketones. Representative samples of alkylated products are given in Table 1 along with their *in vivo* testing data against an experimental infection in mice.

The assignment of the alkylation position on the terminal amine of the spermidine was based on both the nmr spectra (100 MHz, external TMS reference) [*i.e.* N-isopropyl γ_1 and γ_2 mixture (**4** and **5**) in deuterated water exhibited signals at δ 1.33 (d, J=6 Hz, $(CH_3)_2C$), δ 1.17 (d, J=6), δ 1.80, δ 1.98, δ 3.12, δ 5.3, δ 5.79 (d, J=4), δ 6.59 (d, J=16), δ 7.51 (d, J=16), δ 7.20 (d, J=9), δ 7.64 (d, J=9)] of the alkylated antibiotic and in selected cases (*i.e.* the isopropyl derivative) on mass spectra studies of the alkylated spermidine obtained by hydrolysis of the alkylated antibiotic. The alkylated spermidine was acylated with trifluoroacetylchloride. This derivative yielded the ion, $CH_2=\overset{+}{N}\begin{matrix}\diagup C(=O)-CF_3\\ \diagdown CH(CH_3)_2\end{matrix}$ during mass spectral analysis which verifies the position of the alkyl group (isopropyl in this case) on the terminal nitrogen of the spermidine.[3]

The mono isopropyl derivative was selected as the best candidate for additional biological studies. The degree of improvement of this derivative against some bacterial infections in mice was as much as six-fold as compared to the parent antibiotic and the acute lethal toxicity was decreased by a factor of two. Table 1 illustrates the increase in the margin of safety for one of these infections. These new derivatives also show activity against bacteria resistant to other aminoglycoside antibiotics and are bacteriocidal in action.

Di- or trialkylated derivatives, obtained by longer reaction time with the smaller molecular weight aldehydes, yielded products of lower antibacterial potency than the mono alkylated derivatives.

Photolysis of the mixture of $\gamma_1+\gamma_2$ isomers (**2** and **3**) in water for 30 minutes, using a Hanovia high pressure ultra violet lamp results in a quantitative conversion of the *trans* natural products (**2** and **3**) to the *cis* isomers.

The *trans p*-coumaroyl portion of the molecule has a UV maximum at 286 nm and very characteristic pmr (100 MHz in D_2O, external

TMS reference) signals for the vinyl protons at δ 6.60 (1H d's J=16) and 7.50 (1H d's J=16). The *cis p*-coumaroyl moiety has a UV maximum at 276 nm and NMR signals for the *cis* vinyl protons at δ 6.08 (1H d's J=12) and 6.90 (1H d's J=12). The other characteristic PMR signals are common to both isomers.

The mechanism for this transformation must involve the transition of excited π electrons of the double bond to the antibonding orbital which significantly reduces the strength of the C-C bond and permits *cis-trans* isomerization to take place.

Experimental

General Procedure for Alkylation of BM123γ_1 + γ_2 (**2** and **3**)

To a stirred solution of BM123γ (220 mg) in 60 ml of methanol was added a large excess (10 equivalents) of ketone followed by sodium cyanoborohydride (100 mg). The pH of the reaction mixture was maintained at 7 (with methanol saturated with HCl gas) and the reaction mixture was stirred at room temperature for a period varying from a few minutes to a number of days depending on the reactivity of the ketone. The reaction mixture was evaporated to approximately 5 ml under reduced pressure and the inorganic salt that was separated was filtered and the filtrate was diluted with 50 ml of acetone. The product that was separated was filtered and dried.

Photolysis of a BM123γ_1+γ_2 Mixture

A solution of antibiotic mixture (100 mg) in 200 ml of water was photolized with a Hanovia lamp in a water jacketed round bottom quartz flask for 1.5 hours. The solution was freeze-dried yielding *cis* product (>95% pure).

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