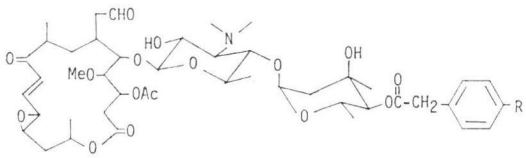
STRUCTURE-ACTIVITY RELATIONSHIPS AMONG 4''-(*p*-SUBSTITUTED PHENYLACETYL)-DELTAMYCINS, THE SEMISYNTHETIC MACROLIDES

Sir:

We have reported that 4''-O-deacyl-4''-O-phenylacetyldeltamycin (PAD) derivatives bearing various electronegative atoms or groups at the *para*-position on the phenylacetyl moiety were prepared (Fig. 1) and that variations of the *para*-substituent yielded compounds with a wide range of antimicrobial activity[1].

Fig. 1. Structures of PAD-derivatives.



Substituent	Compd. name	Compd. No.
R: $MeSO_2$-	; SPAD	**1**
NO_2-	; NPAD	**2**
CN-	; YPAD	**3**
CF_3-	; FPAD	**4**
H-	; PAD	**5**
F-	; LPAD	**6**
Cl-	; CPAD	**7**
MeO-	; MPAD	**8**
m, *p*-$(MeO)_2$-;	DPAD	**9**
OH-	; OPAD	**10**

Concerning relationships between electronegativity and antimicrobial activity, studies on chloramphenicol derivatives were reported in 1956[2]. Antimicrobial activity of PAD derivatives (Table 1) was estimated from the relative electronegativity of the substituents in the chloramphenicol studies (Note 1) but there was no linear relationship between them. Many studies have recently shown that chemical shifts of aromatic ring hydrogen caused by substituents in NMR spectrometry correlate well with their electronegativities[3].

We report in this paper that the antimicrobial activity of PAD derivatives correlates well with the electronegativity obtained from chemical shift in ^{1}H-NMR.

A series of the ^{1}H-chemical shifts of the *p*-substituted phenylacetyl group in the PAD derivatives were shown in Table 2 in the order of

(Note 1) Relative electronegativity* of *p*-substituents. Substituent: Relative electronegativity ($10^5 \times$Ka), NO_2, 37.6, CN, 31.0, Cl, 10.6, F, 7.22, H, 6.3, MeO, 3.38, OH, 2.8.

*: The dissociation constants of *p*-substituted benzoic acids are listed as a measure of the relative electronegativities of the substituents according to LUCAS.

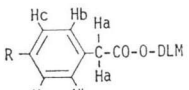
Table 1. Antimicrobial activity (MIC) of PAD derivatives.

Compound	*S. aureus** (μg/ml)	*M. gallisep.*** (μg/ml)
1	0.2	0.0001
2	0.2	0.0002
3	0.4	0.0004
4	0.8	0.0002
5	0.4	0.001
6	0.4	0.002
7	0.4	0.004
8	0.8	0.004
9	3.2	0.008
10	0.8	0.002

* *Staphylococcus aureus* Smith, Medium; BHI.
** *Mycoplasma gallisepticum* KP 13, Medium; PPLO enrichment broth.

Table 2. Chemical shift values of *p*-substituted phenylacetyl group in PAD derivatives.

Compound	Ha (2H, s)	Hb (2H, d, J=8)	Hc (2H, d, J=8)
1	3.82	7.52	7.80
2	3.80	7.42	8.10
3	3.78	7.43	7.62
4	3.77	7.42	7.58
5	3.72	—	—*
6	3.68	6.95	7.12
7	3.64	7.22 (4H, d)	
8	3.63	6.84	7.22
9	3.59	—	—**
10	3.56	6.66	7.06



DLM: 4''-O-deacyldeltamycin residue.

* 7.31 (5H, s-like), no substituent.
** 6.73~6.79 (3H, m) *m*, *p*-disubstituents.
ppm in 100 MHz ($CDCl_3$).

decreasing shift value. Among the ^{1}H-NMR spectra of the derivatives, the signals derived from deltamycin residue were almost same each other, which means that the conformation of the fundamental macrolide skeleton was not changed by *p*-substituted phenylacetylation process.

The shifts of *meta*-protons (Hb) on the aromatic ring of the *p*-substituted phenylacetyl group correlate with the corresponding HAMMETT type substituent constants (σ_{meta}) (Note 2)[5]. But correlation between the shifts of the *ortho*-protons (Hc) and the substituent constants could not be found, since HAMMETT's rule is inapplicable to *ortho*-position. On the chemical shifts of the methylene protons (Ha) at *para*-position of phenylacetyl group in PAD derivatives, relationship between the *para*-substituent constants (σ_{para}) (Note 2) and chemical shifts at methyl protons of corresponding *p*-substituted toluenes[4] as model compounds (Table 3) was examined, because influence of electronegative substituent of aromatic ring on the exocyclic proton has not been reported.

Table 3. Chemical shift values of *p*-substituted toluenes.

Compound	Methyl proton (3H, s)
$MeSO_2$-toluene	2.50
NO_2- 〃	2.50
H- 〃	2.35
F- 〃	2.32
Cl- 〃	2.32
MeO- 〃	2.26
OH- 〃	2.24

ppm in 60 MHz ($CDCl_3$).

As shown in Fig. 2, a linear relationship was obtained between the shift of methyl proton at *p*-substituted toluene and HAMMETT's *para*-substituent constant (σ_{para}). This suggests that the property of the substituent on the ring has an effect on that of hydrogens at benzyl position in correlation with each other. The chemical shifts of the model compounds also correlated with the chemical shifts of the methylene protons

(Note 2) Substituent constant (σ) of HAMMETT's equation. Substituent: σ_{meta}, σ_{para}; NO_2, 0.710, 0.778, $MeSO_2$, 0.647, 0.728, CN, 0.678, 0.628, CF_3, 0.415, 0.551, Cl, 0.373, 0.226, F, 0.337, 0.062, MeO, 0.115, −0.268, OH, −0.002, −0.357.

Fig. 2. Methyl shift of *p*-substituted toluene *vs.* HAMMETT's substituent constant σ_{para}.

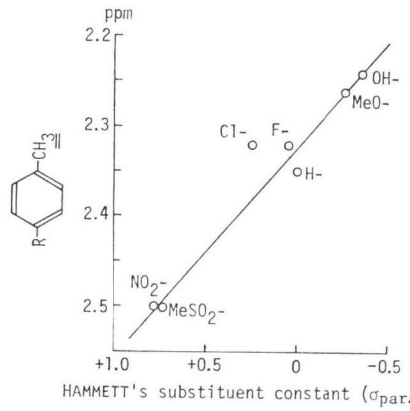
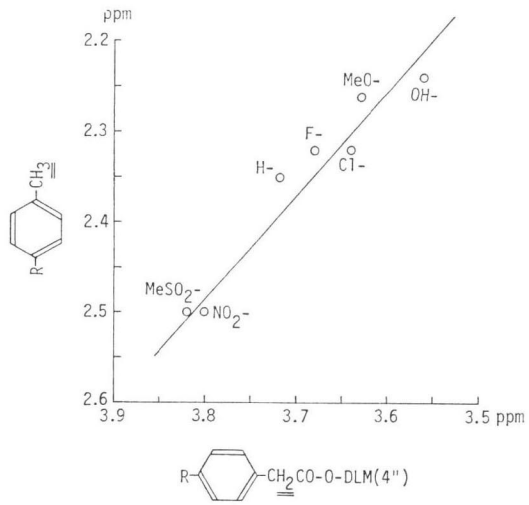


Fig. 3. Chemical shift of benzyl protons (*p*-substituted toluene *vs.* 4″-O-*p*-substituted phenylacetyl-DLM).

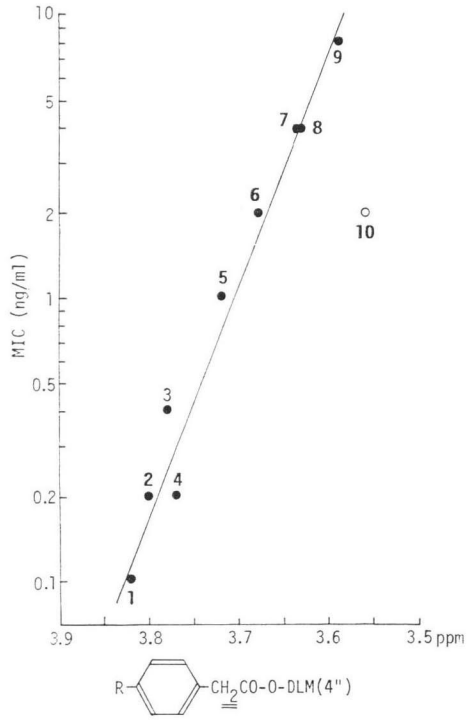


(Ha) in the corresponding 4″-O-*p*-substituted phenylacetyl-4″-O-deacyl-DLM as shown in Fig. 3. The two experimental results indicate that the benzyl proton was influenced by electronegative substituent on the ring and was shifted in proportion to the electronegativity.

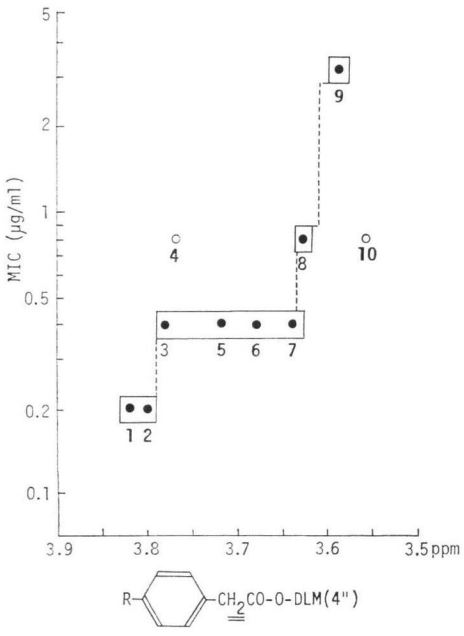
Among the shifts of three protons, Ha, Hb and Hc, Ha proton shift was used to elucidate the activity-electronegativity relationship for the following reasons: Ha proton showed the most narrow shift range but it gave the most proper shift without being influenced by the neighboring group disturbance. The Ha proton had also the following characteristics: (i) multiplicity of the

Fig. 4. Correlation diagram between Ha chemical shift and antimicrobial activity

1) Antimycoplasmal activity.
Test organism: *M. gallisepticum* KP13



2) Antistaphylococcal activity.
Test organism: *S. aureus* Smith



Ha proton was a singlet which was easy to be analysed, (ii) the proton showed a reasonable shift value, even if the *ortho*-, *meta*-position or nothing was substituted on the ring, (iii) the signal of Ha proton was separated from other signals, although those of Hb and Hc overlapped in some substituents.

Minimal inhibitory concentrations (MICs) of the PAD derivatives against some microorganisms were measured by the following methods: the MIC against *Mycoplasma gallisepticum* KP13 was determined by two-fold tube dilution method in PPLO enrichment broth and the MIC against *Staphylococcus aureus* Smith was done by the above-dilution method in Brain heart infusion broth (BHI) at pH 7.5.

On a semilogarithmic plot (the MIC on log scale and the chemical shift on normal scale), a straight line was given in the MICs against *M. gallisepticum* of the derivatives *vs.* the chemical shifts of the Ha protons as shown in Fig. 4, whereas a plot of the MICs against *S. aureus vs.* the shift gave a stepwise linear relationship (Fig. 4). Probably, wide range of the MIC as in Mycoplasma (0.1 ~ 8 ng/ml) may give a straight line, on the other hand narrow range of MIC as in *Staphylococci* (0.2 ~ 3.2 μg/ml) a stepwise line. Among these derivatives, only the phenolic-PAD (compound **10**) did not fall on the line. The derivative had a smaller proton shift or lower electronegativity but higher antimicrobial activity against the two microorganisms. This might reflect a strong germicidal activity of the phenolic-OH group itself.

Our results show that chemical shift in ^{1}H-NMR is a good index of antimicrobial activity in chemical modification of antibiotics and provides a useful tool to obtain more active derivatives.

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