

Since the lifetimes of triplet exciplexes in general, and the one we suggest in particular, are totally unknown, it is not possible to give even an estimate of  $k_Q$ . It does, however, seem safe to say that the lifetime must be much too short to permit  $k_Q$  to be of the order of magnitude of that for  $T_1-S_0$  radiationless transitions in aromatic hydrocarbons of comparable triplet excitation energy,<sup>17</sup> *ca.*  $1 \text{ sec}^{-1}$ . It thus appears probable that new radiationless decay mechanisms must be considered.

Finally, we wish to point out explicitly that the effect we observe may occasionally complicate determination of  $\phi_{ST}$  values by the Hammond-Lamola technique;<sup>2</sup> we would suggest that at least two independent systems be tried when utmost confidence in  $\phi_{ST}$  is required.

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(17) W. Siebrand, *J. Chem. Phys.*, **47**, 2411 (1967).

(18) G. S. Hammond and D. Valentine, *J. Amer. Chem. Soc.*, in press.

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## Microbial Transformation of Antibiotics. V. Clindamycin Ribonucleotides

Sir:

Clindamycin (I) is a clinically useful antibiotic produced by chlorination of lincomycin.<sup>1</sup> Previous papers have described the microbial phosphorylation<sup>2</sup> of clindamycin to clindamycin 3-phosphate (II) and the conversion of clindamycin to *N*-demethylclindamycin or clindamycin sulfoxide by *Streptomyces* species.<sup>3</sup> The present communication describes studies related to the bioconversion of clindamycin to clindamycin ribonucleotides.

Several streptomycete species were found to transform clindamycin to compound(s) lacking *in vitro* antibacterial activity against test organisms. One of these species, *Streptomyces coelicolor*, completely inactivated clindamycin in less than 48 hr when the antibiotic was added to 24-hr cultures of the organism grown in a complex medium. Clindamycin could be regenerated by treatment of the bioinactive fermentation broth with either crude alkaline phosphatase or snake venom phosphodiesterase. This enzymatic behavior suggested that *S. coelicolor* converted clindamycin to compound(s) containing phosphodiester bonds.

(1) B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *Antimicrob. Ag. Chemother.*, **727** (1967).

(2) J. H. Coats and A. D. Argoudelis, manuscript in preparation.

(3) A. D. Argoudelis, J. H. Coats, D. J. Mason, and O. K. Sebek, *J. Antibiot.*, **22**, 309 (1969).

The clindamycin bioconversion products were isolated by adsorption on Amberlite XAD-2 and elution with aqueous methanol. They were purified by chromatography on Dowex-1 (acetate) and counter double current distribution using 1-butanol-water (1:1) as the solvent. Tlc of the obtained material indicated a mixture of bioinactive, uv absorbing compounds which afforded clindamycin by treatment with snake venom phosphodiesterase. Chromatography of the mixture on DEAE-Sephadex (acetate) using Tris-acetate (pH 8.0, 0.1–0.2 *M*) buffer gave eight compounds designated A, B, C, D, E, F, G, and H, in order of elution from the column. Characterization data on compounds A, D, E, G, and H are presented in Table I. Compounds B, C, and F were isolated in small amounts and are not completely characterized.<sup>4</sup>

Compound H was identified as clindamycin 3-phosphate (II) by comparison ( $[\alpha]_D$ , ir and nmr spectra) with an authentic sample.<sup>2</sup> This material afforded clindamycin by treatment with alkaline phosphatase (Table II) but remained unchanged after incubation with either snake venom or spleen phosphodiesterase.

The molecular formulae of compounds A, D, E, and G (specifically the presence of one P atom per molecule), the uv spectra, and the hydrolysis of these compounds by crude alkaline phosphatase to clindamycin and cytidine, adenosine, uridine, and guanosine (Table II) suggested structures for these compounds in which clindamycin is linked to the phosphate group of cytidine phosphate (compound A), adenosine phosphate (compound D), uridine phosphate (compound E), and guanosine phosphate (compound G). The inability of spleen phosphodiesterase to cleave all four clindamycin ribonucleotides, contrasted with hydrolysis to clindamycin and the corresponding nucleoside 5'-phosphates (Table II) by snake venom phosphodiesterase, indicated a nucleoside 5'-phosphate-clindamycin linkage in these compounds.

We propose structures III, IV, V, and VI for compounds A, D, E, and G, respectively. The assignment of the phosphate diester linkage at the C-3 position of the aminosugar moiety of clindamycin is based on periodate oxidation studies. In this oxidation, it was found that cytidine, adenosine, uridine, guanosine, and their respective 5'-phosphates consumed 1 mol of periodate in less than 15 min with no overoxidation. It was also found that clindamycin rapidly consumed 2 mol of periodate by cleavage of the glycol groupings at C-2, C-3, and C-4 and slowly an additional mol by oxidation of the sulfur. Compounds A, D, E, and G consumed 2 mol of periodate (one rapidly and one slowly) which indicates phosphodiester attachment at C-3 since alternative attachments (C-2 or C-4) would require consumption of 3 mol of periodate.

The results obtained by chemical hydrolyses (Table II) support the postulated structures. As expected,<sup>5</sup> the purine ribonucleotides (compounds D and G) afforded purines and ribose by treatment with 1 *N* aqueous HCl,

(4) Data available at present suggest that compound B is clindamycin sulfoxide 5'-adenylate. The formation of this compound is not surprising since clindamycin is converted to clindamycin sulfoxide by *Streptomyces* species (see ref 3). Compound C appears to be clindamycin 4-(5'-adenylate) and it is most probably produced by rearrangement of the adenyl group under the isolation conditions. Similarly, compound F appears to be clindamycin 4-(5'-guanylate).

(5) E. Chargraff and J. N. Davidson, "The Nucleic Acids," Vol. I, Academic Press, New York, N. Y., 1955, Chapter 5.

Table I. Characterization Data

Compd	Mol formula <sup>a</sup>	Mol wt <sup>b</sup>		[M] <sub>D</sub> , ° deg	Uv [ $\lambda_{\max}$ , m $\mu$ ( $\epsilon \times 10^{-3}$ )]		
		Calcd	Found		pH 2.0	pH 7.0	pH 11.0
A	C <sub>27</sub> H <sub>45</sub> N <sub>5</sub> O <sub>12</sub> ClPS	729	742	+445	279 (9.60)	269 (6.80)	271 (6.60)
D	C <sub>28</sub> H <sub>45</sub> N <sub>7</sub> O <sub>11</sub> ClPS	753	726	+473	257 (12.60)	261 (12.50)	261 (12.70)
E	C <sub>27</sub> H <sub>44</sub> N <sub>4</sub> O <sub>13</sub> ClPS	732	764	+578	261 (8.20)	262 (8.40)	262 (6.50)
G	C <sub>28</sub> H <sub>45</sub> N <sub>7</sub> O <sub>12</sub> ClPS	769	750	+530	256 (11.10)	254 (12.50)	259 (10.70)
H	C <sub>18</sub> H <sub>34</sub> N <sub>2</sub> O <sub>8</sub> ClPS	504	530	+458	277 (7.50) (sh)	273 (8.00) (sh)	266 (10.60)
					None		

<sup>a</sup> Satisfactory analyses were obtained on all listed compounds. <sup>b</sup> Molecular weights were determined by vapor pressure osmometry in methanol. <sup>c</sup> Specific rotation was determined in water (*c* 1).

Table II. Compounds<sup>a</sup> Produced by Degradation of Clindamycin Ribonucleotides

Compd	Crude alkaline phosphatase	Enzymatic hydrolysis		Chemical hydrolysis <sup>b</sup>		
		Snake venom diesterase <sup>c</sup>	Spleen diesterase <sup>c</sup>	1 N HCl <sup>d</sup>	6 N HCl or 72% ClO <sub>4</sub> H <sup>e</sup>	0.1 N NaOH <sup>f</sup>
A	Clindamycin; cytidine	Clindamycin; cytidine-5'-P	No reaction	Cytidine	Cytidine Cytosine	Cytidine
D	Clindamycin; adenosine	Clindamycin; adenosine-5'-P	No reaction	Adenine	Adenine	Adenosine
E	Clindamycin; uridine	Clindamycin; uridine-5'-P	No reaction	Uridine	Uridine Uracil	Uridine
G	Clindamycin; guanosine	Clindamycin; guanosine-5'-P	No reaction	Guanine	Guanine	Guanosine
H	Clindamycin	No reaction	No reaction	Ribose		

<sup>a</sup> Identified by tlc (silica gel GF) in three solvent systems. <sup>b</sup> Adenine and guanine were isolated as the crystalline hydrochlorides. Crude alkaline phosphatase (Worthington Biochemicals) exhibited both mono- and diesterase activities. <sup>c</sup> Worthington Biochemicals Corp., Freehold, N. J. <sup>d</sup> 100°, 1 hr. <sup>e</sup> 100°, 1 hr. <sup>f</sup> 100°, 2.5 hr.

while the pyrimidine nucleotides A and E gave the corresponding nucleosides, cytidine and uridine, respec-

purine or pyrimidine bases, while treatment with 0.1 N NaOH gave in all cases the corresponding nucleosides.

Finally, the nmr spectra<sup>6</sup> of compounds A, D, E, and G are in agreement with the assigned structures. Absorptions due to CH<sub>3</sub>CH<sub>2</sub>- ( $\delta$  0.98), CH<sub>3</sub>C(Cl)H- ( $\delta$  1.42), CH<sub>3</sub>S- ( $\delta$  2.25), and CH<sub>3</sub>N- ( $\delta$  2.98) groups of clindamycin were present in all spectra. Absorptions due to the hydrogen(s) of the corresponding purine or pyrimidines were also observed.

As mentioned earlier, the isolated clindamycin ribonucleotides were inactive *in vitro* against several organisms including *Staphylococcus aureus*. However, these compounds were found to protect *S. aureus* infected mice with a CD<sub>50</sub><sup>7</sup> of ca. 30 mg/kg when they were administered subcutaneously.

In some respects the formation of these compounds resembles the adenylation of streptomycin<sup>8</sup> and spectinomycin<sup>9</sup> by an *R* factor carrying *Escherichia coli*. Both antibiotics, like clindamycin, inhibit protein synthesis<sup>10,11</sup> in bacterial systems.

(6) Nmr spectra were determined on 10–15% solutions in DMF-*d*<sub>7</sub> with a Varian A-60 spectrometer.

(7) D. J. Finney, "Statistical Methods in Biological Assay," 2nd ed, Hafner Publishing Co., New York, N. Y., pp 524–530.

(8) H. Umezawa, S. Takasawa, M. Okanishi, and R. Utahara, *J. Antibiot.*, **21**, 81 (1968).

(9) R. E. Benevise, B. W. Ozanne, and J. Davies, *Bacteriol. Proc.*, **48** (1969).

(10) B. Weisblum and J. Davies, *Bacteriol. Rev.*, **32**, 493 (1968).

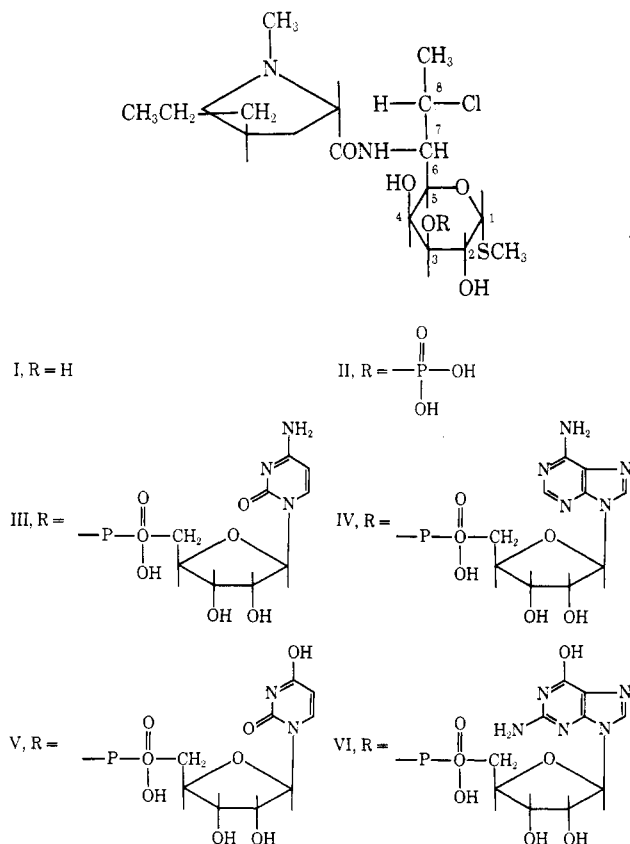
(11) F. Reusser, personal communication.

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Communications to the Editor



tively. Hydrolysis of all four nucleotides with 6 N HCl or 72% perchloric acid resulted in liberation of