

## COMMUNICATIONS TO THE EDITOR

### HYDROLYSIS PRODUCTS OF PSEUDOVITAMIN B<sub>12</sub>

Sir:

Acid hydrolysis of vitamin B<sub>12</sub> yields 5,6-dimethylbenzimidazole,<sup>1</sup> 5,6-dimethylbenzimidazole-1- $\alpha$ -D-ribofuranoside,<sup>2</sup> and its 2- or 3-phosphate,<sup>3</sup> D<sub>g</sub>-1-amino-propanol-2,<sup>4</sup> ammonia,<sup>5</sup> and a red cobalt fraction.<sup>6</sup> Comparable hydrolysis of crystalline pseudovitamin B<sub>12</sub><sup>7</sup> does not give 5,6-dimethylbenzimidazole nor any derivative thereof. Hydrolysis with 1 M HCl at 100° for 2.5–12 hours yields adenine, a purine tentatively identified as hypoxanthine, a ninhydrin reacting material corresponding to D<sub>g</sub>-1-amino-propanol-2, ammonia, inorganic phosphate, and a red cobalt pigment fraction.

A solution of pseudovitamin B<sub>12</sub>, 1.4 mg. in 2.8 ml. of 1 M HCl, was heated in a sealed tube for 2.5 hours at 100°. The resulting red solution was extracted with *n*-butanol to remove the red pigment, and the colorless acid fraction was chromatographed on paper with *n*-butanol-acetic acid-water (4:1:5), *n*-butanol-water, and isoamyl alcohol-5% disodium phosphate.<sup>8</sup> A ninhydrin reacting material which was not an amino acid<sup>9</sup> was demonstrated on the developed paper chromatograms. It could not be differentiated from an authentic sample of D<sub>g</sub>-1-aminopropanol-2<sup>10</sup> on mixed chromatograms. The presence of phosphate was indicated by a positive ammonium molybdate-benzidine test, and ammonia by Nessler test.

Examination of developed paper chromatograms under ultraviolet light (2537 Å.) failed to reveal fluorescent spots characteristic of dimethylbenzimidazole and its derivatives. However, two dark blue absorbent zones characteristic of purines were observed. In butanol-acetic acid, the more intense band had an  $R_F$  value (0.59) corresponding to that of adenine while the  $R_F$  (0.49) of the zone of lesser intensity corresponded to that of hypoxanthine. The hydrolytic fragment ( $R_F$  0.59), eluted from the paper with 0.1 M HCl, gave a positive mercuric nitrate test,<sup>11</sup> negative murexide and diazo tests, and a positive color test<sup>12</sup> specific for adenine. The partition coefficient of the above hydrolytic fragment in butanol and 1 M phosphate buffered to pH 6.5<sup>13</sup> was 2.9. The ultraviolet spectrum of the eluate was characteristic of adenine with a maximum at 262.5  $\mu$  in 0.1 M HCl and at 268  $\mu$  at pH 11.0; the calculated yield was 0.78

mole. Control experiments indicate that the fragment on paper chromatograms corresponding to hypoxanthine is an artifact resulting from deamination of adenine. Similar results were obtained on repetition of the hydrolysis with several samples of pseudovitamin B<sub>12</sub>.

It appears that pseudovitamin B<sub>12</sub> and vitamin B<sub>12</sub> differ in the occurrence of adenine instead of 5,6-dimethylbenzimidazole in the nucleotide portion of the molecule.

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### RELATIONSHIP BETWEEN AUREOMYCIN AND TERRAMYCIN

Sir:

Some X-ray and optical measurements for crystals of aureomycin<sup>1</sup> and terramycin<sup>2,3</sup> hydrochlorides are presented below.

	Aureomycin HCl	Terramycin
<i>a</i> (Å.)	11.22	11.19
<i>b</i> (Å.)	12.89	12.49
<i>c</i> (Å.)	15.55	15.68
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Density (g. cm. <sup>-3</sup> )	1.52	1.51
Molecular weight (Z 4)	515	499
$\alpha$	1.633(∥ to <i>c</i> )	1.639
$\beta$	1.700(∥ to <i>b</i> )	1.686
$\gamma$	1.715(∥ to <i>a</i> )	1.721(∥ to <i>a</i> )

We have noticed a striking resemblance between intensities of corresponding X-ray reflections, (*h*0*l*) and (0*k**l*), for the two compounds. The above results, particularly the intensity correlation, show that the two molecules must be very similar in shape and orientation in closely related isomorphous unit cells. The observed molecular weights are probably accurate to within about 5 units, and are in accord with the view that the non-ionic chlorine atom in aureomycin has been replaced by a hydroxyl group in terramycin. Collection of complete three-dimensional intensity data for a full X-ray structure analysis of aureomycin hydrochloride is now in progress.

The X-ray measurements were carried out at the Gates and Crellin Laboratories, California Institute of Technology, and at Pennsylvania State College, and we wish to acknowledge our indebtedness to Professors Linus Pauling and Ray Pepinsky for the facilities provided.

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