

PREPARATION OF RADIOISOTOPI-  
CALLY LABELED TUBERACTINOMYCIN,  
[ $\beta$ -[ $^{14}$ C]UREIDO-DEHYDROALANINE $^5$ ]-  
TUBERACTINOMYCIN O

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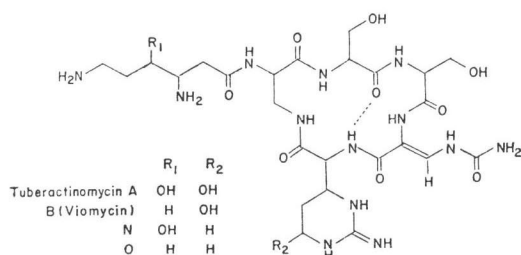
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In our previous paper,<sup>1)</sup> we reported the preparation of [modified  $\beta$ -ureidodehydroalanine $^5$ ]-tuberactinomycin N from the natural antibiotic, tuberactinomycin N (Fig. 1) in practical use as an antituberculous agent, utilizing the ureido-exchange reaction through an equilibrium under acidic condition as shown in Fig. 2. In view of simplicity of the modified procedure, its applicability to small-scale experiments and the stability of the product, this method seemed to be appropriate for the preparation of labeled tuberactinomycin, which would be a useful tool for biochemical investigations, particularly on mechanism of action of this antibiotic. This paper presents a preparation of [ $\beta$ -[ $^{14}$ C]ureido-dehydroalanine $^5$ ]-tuberactinomycin O (Fig. 1). This congener contains  $\beta$ -lysine at the branched part in the place of the  $\gamma$ -hydroxy- $\beta$ -lysine residue in tuberactinomycin N, which readily liberates  $\gamma$ -hydroxy- $\beta$ -lysine lactone on strong acid-treatment.<sup>1,2)</sup>

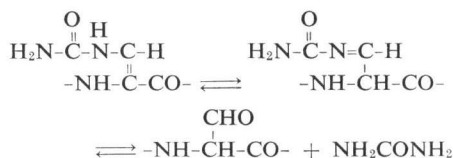
A preliminary experiment was carried out with urea of low radioactivity in a similar manner to that described for cold compound.<sup>1)</sup> Thus, tuberactinomycin O (15.0 mg, 19.3  $\mu$ mol) was treated with a mixture of cold urea (15.0 mg, 250  $\mu$ mol) and [ $^{14}$ C]urea\* (0.10 mCi, ca 2  $\mu$ mol) in 3 N HCl (0.3 ml) at room temperature (5~

Fig. 1. Structures of tuberactinomycins.



\* The radioactive urea (40~60 mCi/mmol) was supplied by The Radiochemical Center Amersham.

Fig. 2. Equilibrium in acidic solution of  $\beta$ -ureido-dehydroalanine residue.

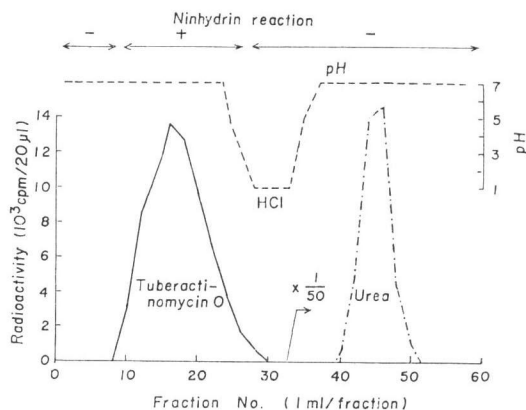


15°C) for 30 days. Isolation of the product in this experiment by ion-exchange column chromatography employed in the previous study<sup>1)</sup> was unfavorable due to loss of some material. Therefore, the reaction mixture was passed through a column (1.2  $\times$  80 cm) of Sephadex G 10 and eluted with water. The desired labeled product was successfully separated from excess urea as well as HCl as demonstrated in Fig. 3.

The synthesis of highly radioactive compound was performed in the same way as described above except changing the ratio of radioactive urea (3.9 mCi, ca 78  $\mu$ mol) and cold urea (11.0 mg, 183  $\mu$ mol). A small amount of dark precipitate was formed during the course of reaction in this case, probably due to either an impurity in the hot urea or decomposition of a very reactive species by radiation. This, however, could be completely removed by chromatography on Sephadex. The fractions containing labeled tuberactinomycin O were collected and lyophilized.

Fig. 3. Elution pattern of labeled tuberactinomycin O in chromatography on a Sephadex G10 column.

Radioactivity was measured in an aliquot of each fraction in a mixture of ethanol (0.5 ml) and a scintillant solution (5 ml) of 2,5-diphenyl-oxazole (6 g) and 1,4-bis[2-(5-phenyloxazolyl)]-benzene (0.1 g) in xylene (1 liter) with a Beckman LS150 Scintillation Counter.



ed to afford a white powder (10.5 mg (69.8%), 15.0 mCi/mmol\*). Purity of the product was ascertained chromatographically, judged by ninhydrin color and also by radioactivity. As a preparative method for a labeled derivative of the natural antibiotic peptide, this procedure is very advantageous since neither protecting groups nor coupling reagents are needed in the reaction. Furthermore, this method could be applied for the preparation of not only even more radioactive tuberactinomycin, but also another labeled tuberactinomycin or capreomycin<sup>3)</sup> in an extremely facile manner.

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#### References

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