# PREPARATION OF ALL-TRANS-11-$^3$H-RETINOL FROM ITS ACETATE

H. H. Kaegi and J. E. Bupp
Bio-Organic Chemistry Laboratory
SRI International
Menlo Park, California 94025, U.S.A.

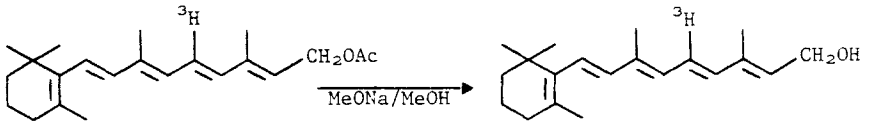
## SUMMARY

Starting with tritiated all-*trans*-retinyl acetate the preparation of tritiated retinol is described.

Key Words: all-*trans*-11-$^3$H-retinol, all-*trans*-11-$^3$H-retinyl acetate

## INTRODUCTION

Retinol or Vitamin A, a substance necessary for vision, reproduction, growth, mucus secretion, and the maintenance of normal differentiation of epithelial tissues, is of particular interest in a labeled form for the study of its mode of action. This rather unstable pentaene alcohol becomes even more unstable with the incorporation of a radioactive label. Because of this inherent instability it is best stored in the form of one of its esters or as retinoic acid, from which it can be prepared by reduction with lithium aluminum hydride.[1,2,3] A quick efficient preparation of small quantities of this compound from a more stable and storable source would therefore be desirable. We would like to report a simple method for the preparation of tritiated all-*trans*-retinol from its acetate. All-*trans*-$^3$H-retinyl acetate was the starting material of choice because it can be prepared easily from all-*trans*-$^3$H-retinal[4] and was found to be quite stable when stored in toluene at -50°C.




1 2

A simple transesterification, in which all-*trans*-11-$^3$H-retinyl acetate (1) in anhydrous methanol was stirred in the presence of a catalytic amount of sodium methoxide at room temperature and under argon for 20-24 h, was used. Analysis of the reaction mixture at that time showed it to be 85% tritiated retinol 2. The product

0362-4803/83/040553-03$01.00

Received April 15, 1982
Revised November 29, 1982

was purified by mini-preparative HPLC and stored in the HPLC solvent. In this manner 11-$^3$H-retinol, with a radiopurity of 95%, was obtained in 42% yield. The somewhat disappointing yield was typical in repeated runs and a result of the inherent instability of tritiated retinol under the HPLC purification conditions.

## EXPERIMENTAL

Radioassays were carred out in 10 ml of Scintisol cocktail (Isolab Inc.) with internal standards and counted with a Beckman LS-250 liquid scintillation system. Analyses by HPLC were obtained from a Waters 6000A solvent delivery system, U6K injector, Model 450 variable wavelength detector and a RCM-100 radial compression module using a Radial-Pak C18 column (8 mm ID x 10 cm). Unless otherwise noted, analyses were done with ethanol-water 75-25 at 2 ml/min with detection at 280 and 325 nm.

### All-trans-11-$^3$H-retinol

With the aid of argon and at room temperature the solvent was blown off gently from a 10 mCi sample of all-trans-11-$^3$H-retinyl acetate (5.95 mCi/mg; 1.68 mg) in 5 ml of toluene. The residue was taken up in 10 ml of dry methanol, and 1 λ of a solution of 0.014 M sodium methoxide in methanol was added. The mixture was allowed to stir under argon and yellow light at room temperature for 24 h. HPLC analyses at this time showed 85% 11-$^3$H-retinol in the sample. The solvent was blown off carefully with argon at room temperature, and the residue dissolved in 1 ml of ethanol. The sample was purified by applying 500-µl aliquots to a Radial-Pak C18 column eluting with ethanol-water (75-25). The combined fractions containing 11-$^3$H-retinol were diluted with ethanol-water 75-25 to a 10-ml total volume for analyses and storage at -50°C. A total of 4.27 mCi (42% yield) of all-trans-$^3$H-retinol was obtained, UV: $\lambda_{max}$ (75% EtOH) 322. The radiochemical purity was found to be 95% by HPLC analysis.

An almost identical run stirred for 24 h and purified by HPLC (Radial-Pak C18) but with acetonitrile:water (80:20) as solvent system yielded 5.1 mCi of 11-$^3$H-retinol (51% yield) with a radiopurity of 97.7%. Removal of the solvent is best carried out by freeze-drying at low temperature because decomposition occurs when the solvent is removed in vacuo at room temperature.

ACKNOLWEDGMENT

This work was supported by Contract No. NO1-CP-05601 from the National Cancer Institute.

REFERENCES

1. Schwarzkopf, O., Cahnmann, H. J., Lewis, A. D., Swidinsky, J., and Wüest, H. M. - Helv. Chim. Acta 32, 443 (1949).
2. Hale, R., Burger, W., Perry, C., and Liebman, A. A. - J. Labelled Comp. Radiopharm. 13, 123 (1977).
3. Kaegi, H. H., and DeGraw, J. I. - J. Labelled Comp. Radiopharm. 18, 1099 (1981).
4. Kaegi, H. H., Bupp, J. E., and DeGraw, J. I. - J. Labelled Comp. Radiopharm., in press.