THE SYNTHESIS OF DISODIUM-3-AMINO-1-HYDROXYPROPANE-1,1-DIPHOSPHONATE-1-^{14}C. (APD·2Na)

Ernest Magnien, Mujahid Shaikh, John T. Suh and
Howard Jones
Revlon Health Care Group
1 Scarsdale Road
Tuckahoe, New York 10707

SUMMARY

Disodium-3-amino-1-hydroxy-1,1-diphosphonate-1-^{14}C (APD 2Na), a powerful calcium binding agent and potential drug for the treatment of Paget's Disease was synthesized. The reaction was carried out by reacting β-alanine (1-^{14}C) with phosphorus acid and phosphorus trichloride in chlorobenzene. Treatment of the resultant APD with two equivalents of sodium hydroxide gave the title compound.

Key Words: Carbon 14, Diphosphonate, APD, Paget's Disease, Calcium Metabolism.

INTRODUCTION

Disodium-3-amino-1-hydroxy propane-1,1-diphosphonate (APD·2Na) is one of a group of diphosphonate compounds which is absorbed by bone tissue. Compounds of this class, such as disodium ethane-1-hydroxy-1, 1-diphosphonate (EHDP), inhibit calcium hydroxyapatite crystal dissolution and prevent immobilization osteoporosis in rats [1,2]. Compounds having this pharmacological profile are potentially useful in the treatment of osteoporosis, Paget's disease, calcinosis universalis and myosites ossificans. It is for this reason that we undertook to synthesize ^{14}C-APD·2Na. Compounds of this type are highly polar and therefore poorly

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absorbed from the gastrointestinal tract. In order to have measurable blood levels of APD we wanted material having a relatively high specific activity in the range of 40 - 60 uCi/mg.

DISCUSSION

A synthetic procedure reported in the patent literature[3] was used with some modification for the synthesis of this compound as shown in Fig. 1.

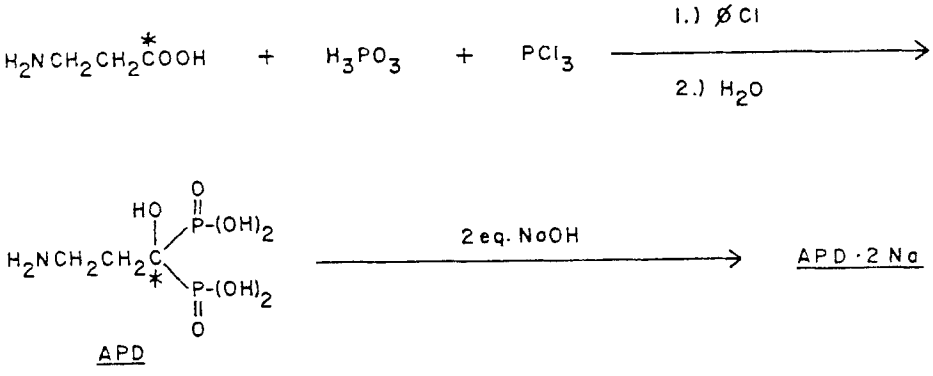

Figure 1

The reactants were combined with chlorobenzene and heated at 100°C for 3 hours. The reaction mixture was cooled, water was added, and the reaction mixture was reheated. Time and temperature of reheating were not specified in the literature reference. It was found that at a temperature of 100°C if the heating time was for one half hour or less, products were obtained which could not be identified. TLC work on these compounds were hampered due to difficulty in visualization of APD and its intermediates. When

the heating time was increased to two hours, a consistent yield of 42% of APD was obtained. In the preparation of pure disodium salt, it should be noted that in principle, compounds containing from one to four sodium atoms can be formed. We have been able to prepare solid derivatives of only salts containing 1 - 3 atoms of sodium. On potentiometric titration one can see end points for the formation of the mono and di-sodium salts and can determine pKa values of these salts, which are respectively 4.15 and 8.2. In order to insure the preparation of only the disodium salt, it was necessary to add exactly two equivalents of sodium hydroxide to a solution of APD in water. On concentration, the pure disodium salt crystallized out. By a similar procedure, the mono and trisodium salts can be obtained.

EXPERIMENTAL

The radiolabelled starting material was β-alanine (1-^{14}C) obtained from the ICN Chemical and Radioisotope Division. The specific activity was 13.9 mCi/mmole. NMR spectra were obtained in D_2O using an EM-390-90MHz spectrometer. Infra-red spectra were done on a Perkin-Elmer Model 621 Spectrophotometer using KBr pellets. A Packard Model 7220/21 scanner was used for the radioscans and specific activity was determined using a LS 9000 Liquid Scintillation Counter. TLC was done using Polygram Cel 300 Cellulose plates.

3-Amino-1-hydroxypropane-1,1-diphosphonic acid-1-^{14}C (APD) In a 100ml round bottomed flask was placed 0.3754g of β-alanine (1-^{14}C). To this was added 1.0g of H_3PO_3 and 5.0 ml of chlorobenzene,

followed by addition of 1.8g of phosphorus trichloride. The reaction mixture was stirred cautiously and then placed in an oil bath at 110°. The reaction mixture was stirred vigorously for 3 hrs. then cooled to room temperature. Ten ml of water was added cautiously and stirring was continued for 2 hrs. in an oil bath at 105°. The reaction mixture was cooled and the aq. layer separated and filtered with Celite and charcoal. The colorless solution was concentrated to a syrup. Two ml of methanol were added slowly as the free acid began to crystallize. After sitting overnight in the refrigerator, the material was filtered off and washed with water, then methanol, then dried at 90° for 2 hrs. to give 0.4334g (42.5%) of colorless crystals.

Disodium-3-amino-1-hydroxypropane-1,1-diphosphonate-1-^{14}C (APD·2Na)

The above free acid was transferred to a beaker with 3ml of water. Two equivalents of 1N NaOH (3.68ml) were added bringing the pH to 7.7. The solution was filtered and then concentrated to a small volume. The disodium salt crystallized out on cooling and was filtered after 2 hrs. to yield 0.4915g (40.6%) of disodium salt. This was recrystallized from 3 ml of hot water by adding methanol to incipient cloudiness. After 2 hrs. the material was filtered and washed with methanol to yield 0.381g (31.5%) of pure disodium salt. Anal. Calcd. for $C_3H_{11}NO_7P_2 \cdot 2Na$: C, 12.91; H, 3.25; N, 5.02. Found: C, 13.23; H, 3.48; N, 4.75.

Sp. Act.: 13.95mCi/mmole

Radiopurity: Single radioactive peak in three solvent systems. Water: ethanol: ammonium hydroxide 80:10:15, r.f. 0.2; water: acetone 70:30; r.f. 0.8; formic acid: water 1:1, r.f. 1.0.

IR: Identical to standard "cold" sample. Broad peak at 2900 - 3200 cm^{-1}. Peak at 1060 and 540 cm^{-1}.

NMR: Identical to standard "cold" sample. Multiplet at 2.2ppm and triplet at 3.3ppm.

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