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NOTE

SYNTHESIS OF [5-^{3}H] HEMIMELLITIC ACID

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SUMMARY

1,2,3-Benzenetricarboxylic acid (hemimellitic acid) tritiated in position 5 has been labelled at a specific activity of 16.2 Ci/mmol, starting from 3-bromo naphthalic anhydride. Attempts at direct bromination of the acid followed by tritiodebromination, or of general labelling by hydrogen-tritium exchange gave poor results.

Key Words: hemimellitic acid, tritium labelling.

INTRODUCTION

1,2,3-Benzenetricarboxylic acid (hemimellitic acid) is a specific inhibitor of the citrate carrier in liver mitochondria as well as an inhibitor of pyruvate carboxylation, as has been shown in small animals like rats, mice and rabbits[1-4]. It blocked citrate transport without affecting malonate or α-heteroglutarate transport.
A radioactive synthesis of [carboxyl-^{14}C]-hemimellitic acid has been described[5] but none previously with tritium.

RESULTS AND DISCUSSION

The synthesis of 5-bromo-1,2,3-benzenetricarboxylic acid, as a suitable precursor for tritiation, was carried out in a two-step procedure, starting with the monobromination of naphthalic anhydride[6] and following by the oxidation of the resulting 3-bromo derivative (scheme I). This synthesis was chosen to prevent any effect of the carboxylic groups which strongly deactivate the accessible ring positions for bromination. The procedure is an adaptation of the synthesis of 1,2,3-benzenetricarboxylic acid[7], starting with naphthalic anhydride.

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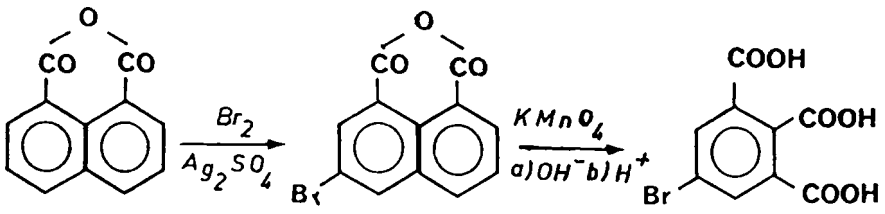

scheme I

Following this method, we synthesized a radioactive molecule, still stable after 3 months (less than 0.1% of radiolytic decomposition), with a high specific activity of 16,2 Ci/mmol.

The NMR spectrum of the brominated molecule has a different print in the aromatic area than that of hemimellitic acid, presenting only one peak at 8.45 ppm. This modification shows that a bromine atom has been introduced in the position 5 (symmetric position) of the benzene ring. The hydrogen ratio of 2:7 also confirms the change in the molecule. The 7 hydrogen atoms are shared as follows: 3 atoms are related to the 3 carboxylic groups and 4 atoms to the 2 molecules of water of crystallization.

As shown by preliminary hydrodebromination, all the bromine atoms were exchanged (tlc and NMR controls). After tritiation, the theoretical specific activity (29 Ci/mmol) was not reached. The low molar specific activity of [5-^{3}H]-hemimellitic acid is probably due to isotopic dilution of the tritium with protium from the solvent used in the tritiation. The reaction was performed in dioxane:NH_4OH (1:1). The large excess of ammonia was necessary to solubilize the tricarboxylic acid and also to neutralize the TBr formed during the tritiodebromination.

EXPERIMENTAL

Tritiation experiments were performed on a vacuum manifold, as previously described[8]. Ultra-violet spectra were recorded on a Perkin-Elmer instrument, model 402 and NMR spectra on a Varian EM 360 spectrometer. Radiochemical and chemical purity were determined by radiochromatogram scanning of thin-layer chromatography (tlc) precoated plates (silicagel 60, F_{254}, Merck) on Berthold Dunnschicht Scanner II, model LB 2722; total and specific activity were measured on Packard Tri-Carb Liquid Scintillation spectrometer, model 3375.

Bromination of naphthalic anhydride[6] To 1 gr (5 mmol) naphthalic anhydride dissolved in 20 mL concentrated H_2SO_4, 0.8 gr Ag_2SO_4 as catalyst and 0.35 mL (12,5 mmol) molecular bromine are added.. A condenser is connected to the vessel and the mixture is stirred for 6

hours at 50-60°C. The solution is allowed to stand overnight at room temperature., and then filtered into a mixture of ice in water. The bromo derivative which precipitates is filtered and washed until pH 7 is reached, then rapidly washed once more with ether and dried at room temperature. A solid is obtained (1.24 gr) and used in the next step of oxidation without any further purification.

Oxidation of 3-bromonaphthalic anhydride[7] 3 gr (11 mmol) 3-bromonaphthalic anhydride and 1.2 gr (30 mmol) NaOH dissolved in 50 mL water are heated with constant stirring to 80-90°C. A hot solution of 14 gr (90 mmol) $KMnO_4$ in 250 mL water is added slowly during 2 hours. Close to the end of addition, the pink color remains and the excess of $KMnO_4$ is reduced by addition of 2 mL hot ethanol. The precipitated MnO_2 is filtered from the hot solution and washed with hot water. The resulting clear solution is reacidified with 3 mL concentrated H_2SO_4, heated to 90°C and another portion of $KMnO_4$ (1.4 gr, 9 mmol) in 30 mL hot water are slowly added with continuous stirring. The color disappeared and MnO_2 precipitated. The filtration is repeated. The volume of the solution is reduced by distillation to 75-80 mL and allowed to cool for 2 hours. The potassium salt of 5-bromobenzenetricarboxylic acid separates as a white precipitate. After filtration, the solid is acidified in an aqueous HCl 1N solution to form the free acid which is extracted by 3 portions of 30 mL ether. The etheral solutions are dried on $MgSO_4$, filtered and the ether is evaporated to dryness. Further purification is obtained by dissolving the acid in 10 mL dioxane:NH_4OH 10% (1:1), the solution evaporated to dryness and the solid washed with methanol. The acidification and the etheral extractions are repeated. 700 mg of pure 5-bromobenzene tricarboxylic acid are obtained. mp.: 186-188°C, tlc system: methanol:water:ammonia (80:15:5), R_f: 0.48
NMR: (DMSO-d^6:DCL_3, 1:1) 5.25 (s, 7H)-2 H_2O + 3 -COOH; 8.45 (s, 2H)-aromatic ring.

Tritiodebromination
The tritiation is performed on a vacuum manifold[8].
5-Bromobenzene-1,2,3-tricarboxylic acid (35 mg, 11 mmol) is dissolved in 0.5 mL dioxane and 0.5 mL NH_4OH 10% and 15mg Pd/C 10% are added as catalyst. The solution is frozen, the reaction system is washed twice with nitrogen gas and evacuated to a residual pressure of 10^{-2} mm Hg. 25 Ci tritium gas are then transferred into the reaction vessel, developing an initial pressure of 350 mm Hg. The solution is allowed to return to room temperature and the suspension is vigorously stirred. After 40 min., 8 Ci (0.14 mmol) of tritium are consumed and

the reaction stops spontaneously. The reaction vessel is frozen, residual tritium is evacuated and the solvent evacuated by cryo-sublimation. The catalyst-substrate mixture is washed twice with 2 mL methanol aliquots which are removed by cryo-sublimation. The vessel is disconected from the system, and the mixture washed once more with methanol which dissolves selectively all the radioactive impurities. The methanol solution is separated by filtration and the solid is separated from the catalyst by dissolution in aqueous solution HCl 1N. The radioactive compound is extracted from the acidic solvent by ether, the organic solution is dried with $MgSO_4$, filtered and evaported to dryness. 335 mCi of 98% chemically and radiochemically pure 1,2,3-[5-^{3}H]-benzene tricarboxylic acid are obtained without any further purification. Specific activity: 16.2 Ci/mmol; tlc systems: n.butanol:acetic acid: water (50:25:25), R_f: 0.61; methanol:water:ammonia (80:15:5), R_f:0.64

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