THE SYNTHESIS AND PURIFICATION OF 2,6-DI-TERT-BUTYL-P-CRESOL-$^{14}C_6$.

B.D Shipp, J.B. Data and J.E. Christian

Departments of Bionucleonics and Medicinal Chemistry-Pharmacognosy,
School of Pharmacy and Pharmacal Sciences,
Institute for Environmental Health, Purdue University,
Lafayette - Indiana, 47906, U.S.A.

Received on September 4, 1972.

SUMMARY

2,6-Di-tert-butyl-p-cresol-$^{14}C_6$ was synthesized by reacting p-cresol-$^{14}C_6$ with isobutylene using concentrated sulfuric acid as catalyst.

Purification of the labelled butylated p-cresol involved very careful recrystallization of this product from absolute alcohol.

Proof of radiochemical purity was determined using thin layer chromatography and autoradiography. Internal liquid scintillation counting techniques were used to determine the specific activity of the compound.

INTRODUCTION

2,6-Di-tert-butyl-p-cresol (BHT) is a well known antioxidant used for many purposes in industry. It is used in the rubber industry to retard the cracking of

rubber, in paints to retard paint oxidation and peeling, in many plastics in the petroleum industry in a variety of its processes, and in the food industry. Probably of the greatest concern, certainly from a health standpoint, is its use in the food industry. Recently, BHT has been shown to be a very active antioxidant in living biological systems[1].

The advent of radiotracer techniques in scientific studies enables one to explore in biological systems many parameters hitherto unattainable for lack of sensitivity of techniques. Therefore, BHT labelled with radioactive $^{14}C_6$ would be extremely useful for investigating in living systems the activity of the compound. While BHT has been used[2] with a carbon of the isobutyl groups labelled (^{14}C) no published synthesis for it has been reported. An exhaustive review of the literature seems to reveal no published procedure for the synthesis of BHT-$^{14}C_6$ either. Thus, this paper is for the purpose of describing the synthesis of the latter compound.

Several procedures have been reported for the synthesis of unlabelled BHT. Paltin and Vitca[3] synthesized BHT in 78.0% yield by bubbling isobutylene through p-cresol in the presence of sulfuric acid. Mai[4] claimed a synthesis for BHT using p-cresol, isobutylene and alkylaluminum halide as catalyst to yield 70.2% of product. Venaka et al.[5] reported that BHT can be produced in 78.0% yield from p-cresol and isobutylene using concentrated sulfuric acid and ferrous or ferric sulfate as catalyst. Isagulyants et al.[6] prepared BHT from p-cresol and isobutylene in the presence of cation-exchange resins KV-1 or KV-2 as catalysts in 42.9-46.3% yield. Kalav[7] reported a preparation to yield 93.5% of BHT using p-cresol, isobutylene and concentrated sulfuric acid as catalyst.

The procedure of Kalav[7] was used for the radiosynthesis of BHT reported herein. Several parameters were investigated using unlabelled p-cresol to learn the optimum conditions to give the best yield in a microsynthesis. It was found by thin layer chromatography that at the end of 6 hours the reaction was 85-90%

complete, at 9 hours 90-95% and at 12 hours 95-99%. Yields for reaction temperatures of 60°, 70°, 75° and 90° C were determined. Using labelled p-cresol there was obtained, at the end of 12 hours at a temperature of 75-77° C, 91.9% of pure, recrystallized product which showed 99+% radiochemical purity. Two solvent systems, (a) benzene and (b) toluene-acetone (20:1), were used for its thin layer chromatographic analysis. Benzene gave an R_f value of 0.73 and toluene-acetone gave 0.89. During the preparation there was no loss of radioactive material from the mixture as found from an examination of the trapped gases collected with a methylene chloride-dry ice bath. The flow of gaseous isobutylene through the reaction mixture should be sufficiently rapid to bring about gentle agitation and should be introduced at the bottom of the mixture.

EXPERIMENTAL

2,6-Di-tert-butyl-p-cresol-$^{14}C_6$ (7).--Into a 10 ml 2-necked, pear-shaped flask there was placed 0.9530 g (8.8 millimoles) of dried crystalline p-cresol, 62 mg (0.57 millimole) of p-cresol-$^{14}C_6$* and 0.018 g of concentrated sulfuric acid as catalyst. The labelled p-cresol was so placed by transferring it from the shipping container into the reaction flask by using 10 ml of anhydrous ether; the ether was removed by evaporation while passing a stream of dry nitrogen across the mouth of the flask. The flask and its content was placed in an oil bath heated to maintain 76° C ±1° (inside temperature). The flask was equipped with a capillary delivery tube drawn to a fine point and curved at the tip to the curvature of the pear-shaped flask. The flask was fitted with a reflux condenser connected at the top to a trap in a methylene chloride-dry ice bath. Isobutylene was introduced at the rate of 6.5 ml/min. The reaction was allowed to continue for 12 hours

*Mallinckrodt/Nuclear, St. Louis, Missouri.

undisturbed. A slightly yellowish, oily product resulted which completely solidified upon seeding with a single crystal of BHT. A very small sample was removed for analysis (See below.). The crude, crystalline BHT-$^{14}C_6$ was dissolved in 10 ml of anhydrous ether and transferred to a 50 ml erlenmeyer flask. The ether was then removed with dry nitrogen as before.

Purification of the Crude BHT-$^{14}C_6$.--The crude, labelled BHT was dissolved in 10 ml of ethyl alcohol (99.6%) and heated on a steam bath to promote dissolution. When the solution began to reflux distilled water was added dropwise until the solution just became cloudy. The flask was immediately placed in an ice bath, seeded and then placed in the freezing compartment of a refrigerator (temperature -15° C) for 3 hours for crystallization. This procedure is very critical as the impurities apparently have similar structures and readily co-precipitate. The crystals were filtered off while cold through Sargent No. 500 filter paper using a "glass button" filter funnel[8], and the filtrate removed. The crystals were washed several times with 5 ml portions of distilled water and then air dried. There was obtained 1.81 g (88.0%) of white, crystalline product. The filtrate was heated on a steam bath until the solution just became cloudy. Ethyl alcohol (99.6%) was added dropwise until the solution cleared. The flask was then immersed in an ice bath, seeded and placed in the refrigerator for 3 hours as before. After filtering and air drying there was obtained from the filtrate 0.08 g (3.9%) of pure, white crystals of labelled BHT. The combined yields totaled 1.89 g (91.9%).

Thin Layer Chromatographic and Autoradiographic Analysis.--Thin layer plates were prepared by coating 20 x 20 cm glass plates with a 200 μ layer of Silica Gel G a fluorescent indicator* in the form of a slurry. The preparation of 5

*Adsorbasil-P with 10% Binder----Applied Scientific Laboratories, State College, Pennsylvania.

plates required a slurry of 25 g of absorbent and 32.3 ml of distilled water. The chromatographic plates were allowed to air dry and then heated to 100° C for 1 hour prior to use.

Two thin layer plates so prepared were each spotted with 1 µl portion of a 200 µg/µl concentration of unlabelled BHT in ethanol (99.6%). The two plates were then each spotted (two spots) with a 1 µl portion of a 200 µg/µl concentration of unpurified BHT-$^{14}C_6$. The two plates were each further spotted (two spots) with a 1 µl portion of a 200 µg/µl concentration of purified BHT-$^{14}C_6$. One plate was developed in benzene and the other plate in a 20:1 toluene-acetone solution. In both systems the unlabelled BHT, the unpurified BHT-$^{14}C_6$ and purified BHT-$^{14}C_6$ spots compare to each other in position and size when observed after development in iodine vapors. The unpurified BHT-$^{14}C_6$ showed a slight evidence of contamination.

A portion (1 µl) of purified BHT-$^{14}C_6$ was diluted with ethanol (99.6%) 100 times. A 1 µl portion of the diluted solution was spotted on each of two plates previously spotted thin layer plates for the purpose of showing the intensity of a 1% impurity on an autoradiogram. Autoradiograms were made using No Screen Kodak Medical X-Ray Film. The thin layer chromatogram of the labelled product on both plates were sprayed with Neatan New*, allowed to dry and then placed in a 10 x 12 inch cassette. After the proper exposure time (4 days) the film was developed with Kodak chemicals in accordance to the manufacturers instruction. The autoradiogram revealed impurities in the unpurified BHT-$^{14}C_6$; however, no spots were noted in the purified product. The radio purity of the purified BHT-$^{14}C_6$ was found to be greater than 99.0%.

The specific activity was determined using internal liquid scintillation counting. The specific activity was found to be 0.35 µCi/mg.

*Neatan New----E. M. Reagents Division, Brinkman Instrument, Inc., Westbury, New York.

ACKNOWLEDGMENT

This investigation was supported by PHS Training Grant No. 5-T01-EC00064 from the Bureau of Radiological Health.

REFERENCES

1 - Ershoff, B. H. and Steers, C. W. - Proc. Soc. Exp. Biol. Med. 104 : 274 (1960).

2 - Landomery, L. G., Ryan, A. J. and Wright, S. E. - J. Pharm. Pharmacol. 19 : 383 (1967).

3 - Paltin, E. and Vitca, V. - Rev. Chim. 16 : 546 (1965).

4 - Mai, K. L. - Belg. Patent No. 629,537 (October 21, 1963); through Chem. Abstr. 60 : 10,601 (1964).

5 - Venaka, K., Hamada, T., Tamura, T. and Tanimoto, K. - Japan. Patent No. 6,902,974 (February 7, 1967); through Chem. Abstr. 70 : 77,571 (1970).

6 - Isagulyants, V. I., Favorskaya, N. A. and Tishkova, V. N. - Zhur Priklad. Khim. 34 : 693 (1961); through Chem. Abstr. 55 : 17,558 (1961).

7 - Kalav, C. - Brit. Patent No. 1,191,769 (May 13, 1970).

8 - Gattermann, L. and Wieland, H. - " Laboratory Methods of Organic Chemistry ", MacMillan and Co., Limited, London, England, 1943, p. 11, Fig. 8.