

Universal Method for Obtaining of any Polyhalogenated Dioxins labeled by Tritium

Irina V. Romannikova, Yuriy Ya. Kit, Vladimir V. Litvak* and Vladimir A. Richter

Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, Pr. Lavrent'eva, 8, Novosibirsk, 630090, Russia
*CoLTD "Synthesis", Pr. Lavrent'eva, 8, Novosibirsk, 630090, Russia

Introduction

Radioactivity labeled dioxins are often used in different dioxins studies - migration, bioconcentration, transformation, degradation, toxicology, etc. (1, 2). Only two radioactive isotopes (^3H and ^{14}C) is adaptable to labeling of all dioxin's congeners. Using of ^3H is favoured because it have greater theoretical specific activity in comparison with ^{14}C . But only several tritium labeled chlorinated dioxins are commercially available.

Development of universal method for preparation of any dioxins with different halogen substitutes in any locations of dioxin's molecule was the main objective of this investigation.

Material and Methods

Chemicals. All reagents and solvents were of analytical grade.

Polyhalogenated dioxins were synthesized by heating of dipotassium salt of catechol with various polyhalobenzenes in dimethyl sulfoxide (3). Products were characterized by using data of HPLC, IR-, NMR- and UV-spectroscopy, temperature of melting, element analyses.

Tritium labeling procedure. The tritium labeling procedure was performed by using of thermally activating tritium method and a device for tritium labeling (4). Solution of sample for labeling in toluene was spread on the surface of reaction vessel. The tritium labeling was confirm under cooling of the reaction vessel by liquid nitrogen. The system was previously evacuated than filled with gaseous tritium to the operating pressure by the heating of titanium tritide. Tritium was activated on a red-hot tungsten spiral (T 1500 - 2000 °K) during 15 s. The process was repeated five times. The labeling substance was extracted from surface of the reaction vessel with 1 ml of toluene three times. The extracts was combined and evaporated. The pellet of the labeling substance was dissolved in 1 ml of dioxane-water mixture (25:75 v/v) and used for HPLC-purification.

Purification of tritium labeled dioxins. Purification of labeled products was performed with using of high performance liquid chromatography (HPLC) (5). The HPLC system consisted of two Waters Model 590 pumps, Waters Lambda-max Model 481 detector, Waters Automated Gradient Controller and BBC Goerz Metrawatt SE-120 recorder. Samples were

injected manually using a Rheodyne 7125 with 1ml sample loop. The HPLC apparatus was connected to Vydac Protein & Peptide C18 column (4.6 mm x 25 cm). All chromatographic procedures were carried out at room temperature (about 25°C). A mixture of water:dioxane (25:75 v/v) as solution A and methanol:water:dioxane (20:5:75) as solution B were used in all chromatographic procedures as mobile phase. Dissolved in 1 ml of solution A samples were injected into the column. Gradient elution was used: 10 min. - 100% of solution A; 60 min. - from 100% of solution A to 100% of solution B. Flow rate was 0.5 ml/min. UV detection was carried out at characteristic wave length for analyzing compounds. The interesting chromatography picks were collected and used for further analysis.

Analysis of tritium labeled dioxins. Radiochemical purity of labeled dioxins was determined by analytical HPLC and thin-layer chromatography (TLC).

Analytical HPLC system "Millichrom" (Nauchpribor, Russia) with dual (UV and Radiation) detection of elute was used for examination of radiochemical purity and for UV-spectrometry of tritium labeled dioxins.

Kieselgel 60 (Merck, Germany) TLC plates and benzene:acetone (20:5) as mobile phase were used for TLC. The visualization of product spot was conducted under UV-irradiation and autoradiography.

Results and Discussion

The suggesting method of obtaining tritium labeled dioxins is based on the substitutions of ^1H -atoms on ^3H in the dioxin molecule. High performance of this substitution is provided by thermal activating of tritium. Interaction of activated tritium atoms with dioxin molecules take place in the thin layer of substance cooled by liquid nitrogen. Different modifications of this method are well-known and were used for obtaining of wide class of organic compounds (6). It is known that labeling procedure is not "mild" and some substances are strongly decomposed in process of labeling and many radioactive impurities are accumulated in reaction mixture. The decomposition of compounds is a result of many secondary chemical reactions of activated tritium with these compounds. For polyhalogenated dioxins we found following main secondary reaction that decrease yield of target product and lead to accumulation undesirable radioactive impurities: hydrogenation of double carbon bonds; substitution of halogen atoms by tritium and destruction of dioxin molecules. We found that all decomposition processes intensify with increasing time of action by activated tritium and quantity of labeling cycles. To produce the tritium polyCl- and polyBr dioxins with specific activity 20 - 50 Ci/mmol optimal conditions was five labeling cycles at 15 s each. Labeling of dioxins with F-substitutes under these reaction conditions lead to considerable decreasing of yield. In cases of labeling 2,3,7,8-tetrafluorodioxin we observed a total decomposition of it in these conditions. Therefore we used more mild conditions for obtaining of tritium dioxins with fluoro-substitutes. Usually specific activity of fluorodioxins does not exceed 1 - 5 Ci/mmol.

As already noted many radioactive impurities were accumulated in reaction mixture under labeling process. These impurities prevented using of tritium dioxins in analytical purposes. Therefore elaboration of methods for purification of dioxins after the labeling was a special task of our investigation. Earlier we determined that reverse phase high performance liquid chromatography (RP HPLC) was a best method for purification of any organic compounds labeling by tritium in our conditions. RP HPLC of dioxins is usually based on using of

acetonitrile-water and acetonitrile-alcohol mixtures (7-9). But solubility of dioxins in this systems is very small and not exceed some mkg/ml as a rule. In our case it was necessary to increase solubility of dioxins to level of some hundreds mkg/ml for its purification after the tritium labeling. Dioxane is known to be a fine nonpolar solvent for many dioxins. We attempted to use dioxane-water and dioxane-methanol solutions as mobile phases for reverse phase HPLC and found that this systems was a suitable for purification of tritium labeled dioxins in preparative quantities (5). All obtaining tritium labeled dioxins after one stage purification in this system were homogeneous according to the data of analytical HPLC and TLC.

The identity of obtained tritium labeled dioxins with initial compounds was determined by coincidence of three parameters: retention time in HPLC; mobility in TLC and UV-spectrum.

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