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Synthesis of C-13-labeled atrazine

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Atrazine is a long-lasting herbicide that has been shown to affect hormone levels in amphibians. Using the C-13 labeled atrazine to detect its residue is effective and essential. This study presents three steps for the synthesis of [$^{13}C_3$]atrazine, which starts from [^{13}C]urea, and results in the incorporation of C-13 atoms at the 1, 3 and 5 positions of the *S*-triazine ring of atrazine. The method prepares the product in an overall yield of 57.6% and chemical purity of 98.6%, for use as an internal standard.

Keywords: isotopically labeled synthesis; C-13; cyanuric acid; cyanuric chlorine; atrazine synthesis; internal standards

Introduction

Atrazine is a widely used herbicide for control of broadleaf weeds in sugarcane and maize fields. For about 40 years, atrazine was considered safe because it has not caused any fatal disease based on human health issues. However, the long-lasting residues of atrazine can be detected in surface water, including drinking water. In 2001, Tyrone B. Hayes and his colleague found that atrazine can affect the hormone level and promotes the conversion of testosterone to estrogen, which may be a reason for the decline of the global amphibian population.[1] There are also some other effects on human central nervous system, immune system, and cardiovascular function reported in Environmental Protection Agency (EPA) documents.[2,3] So, to find an effective way to detect the trace residue of the atrazine in surface water became an important issue in atrazine toxicology. In 1993, D.A. Cassada and R.F. Spalding's paper, solid-phase extraction coupled with isotope dilution gas chromatography-mass spectrometry has been reported sensitive and reliable for the determination of atrazine, in which [$^{13}C_3$]atrazine has been used as the internal standard.[4] The labeled atrazine not only can be used for residue analysis but also can be used to measure the trace atrazine residue that exists in human body and to detect the urinary metabolites of the atrazine.[5]

Although labeled atrazine has been frequently used, there is no paper reporting the method to synthesize the labeled atrazine yet. In this paper, we developed a synthesis of [$^{13}C_3$] atrazine starting from [^{13}C]urea, in high yield and purity.

Experimental

Materials and instruments

[^{13}C]Urea (99 atom% ^{13}C) was bought from Sigma-Aldrich. All other purchased starting reagents were of analytical grade and used without further purification. Infrared spectra were recorded in KBr (also provided by Thermo-Fisher Scientific) disks on a Thermo Nicolet 6700 FT-IR spectrophotometer; the ^{1}H-NMR (300 MHz) spectra were recorded on a Bruker DPX 300 MHz NMR spectrometer (Bruker Corporation, USA) and chemical shifts values are given in parts per million relative to TMS in DMSO-d6; HRMS was performed on a Bruker Apex IV FTMS (Bruker Corporation, USA).

Synthesis

*Synthesis of [$^{13}C_3$]cyanuric acid **2***

A total of 12 g sulfolane (0.100 mol) was mixed with 4 g cyclohexanol (0.040 mol) in a 50-mL three-neck flask, equipped with stirrer and reflux condenser, was heated in an oil bath. The labeled 4 g urea (0.067 mol) was added into the flask when the temperature of the mixture reached 60 °C. The gas collection plant (vacuum pump) was installed to the end of the reflux condenser so as to form a reduced pressure environment and also to absorb the ammonia generated by the reaction. The mixture was then heated to 200 °C, and this temperature was kept for about 3 h, until the system did not produce ammonia any more. The product was filtered after the system was cooled to about 50 °C. Crude product was washed by ethyl alcohol, and then the resulting product was heated in vacuum drier under 60 °C for 2 h to give 2.5 g (0.019 mol) light yellow powder. The yield of this reaction was about 87.0%.

*Synthesis of [$^{13}C_3$]cyanuric chloride **3***

A total of 0.90 g [$^{13}C_3$]cyanuric acid (0.007 mol) in a three-neck-flask, equipped with the reflux condenser and a drying tube in order to preclude atmospheric moisture, 4.37 g phosphorus pentachloride (0.021 mol), and 3.22 g phosphorus oxychloride (0.021 mol) was added, heated to 115 °C, and kept for 24 h until release of HCl ceased. The residue was distilled at 120 °C to remove the phosphorus oxychloride. The crude product was dissolved in the chloroform, washing down from the inside of the condenser, and the evaporated solution reduced pressure giving about 0.91 g white solid (0.005 mol). The yield in this step was 71.4%.

*Synthesis of [$^{13}C_3$]atrazine **5***

A total of 4.07 g [$^{13}C_3$]cyanuric chloride(0.020 mol) was placed in a three-neck-flask with 40 mL chlorobenzene and cooled below 0 °C, and 1.32 g (0.020 mol) of isopropylamine was added. The temperature was

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controlled under 10 °C and stirred for 2 h, when TLC presented a clear and centralized spot, and 10% sodium hydroxide solution was added to neutralize the acid. About 1–2 h later, 0.99 g (0.020 mol) ethylamine was added, and the temperature maintained at 15–20 °C. After 1 h, 0.88 g (0.020 mol) NaOH was added to neutralize the acid, and the reaction was stirred for 2 h. The layers were separated and crude product was obtained from evaporating the chlorobenzene and triturating the residue with petroleum ether. The product was a white powder obtained in 95.4% yield (4.11 g, 0.019 mol). FT-IR (KBr) cm^{-1}: 3260, 3117, 2974, 1620, 1551, 1439, 1403, 1384, 1346, 1261, 805. ^{1}H-NMR (300 M, CDCl3) δ: 1.19–1.38 (m, 9H), 3.41–3.50 (m, 2H), 4.13–4.20 (m, 1H), 5.20–5.29 (m, 1H), 5.73–6.01 (m, 1H). HRMS (ESI, *m/z*): calcd mass for $^{12}C_5^{13}C_3H_{14}ClN_5$, $[M + H]^+$: 219.10919; found: 219.11069. The ^{13}C abundance of the product was 99.05%.

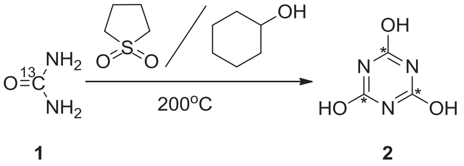
Results and discussion

Many papers and patents have reported synthesis of cyanuric acid by the pyrolysis of urea in solvents solvent. An appropriate solvent for this reaction must fit the following principles: the solvent has a boiling point within the pyrolysis temperature range; it can dissolve the urea but do not or slightly the cyanuric acid; it can be well recovered and recycled.[6] We finally choose the sulfolane–cyclohexanol (Scheme 1) mixture as the solvent because it could provide an appropriate reaction temperature and the final product's purity was higher than the normal industrial method.[7] It has been reported that the presence of ammonia produced by urea decomposition would cause a higher content of aminated by-product.[8] Reduced pressure was reported favorable for getting satisfying yield of the product.[9] So, a vacuum pump was equipped at the end as a gas collecting plant and to form a reduced pressure reaction environment.

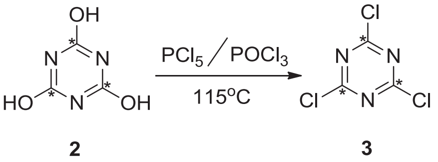
Cyanuric chloride is a common reagent frequently used in syntheses of the cyanuric acid.[10] However, the reverse reaction has seldom been reported. B.P. Bandgar and S.S. Sawant describe the conversion of the cyanuric acid, recovered from another reaction, to cyanuric chloride by treatment of a mixture of the acid and *N*,*N*-diethylaniline with $POCl_3$.[11] In our method, this step of reaction could be finished in one reactor, so it could reduce the wastage of material and crude product during the procedure. PCl_5-$POCl_3$ mixture has been reported as a well chlorine provider for many compounds whose structures were very similar with cyanuric acid.[12–14] In this study, the mixture was proved facilitating the cyanuric acid converting to cyanuric chlorine as well and helped furnishing the product in yield of 71.4% (Scheme 2).

Benita Barton described a method to synthesis the atrazine from cyanuric chloride as shown in Scheme 3 and also investigated the reaction calorimetry so as to find an optimal addition sequences and rates.[15] It seems that this step from cyanuric chloride to atrazine is quite straightforward, but labeled cyanuric chloride is expensive and difficult to obtain. On the contrary, labeled urea is a common material that is often employed to label compounds and is much cheaper. In this paper, we describe a simple method to label the atrazine from [^{13}C]urea.

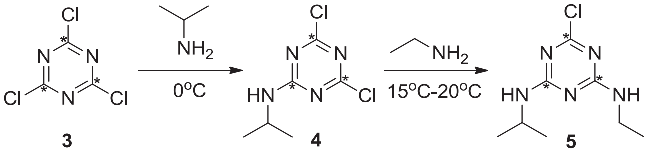
In summary, our work has developed a simple way to label the widely used herbicide atrazine with overall yield of 57.6% and chemical purity of 98.58%. The product is qualified to the internal standard and could be used in detecting the ground and underground water.



Scheme 1. Synthesis of [$^{13}C_3$]cyanuric acid.



Scheme 2. Synthesis of [$^{13}C_3$]cyanuric chloride.



Scheme 3. Synthesis of [$^{13}C_3$]atrazine.

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Conflict of Interest

The authors did not report any conflict of interest.

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