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ORIGINAL ARTICLE

Preparation of 3-bromo-L-tyrosine and 3,5-dibromo-L-tyrosine

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Abstract L-Tyrosine is converted to 3-bromo-L-tyrosine in good yield by reaction with 1.2 equiv. of DMSO in HBr/AcOH, while reaction with 2.2 equiv. of DMSO under comparable conditions results in formation of 3,5-dibromo-L-tyrosine in good yield. This is the simplest, safest and most efficient method for the preparation of gram quantities of either 3-bromo-L-tyrosine or 3,5-dibromo-L-tyrosine.

Keywords Bromination · Bromotyrosine · Dibromotyrosine · DMSO/HBr · Tyrosine

Abbreviations
DMSO Dimethyl sulfoxide
AcOH Acetic acid

Introduction

3-Bromo-L-tyrosine and 3,5-dibromo-L-tyrosine are common metabolites of L-tyrosine in marine organisms, particularly in sponges, and they serve as the basic structural element for an important class of marine bromotyrosine alkaloids, with potent antimicrobial, antitumor and antimalarial activities (Yin et al. 2011; Xu et al. 2011; Kon et al. 2010; Mukai et al. 2009). Examples of 3,5-dibromotyrosine-derived alkaloids include pseudoceramine A (**1**) (Yin et al. 2011), psammaplysin H (**2**) (Xu et al. 2011), and ceratinadin A (**3**) (Kon et al. 2010) which are shown in Scheme 1. Thus, 3-bromo-L-tyrosine and 3,5-dibromo-L-tyrosine are useful intermediates in the synthesis of some of these alkaloids. Furthermore, 3-bromo-L-tyrosine and 3,5-dibromo-L-tyrosine are produced by the action of myeloperoxidase in humans, and serve as markers in urine for immune stimulation and oxidative stress (Wu et al. 1999; Senthilmohan and Kettle 2006). However, there are relatively few synthesis of 3-bromo-L-tyrosine reported in the literature, and none of them are particularly convenient and efficient for multigram-scale preparation. In conjunction with our mechanistic studies of tyrosine phenol-lyase (Phillips et al. 2006), we required samples of pure 3-bromo-L-tyrosine. Furthermore, we were interested whether 3-bromo-L-tyrosine and 3,5-dibromo-L-tyrosine produced in vivo by oxidative stress (Wu et al. 1999; Senthilmohan and Kettle 2006) may be substrates or inhibitors of human DOPA decarboxylase.

Methods

3-Bromo-L-tyrosine (4)

L-Tyrosine (5.0 g, 27.6 mmol) was suspended in 50 mL AcOH, and 12 mL 48 % HBr (108 mmol) was added. Then, 2.4 mL DMSO (33.1 mmol) was added, and the colorless suspension immediately turned yellow–orange. The suspension was warmed to 65 °C for 2 h, giving a clear light yellow solution. It was then removed from the

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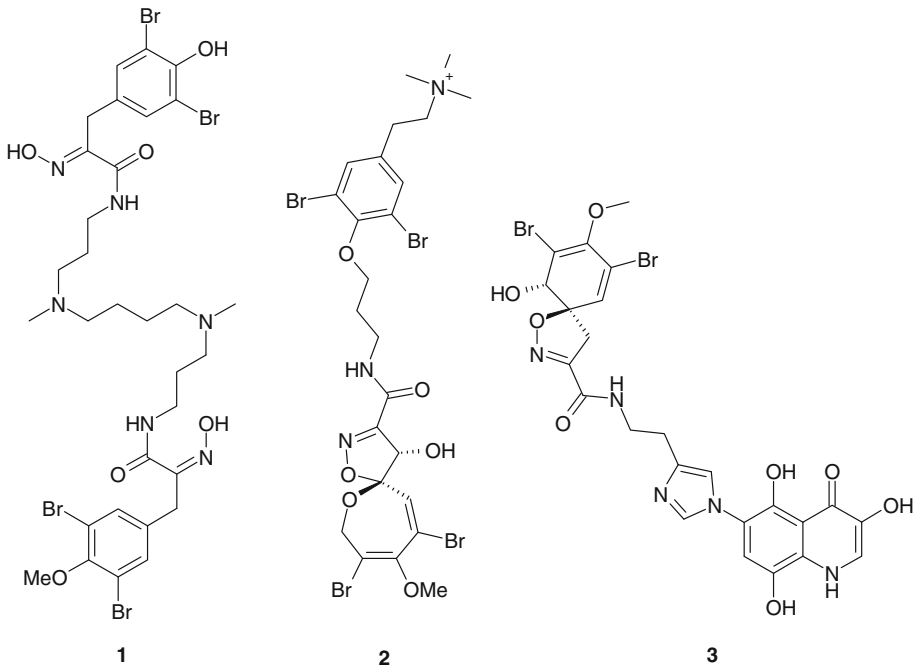
Scheme 1 Structures of some bromotyrosine alkaloids



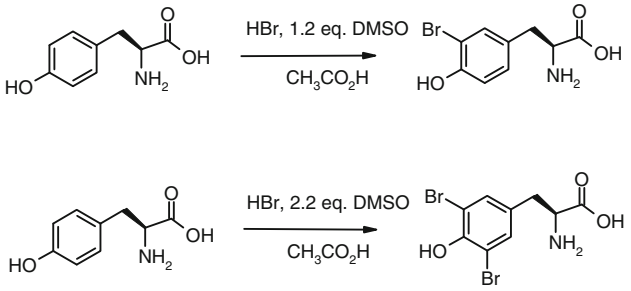
heat and left stirring at room temperature overnight. The clear yellow solution was then evaporated in vacuo to give a white solid, which was dissolved in 100 mL hot water, the pH was adjusted to 6 with solid $NaHCO_3$, and it was left at 4 °C for crystallization to complete. The crystalline suspension was filtered, the white rosettes washed with a little cold water, and left to dry. The filtrate was concentrated to 50 mL, and after standing, filtered to collect the crystals, which were dried and combined with the previous crop, giving a total of 4.68 g (65.2 %), m p. 232–234° C (lit., 232–234 °C, Yokoyama et al. 2006). 1H-NMR: (D_2O) δ 7.27 (1H, d, $J = 2.0$ Hz), 6.99 (1H, dd, $J = 2.0$, 8.4 Hz), 6.82 (1H, d, $J = 8.3$ Hz), 3.76 (1H, dd, $J = 4.8$, 8.0 Hz), 3.01 (dd, $J = 5.6$, 14.8 Hz), 2.88 (1H, dd, $J = 7.6$, 14.8 Hz). $[\alpha]_D^{23} = -2.4^\circ$ ($C = 5.0$, 1.0 M HCl). Surprisingly, we could find no published value of the specific rotation of 3-Br-L-Tyr. MS(ESI): 260, 262 (M + 1).

3,5-Dibromo-L-tyrosine (5)

L-Tyrosine (5.0 g, 27.6 mmol) was suspended in 50 mL AcOH, and 12 mL 48 % HBr (108 mmol) was added. Then, 4.4 mL DMSO (60.7 mmol) was added, and the colorless suspension immediately turned yellow-orange. The suspension was warmed to 65 °C for 2 h, giving a clear light yellow solution. It was then removed from the heat and left at room temperature overnight. The clear yellow solution was then evaporated in vacuo to give a light yellow solid, which was dissolved in 200 mL of hot water, the pH adjusted to 5 with solid $NaHCO_3$, and it was left at ambient temperature overnight for crystallization to complete. The suspension was filtered, the sparkling white needles washed with a little cold water, and left to dry, giving 4.95 g (52.9 %), m p. 230–234 °C (lit., 221–224 °C, Yokoyama et al. 2006). 1H-NMR (D_2O, DCl) δ 7.18 (s, 2H), 4.05 (t, 6.7 Hz, 1H), 2.98 (dd, 5.8, 14.9 Hz, 1H), 2.86 (dd, 7.6, 14.8 Hz). $[\alpha]_D^{23} = +2.6^\circ$ ($C = 5.0$, 1.0 M HCl) (lit, +1.3°, Ding et al. 2004). MS(ESI): 338, 339.8, 341.8 (M + 1).

Results and discussion

The first reported synthesis of 3-bromotyrosine was of the racemate, which was prepared from 3-bromoanisaldehyde using the classic hydantoin condensation method (Johnson and Bengis 1912). The direct bromination of L-tyrosine is attractive, but one of the problems encountered in bromination of L-tyrosine, as is common with any phenol, with Br_2 to give 3-bromo-L-tyrosine (**4**) is concomitant and uncontrolled production of 3,5-dibromo-L-tyrosine (**5**). Indeed, reaction of L-tyrosine with 2 equiv. of Br_2 in glacial acetic acid provides **5** in excellent yield (Ding et al. 2004). In 1975, monobromination of DL-tyrosine with a mixture of BrO_3^- and Br^- in aqueous HCl was reported by McCord et al. (1975). More recently, treatment of L-tyrosine with BrO_3^- in 0.08 N HCl was shown to produce a mixture of **4** and dityrosine (Tilley et al. 2004). However, the purification required HPLC to separate **4** and dityrosine, and the yield was only 7.55 mg of **4** from 10 mg L-tyrosine. An alternative bromination procedure of L-tyrosine uses sodium bromoisocyanurate in 60 % aqueous H_2SO_4, but

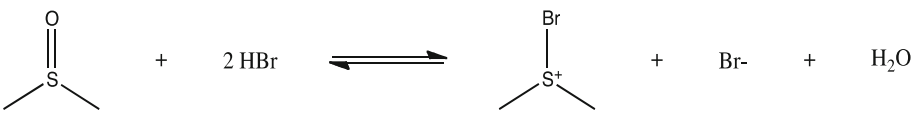


Scheme 2 Bromination of L-tyrosine with HBr/DMSO

the yield of **4** was only 29 % after chromatography (Yokoyama et al. 2006). The best procedure published to date for selective monobromination of L-tyrosine uses a relatively dilute solution (9 g L-tyrosine/2.15 L water) to reduce the likelihood of dibromination, reacting with a mixture of $KBrO_3$ and KBr in 0.5 M HCl (Sano et al. 1986), and resulting in 88 % yield. However, product isolation with this method requires inconvenient evaporation of a large volume of water, followed by chromatography. A recent paper described a procedure for selective monobromination of *N*-acetyltyrosine with *N*-bromosuccinimide in acetonitrile in the presence of *p*-toluenesulfonic acid (Bovonsombat et al. 2008). However, these authors did not report hydrolysis of the resultant *N*-acetyl-3-bromo-L-tyrosine to give the free amino acid. None of these published procedures lend themselves readily to the convenient production of multigram-scale quantities of **4**. Hence, we decided to apply the mild and selective HBr/DMSO brominating system (Majetich et al. 1997) to the problem of **4** and **5** synthesis.

To our delight, we found that L-tyrosine can indeed be selectively monobrominated with aqueous HBr in glacial acetic acid using 1.2 equiv. of DMSO, as shown in Scheme 2. We made some modifications of the published conditions in order to optimize yields. Under the standard conditions in the paper (Majetich et al. 1997) (large excess of DMSO, room temperature) the reaction gave mixtures of unreacted tyrosine, **4**, and **5**. Limiting the amount of DMSO to a slight stoichiometric excess and raising the temperature at the beginning of the reaction resulted in nearly complete conversion of tyrosine to the desired mono or dibromo product. This reaction is not performed at high dilution (5 g L-tyrosine in 50 mL AcOH), so it is unnecessary to evaporate large volumes of solvent in the workup. Furthermore, no chromatography is necessary to isolate the product, and the isolated yield of **4** after crystallization of the crude product from water is about 65 %. Small amounts of contaminating **5** in the product are easily removed by recrystallization at pH 6, taking advantage of the lower p*I* for the dibrominated product. On the other hand, reaction with 2.2 equiv. of DMSO under identical conditions provides a good isolated yield (53 %) of **5** (Scheme 2), which can be easily separated from any residual contaminating **4** by recrystallization from water at pH 5. The excess of HBr presumably acts to protect the α-amino group from oxidation by formation of the salt. The future extension of this approach to tyrosine derivatives such as L-Dopa or L-tyrosinol may be possible, since the amount of oxidant can be easily controlled by the limited addition of DMSO, and catechol is cleanly brominated in glacial acetic acid with Br_2 to give 4,5-dibromocatechol (Kohn 1951).

The mechanism of bromination under these conditions has been proposed to involve the formation of bromodimethylsulfonium bromide by the reaction of DMSO with 2 mol of HBr (Eq. 1) (Majetich et al. 1997). The resulting bromodimethylsulfonium cation serves as the selective source of Br^+ for the aromatic bromination, thus allowing for the preparation of **4** in good yield. Furthermore, this reaction uses economical reagents, and avoids the health and environmental hazards associated with the use of Br_2 for the preparation of **5**. The extension of this procedure to preparation of chloro and iodotyrosines can be envisioned. However, Majetich et al. (1997) found chlorination with HCl/DMSO to be much less effective, and iodination with HI/DMSO fails completely. This is likely due to different redox properties of the halogens. In the case of chlorine, the equilibrium lies toward the side of HCl, while for iodine, the iodosulfonium iodide undergoes competing decomposition to elemental iodine. Only for bromine is the bromosulfonium reagent relatively stable.



(1)

In conclusion, the bromination of L-tyrosine by HBr/DMSO in glacial acetic acid provides a convenient and practical method for the synthesis of either 3-bromo-L-tyrosine (**4**) or 3,5-dibromo-L-tyrosine (**5**), by simply changing the L-tyrosine/DMSO molar ratio. The product is isolated simply by crystallization of the crude product from water, without any need for time-consuming and inefficient chromatography. This process is

thus easily performed to obtain multigram quantities of either **4** or **5**.

Conflict of interest The authors declare that they have no conflict of interest.

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