Synthesis, characterization and bioactivity evaluation of diallyl disulfide

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Abstract: Diallyl disulfide was synthesized by phase transfer catalyst (PTC) during microwave irradiation. The effects of different factors, such as the power of microwave irradiation, the time of microwave irradiation, PTC reagents amount and the mole ratio of reactants, on the yield of product were investigated. The structure of diallyl disulfide was characterized by infrared spectra, mass spectra and ¹H nuclear magnetic resonance. The bioactivity of diallyl disulfide was evaluated by cell viability assay on HepG2 hepatoma cells. The results show that the optimal reaction conditions are as follows: tetrabutylammonium bromide (TBAB) selected as a PTC, the mass ratio of TBAB to sodium disulfide of 0.021: 1, the power of irradiation of 195 W, the reaction time of 12 min, and the mole ratio of sodium disulfide to allyl chloride of 0.65: 1. The yield of diallyl disulfide is 82.2%. The synthetical diallyl disulfide appears to be cytotoxic to HepG2 hepatoma cells in a dose-dependent manner.

Key words: diallyl disulfide; microwave irradiation; phase transfer catalyst; bioactivity

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1 INTRODUCTION

Garlic, one of the most frequently used spices, has been known for its diverse biological activities, such as antithrombosis, antiatherosclerosis, antimutagenesis, and antibacterial. It is recently known that diallyl disulfide, a major organosulfur compound in garlic inhibits the growth of human tumor cells from colon, lung, skin, and breast origins. Smell et al. reported that iodine and allyl mercaptan could form diallyl disulfide. Moreover, Challenger et al. reported that allyl hyposulphite and oxydol could form diallyl disulfide too. There are many disadvantages, such as low yield, long reaction time and bad odor of some materials in these ways.

Phase transfer catalysis (PTC) technology has recently been widely applied to synthesize specialty chemicals from organic reactions. The primary advantage of this technique is to elevate the reaction rate and increase the selectivity under mild conditions. Microwave irradiation technique has received increasing interest in organic synthesis because of its rapid reaction rates, higher yields and cleaner reaction conditions. In this study, diallyl disulfide was synthesized by phase transfer catalyst during microwave irradiation. Its inhibitory effects on human hepatoma cells (HepG2) were evaluated as well.

2 EXPERIMENTAL

2.1 Reagents and instruments

All reagents were obtained from commercial sources and used without purification. Infrared (IR) spectra were recorded on an AVATAR 360 FTIR spectrometer. ¹H nuclear magnetic resonance (¹H-NMR) spectra were measured on INOVA 400 (Varian) NMR spectrometer. Mass spectra (MS) were obtained by a GC/MS QP-2010 spectrometer (EI, 70 eV). The microwave oven used was a commercial household microwave oven (WP650, 650W). Analysis of diallyl disulfide was carried out on a GC/MS QP-2010 spectrometer. The capillary column used was OV-1 with 30 m in length and 0.25 mm in diameter. Conditions were as follows: column temperature began at 60 °C, then increased to 220 °C; Helium was used as carrier gas at a linear flow of 1 mL/min.

2.2 Preparation of sodium disulfide solution

Sodium disulfide solution was prepared by sodium sulfide and sulfur as follows: 31.2 g (0.13 mol) sodium sulfide, 4.16 g (0.13 mol) sulfur and 100 mL distilled water were placed in a flask.
The flask was then placed into the cavity of a microwave oven and irradiated at 30% power (195 W) for 15 min. The resulting solution was cooled to room temperature by cold water. The color of solution was brownish red.

2.3 Synthesis of diallyl disulfide

The 0.3 g tetrabutylammonium bromide (TBA-B) selected as a PTC was added to the sodium disulfide solution, while 16.3 mL (0.2 mol) allyl chloride added dropwise in 30 min. After addition of allyl chloride, the flask was placed into the cavity of microwave reactor again and irradiated at 30% power (195 W) for 12 min. After being cooled to room temperature, the resulting mixture was extracted with 200 mL ether. The combined organic phase was dried over anhydrous Na2SO4. The organic phase was performed on rotary evaporator under aspirator pressure to eliminate ether. Then the residue was evaporated in low vacuum. The fraction at 39–43 °C and 266.7 Pa was gathered. 12 g light yellow oil obtained was diallyl disulfide with the yield of 82.2%. The functional groups were identified by IR: 3 081 cm⁻¹ (νC=H), 985 cm⁻¹ (νC=H), 3 009 cm⁻¹ (νCH2), 2 978 cm⁻¹ (νCH2), 720 cm⁻¹ (νCH2), 1 634 cm⁻¹ (νC=C), 1 422 cm⁻¹ (νC=S); MS(70 eV) m/z (%): 146 (M⁺, 6), 113 (M⁺-SH, 11), 105 (M⁺-C₂H₅, 9), 85 (M⁺-C₃H₇S, 8), 41 (100); H-NMR (CDCl₃, 400 MHz): 3.341 (d, J = 7.6 Hz, 4 H), 5.139-5.220 (m, 4 H), 5.848 (m, 2 H); Calculated values of elementary analysis for C₆H₁₀S₂: C, 49.27; H, 6.89%; Measured values: C, 48.76; H, 6.92%.

2.4 Cell viability assay

To assess the cytocotoxic effects of diallyl disulfide in HepG2 hepatoma cells, we used a tetrazolium salt, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphe nyltetrazolium bromide (MTT) reduction assay. HepG2, a human hepatoma cell line, was cultured in Dulbecco’s modified eagle medium (DMEM) with 10% heat-inactivated fetal bovine serum (FBS), benzylpenicillin (100 kU/L) and streptomycin (100 mg/mL) at 37°C in an incubator containing humid air with 5% CO₂. HepG2 cells were plated in 24-well plates and grown to 80% confluence. After being treated with 0, 10, 20, 50 and 100 μmol/L diallyl disulfide for 24 h, cell viability was determined, and the results were indicated with the suppression ratio of cell growth.

3 RESULTS AND DISCUSSION

3.1 Choice of phase transfer catalyst

Diallyl disulfide was synthesized by Na₂S + 9H₂O, S and allyl chloride (Fig. 1). Because of the presence of aqueous phase including sodium disulfide and organic phase including allyl chloride, the substitution of Cl⁻ by S⁻ was very difficult. The presence of phase transfer catalyst (PTC) can solve this problem. Table 1 shows the effects of the presence of different PTC on the yield of diallyl disulfide. It can be seen that the activity of tetrabutylammonium bromide (TBA-B) is the best, the activities of cetyltrimethylammonium bromide (CTMAB) and polyethylene glycol (PEG) are not good. According to the principle of phase transfer catalysis, the reactive rate is correlated with the number of cation of PTC entering the organic phase from the aqueous phase. The number of cation of PTC can be expressed by the extraction constant. Comparing the extraction constant of aqueous phase with that of organic phase, it can be found that the logarithm value of extraction constant increases with the increase of one carbon atom in various substitution group of quaternary ammonia salt. Comparing CTMAB (19 C) with TBA-B (16 C), CTMAB is better than TBA-B. But the ammonium cation with symmetric, long, linear carbon group is more effective than that only with one long carbon chain. Bigger basis groups around the quaternary heteroatom with positive charge can strengthen this effect. So the catalysis of TBA-B is much better than that of CTMAB on the whole.

\[
\text{Na₂S} + 9\text{H₂O} \xrightarrow{\text{Microwave}} \text{Na₂S} + 9\text{H₂O}
\]

\[
\text{Na₂S} + 2\text{Cl}^- \xrightarrow{\text{PTC-Microwave}} \text{S} \quad \xrightarrow{+ 2\text{NaCl}}
\]

Fig. 1 Schematic diagram of synthesis for diallyl disulfide

Comparing PEG with TBA-B, PEG is de-banced, which is ascribed to its favorable water-solubility which induces mass loss of PEG. On the contrary the low water-solubility of TBA-B can reduce its mass loss. Consequently TBA-B can improve the yield of diallyl disulfide and it is chosen as a catalyst.

| Table 1 Effect of phase transfer catalyst on yield of diallyl disulfide |
|---------------------|----------------------|----------------------|---------------------|---------------------|
| Catalyst            | CTMAB    | PEG 8000     | PEG 1450    | TBA-B       |
| Yield (%)           | 27.3     | 31.2          | 37.5        | 73.0        |

3.2 Effects of microwave parameters

Fig. 2 shows the effect of the mole ratio of sodium disulfide to allyl chloride on the yield of diallyl disulfide. It can be seen that the yield of diallyl disulfide increases with the increase of the mole ratio of sodium disulfide to allyl chloride before the
mole ratio is 0.65: 1. When the mole ratio is over 0.65: 1, the yield decreases on the contrary. It is attributed to the increase of secondary reactions produced in the process of synthesis of Na₂S₂ and the serious carbonization of diallyl disulfide.

**Fig. 2** Effect of mole ratio of sodium disulfide to allyl chloride on yield of diallyl disulfide

Fig. 3 shows the effect of time of microwave irradiation on the yield of diallyl disulfide. When the reaction time is too short, the reaction is incomplete. But when the reaction time is too long, the yield would decrease. The higher yield is obtained while the reaction time is 12 min.

**Fig. 3** Effect of time of microwave irradiation on yield of diallyl disulfide

Fig. 4 shows the effect of the mass ratio of TBAB to sodium disulfide on the yield of diallyl disulfide. It can be seen that the yield increases before the mass ratio is 0.021: 1. When the mass ratio is over 0.021: 1, the yield decreases owing to the carbonization of diallyl disulfide caused by high temperature which is caused by the accelerating of reactive speed.

**Fig. 4** Effect of mass ratio of TBAB to sodium disulfide on yield of diallyl disulfide

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**Table 2** Effect of power of microwave irradiation on yield of diallyl disulfide

| 3.3 1H-NMR spectra of diallyl disulfide |
Elemental analysis and 1H-NMR spectra clearly establish structure of diallyl disulfide. In the 1H-NMR spectra (Fig. 5) the multiplet at chemical shift 5.848 represents -CH= protons of the allyl group. The second multiplet centered at 5.139-5.220 shows -CH₂ proton signals. Protons of the allylic carbon give a doublet at 3.341.

**Fig. 5** 1H-NMR spectra of diallyl disulfide

3.4 Gas chromatogram

It could be known that the purity of synthetic diallyl disulfide was more than 98% by comparing gas chromatogram (GC) of diallyl disulfide synthesized during microwave irradiation with that of diallyl disulfide sold in the market (Fluka Co. 80%
purity) (Fig. 6). This indicates that there is little secondary reaction in the process of synthesis of diallyl disulfide by phase transfer catalyst during microwave irradiation. Therefore purer diallyl disulfide can be obtained through synthesis.

3.5 Cell viability assay

To test the effect of diallyl disulfide on the cell viability of HepG2 hepatoma cells, the MTT reduction assay was used. As shown in Fig. 7, exposure of HepG2 hepatoma cells to diallyl disulfide at concentrations of 20, 50, 100 μmol/L for 24 h decreases cell viability by 12.17%, 18.53% and 27.11%, respectively, compared to the control (0 μmol/L). This result suggests that synthetical diallyl disulfide appears to be cytotoxic to HepG2 hepatoma cells in a dose-dependent manner.

4 CONCLUSIONS

1) Diallyl disulfide is synthesized efficaciously by phase transfer catalyst during microwave irradiation. The yield of diallyl disulfide is 82.2%. The optimal reaction conditions are as follows: tetrabur-

REFERENCES


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