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Original Article

Laboratory-Scale Synthesis of Theophylline and Caffeine at BehanSar Pharmaceutical Factory

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Abstract
An important issue in manufacturing theophylline and caffeine for industrial-scale production is identifying the most efficient method. This process involves achieving high efficiency, minimizing costs, utilizing affordable raw materials, and ensuring compatibility with existing equipment. This study presents a simple and cost-effective method for synthesizing theophylline and caffeine. To achieve this goal, the study focused on optimizing the laboratory-scale synthesis of these compounds straightforwardly and cost-effectively. Theophylline and caffeine were synthesized through a multi-step process using dimethylurea and cyanoacetic acid as starting materials. The synthesis was successful, and the yield of these two substances was relatively acceptable. In addition, the structures of the prepared theophylline and caffeine were confirmed through *Fourier transform infrared*, hydrogen-1 *nuclear magnetic resonance*, and mass spectrometry. The physical and spectrometric data for these two substances were entirely consistent with the information found in the reference books. This research represents the initial phase in synthesizing these compounds on a semi-industrial scale. However, further investigation is necessary in this regard.
Keywords: Caffeine, Theophylline, Laboratory-scale synthesis

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Introduction

Theophylline was first extracted from tea leaves and chemically identified by the German biologist Albrecht Kossel around 1888 (1).

Theophylline, a member of the methylxanthine group, is a bronchodilator referred to as 1,3-dimethylxanthine. It works by relaxing the muscles in the chest and lungs, making breathing easier. Additionally, it decreases the sensitivity of the bronchi to allergens and other triggers that can lead to bronchospasm (2). This drug is also used to treat shortness of breath and symptoms of asthma, bronchitis, emphysema, chronic obstructive pulmonary disease, and other respiratory problems (3). Theophylline is also a phosphodiesterase inhibitor. It competitively inhibits phosphodiesterase types III and IV, the enzymes responsible for the breakdown of cyclic adenosine monophosphate (cAMP) in smooth muscle cells, ultimately increasing intracellular cAMP (4). By inhibiting the breakdown of cAMP, theophylline allows for the accumulation of the second important messenger within the cells. Elevated levels of intracellular cAMP trigger a series of downstream signaling events that ultimately lead to the relaxation of smooth muscle (5). In terms of chemical structure and pharmacology, it is similar to theobromine and caffeine (6).

Caffeine is a naturally occurring compound found in several plants, such as coffee, cocoa, and tea. As a stimulant, it can interfere with sleep patterns (7). Caffeine is an alkaloid from the methylxanthines family, and its properties are similar to those of theophylline and theobromine (8). Increasing the body's metabolism, stimulating the central nervous system, and enhancing alertness, concentration, memory, and environmental awareness are among the most important effects of caffeine (9). In addition, caffeine is available in tablet form, which has the same properties and effects as natural caffeine. It can be prescribed topically, orally, inhaled, or injected (10). Caffeine alone is also used by injection in the prevention and treatment of pulmonary dysplasia in premature babies (11). The strong stimulating effect of caffeine on the respiratory system makes this

compound a valuable antidote for respiratory depression caused by excessive use of drugs such as morphine and barbiturates (12).

While caffeine can be extracted from various plant sources, theophylline is typically present in much smaller quantities (13-15). Given that caffeine can be synthesized from theophylline, developing an efficient synthesis method for theophylline followed by its conversion to caffeine holds significant value. Consequently, over the past few decades, numerous studies have been conducted to synthesize caffeine and theophylline in laboratory settings (16, 17).

Various methods have been documented for the synthesis of theophylline. For instance, You et al successfully synthesized theophylline through the reaction of 5,6-diamino-1 and 3-dimethylpyrimidine with formic acid under reflux conditions (16). Similarly, Balssa and Bonnaire achieved the synthesis using triethylformate in a reflux setup with the same starting materials (18). In addition, several reports have provided details regarding the methylation of theophylline as a pathway to synthesize caffeine. Zajac et al reported the successful synthesis of caffeine by reacting methyl iodide with theophylline in the presence of sodium hydride by utilizing dimethyl sulfoxide as the solvent (17). Meanwhile, Manthorpe and Lockley conducted this methylation reaction using potassium carbonate in methanol at room temperature (19).

Here, we would like to present our work on optimizing a laboratory-scale synthesis method for theophylline and caffeine.

Experimental Phase

General Information

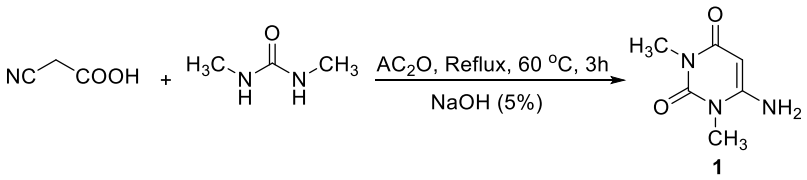
All reagents and solvents were purchased from the Aldrich-Merck Company and utilized without any further purification. Thin-layer chromatography was employed to monitor the progress and completion of the reaction. Aluminum plates coated with silica gel F_{254} (Merck) served as the stationary phase. A 50:50 mixture of methanol and n-hexane was used as the mobile phase. It should be noted that all reported melting points are uncorrected and given in degrees Celsius. The melting points were measured using a digital apparatus from Thermo Scientific, employing capillary tubes for the process. *Fourier transform infrared* (FT-IR) spectra were recorded on a Bruker Alpha instrument with KBr pellets. Hydrogen-1 *nuclear magnetic resonance* (^{1}H-NMR) spectra were recorded on a BRUKER AVANCE 301 MHz instrument by utilizing DMSO-d_6 as a solvent. Chemical shift values are expressed on the δ scale. In reports concerning 1H NMR spectra, several symbols were used, including s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were acquired using an Agilent Technology 5975 instrument. The reported yields refer to isolated products.

Synthesis of 6-Amino-1,3-Dimethylpyrimidine-2,4-Dione (1)

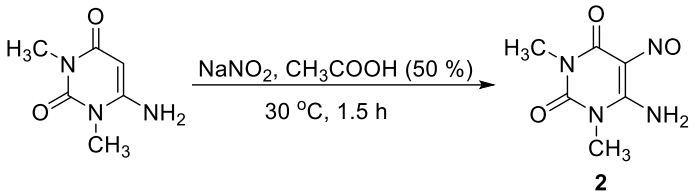
Cyanoacetic acid (100 mmol; 8.5 g) and dimethylurea (100 mmol; 8.8 g) were refluxed in 12.5 mL of the acetic anhydride solvent at 60 ˚C for 3 hours. Then, a 5% cold sodium hydroxide solution (50 mL) was added drop by drop. Finally, the resulting 6-amino-1,3-dimethylpyrimidine-2,4-dione was washed with deionized water and recrystallized using methanol (Scheme 1) (20).

Synthesis of 6-Amino-1,3-Dimethyl-5-Nitroso-Pyrimidine-2,4(1H,3H)-Dione (6-Amino-1,3-Dimethyl-5-Nitrosouracil) (2)

To this end, 6-amino-1,3-dimethylpyrimidine-2,4-dione (31.5 mmol; 4.88 g) was dissolved in 250 mL of water. The temperature was slightly raised to accelerate the dissolution, but the reaction was conducted at room temperature. Then, sodium nitrite (113 mmol; 7.8 g) was slowly added over 15 minutes. Next, the mixture was acidified by adding glacial acetic acid (6.8 g) drop by drop. The mixture was stirred at room temperature for 1.5 hours and then cooled. The resulting pink precipitate was 6-amino-1,3-dimethyl-5-nitroso-pyrimidine-2,4(1H,3H)-dione, which was filtered and washed with deionized water (Scheme 2) (21).



Scheme 1. Synthesis of the 6-Amino-1,3-Dimethylpyrimidine-2,4-Dione.
Yield 77%. M.p. 290-293˚C. IR(KBr)/cm^{-1} 3329 and 3397,1656. ^{1}H-NMR (301 MHz, DMSO-d_6); δ(ppm) 3.09 (s, 3H), 3.26 (s, 3H), 4.7 (s, 1H), 6.8 (s, 2H); MS (EI), m/z: 156 (M+1)



Scheme 2. Synthesis of the 6-Amino-1,3-Dimethyl-5-Nitroso-Uracil.
Yield 75%. M.p. 240-241˚C. IR(KBr)/cm^{-1} 3548, 1663. ^{1}H-NMR (301 MHz, DMSO-d_6); δ(ppm) 3.26 (s, 3H), 3.37 (s, 3H), 9.09 (s, 1H); 12.97 (s, 1H), MS (EI), m/z: 185 (M+1)

Synthesis of 5,6-Diamino-1,3-Dimethylpyrimidine-2,4(1H,3H)-Dione (5,6-Diamino-1,3-Dimethyl Uracil) (3)

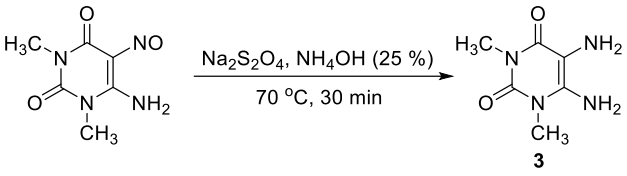
For this purpose, 6-amino-1,3-dimethyl-5-nitrosouracil (54.13 mmol; 9.95 g) was dissolved in 125 mL of 25% ammonia (NH_4OH), and the temperature was then raised to 70˚C. The reaction was performed under a fume hood. Sodium dithionite ($Na_2S_2O_4$, 162.4 mmol; 28.2 g) was slowly added over 30 minutes. The reaction mixture was then allowed to evaporate at 70˚C. The color of the reaction changed from dark green to light green. Subsequently, it was placed under a vacuum desiccator to form crystals of the product (5,6-diamino-1,3-dimethyl uracil). Considering that the product was light-sensitive, the foil was wrapped around all containers. For most crystals, the reaction mixture was placed in the refrigerator. After several hours, the resulting crystals were filtered and kept away from light and under vacuum conditions to dry (Scheme 3) (21).

Synthesis of 1,3-Dimethyl-3,7-Dihydro-1H-Purine-2,6-Dione (Theophylline) (4)

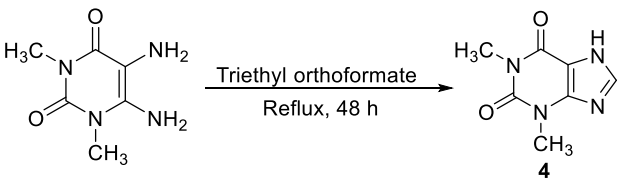
To this end, 5,6-diamino-1,3-dimethyl uracil)16 mmol; 2.9 g) and triethyl orthoformate (102 mmol; 15.16 g) were refluxed in an oil bath for 48 hours. After this time, a precipitate was obtained. The precipitate was filtered and washed with ether. After washing, its color turned green. Due to its low solubility in water, the precipitate was dissolved in boiling water, and activated charcoal was added. Following filtering, the solution became completely colorless and was placed in the refrigerator for crystallization (Scheme 4) (22).

Synthesis of 1,3,7-Trimethyl-3,7-Dihydro-1H-Purine-2,6-Dione (Caffeine) (5)

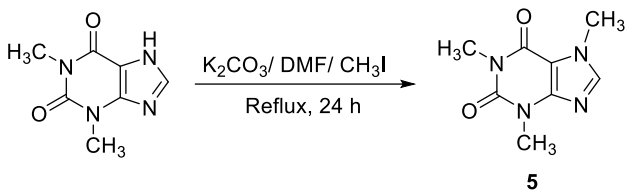
Theophylline (50 mmol; 9 g), methyl iodide (75 mmol; 10.64 g), and K_2CO_3 (75 mmol, 10.36 g) were dissolved in 250 mL of dimethylformamide and stirred at room temperature for 24 hours. The solvent was evaporated, and the product was crystallized in acetone. A milky-colored caffeine was obtained, which was dried in an oven at 105˚C (Scheme 5) (23).



Scheme 3. Synthesis of the 5,6-Diamino-1,3-Dimethyl Uracil.
Yield 79.5%. M.p. 293-295˚C. IR(KBr)/cm^{-1} 3521, 3362, 1605. ^{1}H-NMR (301 MHz, DMSO-d_6); δ(ppm) 3.15 (s, 3H), 3.3 (s, 3H), 3.61 (s, 2H), 6.2 (s, 2H). MS (EI), m/z: 171 (M+1)



Scheme 4. Synthesis of Theophylline.
Yield 52%. M.p. 273-275˚C. IR(KBr)/cm^{-1} 3121, 1717, 1667. ^{1}H-NMR (301 MHz, DMSO-d6); δ(ppm) 3.25 (s, 3H), 3.45 (s, 3H), 8.03 (s, 1H, CH), 13.57 (s, 1H, NH). ^{13}C NMR (76 MHz, DMSO-d6) δ 154.92, 151.69, 148.35, 141.00, 106.89, 30.21, 28.21. MS (EI), m/z: 181 (M+1)



Scheme 5. Synthesis of Caffeine.
Yield 34%. M.p. 234-236˚C. IR(KBr)/cm^{-1} 1700, 1658. ^{1}H-NMR (301 MHz, DMSO-d6); δ(ppm) 3.18 (s, 3H), 3.37 (s, 3H), 3.85 (s, 3H), 7.97 (s, 1H, CH). ^{13}C NMR (76 MHz, DMSO-d6) δ 154.88, 151.40, 148.46, 143.18, 106.93, 33.53, 29.76, 27.87. MS (EI), m/z: 195 (M+1)

Results and Discussion

The structures of all synthesized compounds were confirmed using suitable instrumental techniques. ^{1}H-NMR, mass spectrometry, and FT-IR spectra of the compounds based on the presence of amine, carbonyl, and methyl functional groups indicated that the compounds were satisfactorily synthesized.

Characterization of 6-Amino-1,3-Dimethylpyrimidine-2,4-Dione

In the IR spectrum of 6-amino-1,3-dimethylpyrimidine-2,4-dione, the stretching vibrations of the C=O bond appeared in the region of 1656 cm^{-1}. Two sharp peaks appearing in the regions of 3397 cm^{-1} and 3329 cm^{-1} were related to the stretching frequency of the NH_2 group (Figure S1) (17, 22).

In the ^{1}H-NMR spectrum of this compound, the protons of the CH_3 attached to nitrogen atoms appeared at δ(ppm) 3.09 (s, 3H) and 3.26 (s, 3H), while peaks at 4.7 (s, 1H) and 6.8 (s, 2H) belonged to the one aromatic ring proton and two NH_2 protons, respectively (Figure S2) (18, 22). Moreover, according to mass spectrometry, the value of M+1 of this compound was equal to 156.05, confirming the structure of this compound (Figure S3).

Characterization of 6-Amino-1,3-Dimethyl-5-Nitroso-Uracil

In the IR spectrum of 6-amino-1,3-dimethyl-5-nitroso-uracil, the stretching vibrations of the C=O bond appeared in the region of 1663 cm^{-1}. The stretching vibrations of the N=O bonds appeared in the region of 1517 cm^{-1}. A sharp peak appearing in the region of 3548 cm^{-1} was related to the stretching frequency of the NH_2 group (Figure S4) (17).

In the ^{1}H-NMR spectrum of this compound, the protons of the CH_3 groups attached to nitrogen atoms appeared at δ(ppm) 3.26 (s, 3H) and 3.37 (s, 3H), while two peaks at 9.09 (s, 2H) and 12.96 (s, 1H) belonged to the NH_2 protons. The observed separation of the NH_2 peaks was likely the result of hydrogen bonding between the nitroso group and one of the hydrogen atoms of NH_2 (Figure S5) (17).

The value of M+1 of 6-amino-1,3-dimethyl-5-nitroso-uracil was equal to 185.12, demonstrating the structure of this compound (Figure S6).

Characterization of 5,6-Diamino-1,3-Dimethyl Uracil

In the IR spectrum of 5,6-diamino-1,3-dimethyl uracil, the stretching vibrations of the C=O bond appeared in the region of 1635 cm^{-1}. Additionally, two sharp peaks appearing in the regions of 3362 cm^{-1} and 3521 cm^{-1} were related to the stretching frequency of the NH_2 group (Figure S7) (17, 19).

In the ^{1}H-NMR spectrum of this compound, the protons of the CH_3 groups attached to nitrogen atoms appeared at δ(ppm) 3.15 (s, 3H) and 3.3 (s, 3H), while the chemical shifts of 6.17 and 3.61 (s, 2H) were related to NH_2 protons (Figure S8) (17, 19). Based on mass spectrometry, the value of M+1 of 5,6-diamino-1,3-dimethyl uracil was equal to 171.11 and confirmed the structure of this compound (Figure S9).

Characterization of Theophylline

In the IR spectrum of theophylline, the stretching vibrations of the two C=O bonds appeared in the regions of 1717 cm^{-1} and 1667 cm^{-1}. Further, a sharp peak appearing in the region of 3121 cm^{-1} was related to the stretching frequency of the NH_2 group (Figure S10) (17,20,23).

In the ^{1}H-NMR spectrum of theophylline, the protons of the CH_3 groups attached to nitrogen atoms appeared at δ(ppm) 3.25 (s, 3H) and 3.45 (s, 3H). The peaks that appeared at 8.4 (s, 1H) and 13.57 (s, 1H) belonged to the C-H bond of the imidazole ring and the NH proton, respectively (17,20). Based on the results of mass spectrometry, the value of M+1 of theophylline was equal to 181.09, approving the structure of this compound.

Characterization of Caffeine

The stretching vibrations of the two C=O bonds appeared in the regions of 1700 cm^{-1} and 1658 cm^{-1} in the IR spectrum of caffeine (17,21,24).

In the ^{1}H-NMR spectrum of caffeine, the protons of the CH_3 attached to the nitrogen atoms appeared at δ(ppm) 3.18 (s, 3H), 3.37 (s, 3H), and 3.85 (s, 3H), while the peak at 7.97 (s, 1H) was related to the C-H bond of the imidazole ring (17,21). Moreover, according to mass spectrometry, the value of M+1 of caffeine was equal to 195.08 and confirmed the structure of this compound (Figure S11).

Conclusion

In this study, a simple and cost-effective approach was introduced for the laboratory-scale synthesis of theophylline and caffeine. The synthesis involved a multi-step process using dimethylurea and cyanoacetic acid as starting materials, resulting in acceptable yields of the target compounds. The structures of the prepared theophylline and caffeine were confirmed through spectroscopic techniques, and the data matched reference information. While this research represented an initial phase in the synthesis of these compounds on a semi-industrial scale, further investigation is necessary to optimize the process for potential industrial-scale production.

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Authors' Contribution

Zeynab Hashemi synthesized all compounds. Zahra Najafi, Seyed Omid Hashemi, Jamil Eiri were the scientific advisor for this research. Gholamabbas Chehardoli designed, analyzed, and edited the manuscript.

Competing Interests

The authors declare that they have no competing interests.

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Supplementary Files

Supplementary file contains Figures S1-S17.

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