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THE NOVEL METHOD TO SYNTHESIS OF CANTHARIDIN INTERMEDIATE

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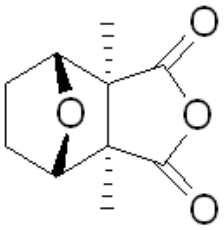
Sulfur-containing dehydrocantharidin(SD) was yielded (76% to 96%) by Diels-Alder reaction in an ionic-liquid system under ordinary pressure and temperature. We explored the influences of different ionic-liquid types, reaction temperatures, and reaction times in this reaction. We found that the optimal reaction temperature was about 35°C, the reaction time was 20 h, and the most suitable ionic liquid was 1-butyl-3-methylimidazolium tetrafluoroborate. Furthermore, in the recycling process of ionic liquid, we found that CH_3CN was the most suitable extraction solvent. We explored four steps in the synthetic route to SD and achieved a good yield of 38% in total. We envisage that this process could be further developed at an industrial scale for the synthesis of Cantharidin and is destined to be an environmentally friendly way to solve the lack of cantharis as a natural resource.



INTRODUCTION

Cantharidin, which can be extracted from cantharis[1], is an antineoplastic drug that can have an effect on liver cancer, cervical cancer, skin cancer, bone marrow cancer, and leukemia.[2-5] Cantharidin causes less damage to normal cells in cancer patients and elevates their white cell, without suppressing the immune systems.[6,7] Cantharidin has been identified as one of the research hot spots for antitumor drugs. Because of the lack of natural resources for cantharis, the Cantharidin tablets or other pharmaceutical preparations cannot be realized at an industrial scale.

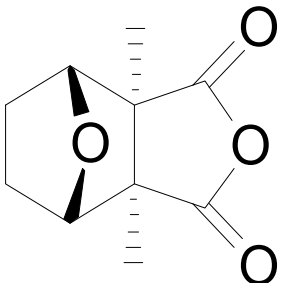


Fig. 1 – The structure of Cantharidin.

The three-dimensional structure of Cantharidin was given by Gadamer in 1914 (Fig. 1).[8] Following this work, its national synthesis was committed to the pincers element of chemical synthesis, and some chemists achieved its total synthesis. In 1951, Stork synthesized Cantharidin for the first time using 11 steps.[9] In 1953, Schenk improved the synthetic route, reducing it to six steps.[10] In 1976, Dauben Synt-hesized it and used just two key steps.[11] Cantharidin cannot be obtained in high total yield after a long synthesis route, however, and some intermediates were difficult to synthesize under ordinary conditions. Although Dauben synthesized Sulfur-containing dehydrocanthari- din(SD) by Diels-Alder (D-A) reaction under 15000 atmospheric pressure, it is difficult to achieve this in industrial production.

The D-A reaction represents an important reaction in the synthesis of Cantharidin. Over the past decade, it has been a focus of research to ease the harsh conditions in the D-A reaction to prepare Cantharidin and to improve the yield of Cantharidin. In 1990, Grieco synthesized SD by

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D-A reaction in lithium perchlorate ($LiClO_4$), improving the target yield,[12] but this D-A reaction requires strict water-free conditions. In 1999, Earle used ionic liquids in common D-A reactions.[13] In 2003, Idiko found that zinc or aluminum salts could be used to change the ratio of endo/exo in a D-A reaction in ionic-liquid systems.[14]

In this paper, we show the newly touching to the SD synthesized by the D-A reaction in an ionic-liquid system under ordinary conditions (Fig. 2). In this process, SD can be obtained at a high yield from the D-A reaction under ordinary pressure and normal temperature. At the same time, the ionic liquids can be recycled to reduce costs in an environmentally friendly manner. High yield and good exo/endo selectivity, high ionic-liquid recycling efficiency, and mild reaction conditions are some of the advantages of this green process to synthesize SD. These advantages also offer good opportunities for the industrial production of Cantharidin.

EXPERIMENTAL

Reagents and Chemicals Instrumentation

Furan was dried using magnesium sulfate. Nuclear magnetic resonance (NMR) data were recorded on an Agilent Technologies 600 MHz DD2 (Santa Clara, CA). Electrospray ionization–mass spectrometry (ESI-MS) data were recorded on a Waters Acquity® SQD (Milford, MA).

2,5-dihydrothiophene-3,4-dicarboxylic acid anhydride followed the route in refs.[11,15,16] The final and key step to prepare **SD** followed refs.[13,14]

Synthesis of Methyl Thioglycolate (Ⅰ)

A mixture of methanol(0.4mol) and mercapto acetic acid (1 mol) was refluxed while stirred and concentrated H_2SO_4 (0.8 g) was added to the solution. Then the mixture was refluxed for 4h. After, the mixture was extracted three times with Cyclohexane (80 mL) and toluene (40 mL), then the extractive solution was dried using $CaCl_2$, the solvent was evaporated in a vacuum to yield the colorless liquid (34g, 94%).

Synthesis of α，β-dicarbomethoxymethyl ethyl sulfide (Ⅱ)

I (0.1 mol) and pyridine (0.2 mL) was cooled in an ice bath while stirred 30min. Additionally, methyl acrylate (1.1 mol) was added to the solution and it was maintained at room temperature, after 5h the mixture solution was washed with water and dried using $NaSO_4$. The dried oil was distilled under vacuum to give a viscous oil of **II**, 17g.Yield: 88.8%; b.p: 168~170°C under 6KPa; ^{1}H NMR ($CDCl_3$) δ : 2.61 (t, 2H, J=6 Hz), 2.86 (t, 2H, J=9 Hz), 3.21 (s, 2H), 3.65 (s, 3H), 3.69 (s, 3H); ^{13}C NMR ($CDCl_3$) δ : 27.51, 33.41, 34.03 , 51.78, 52.38, 170.66, 172.00 ppm; ESI-MS: m/z 215.03 ($M+Na^+$, 100%).

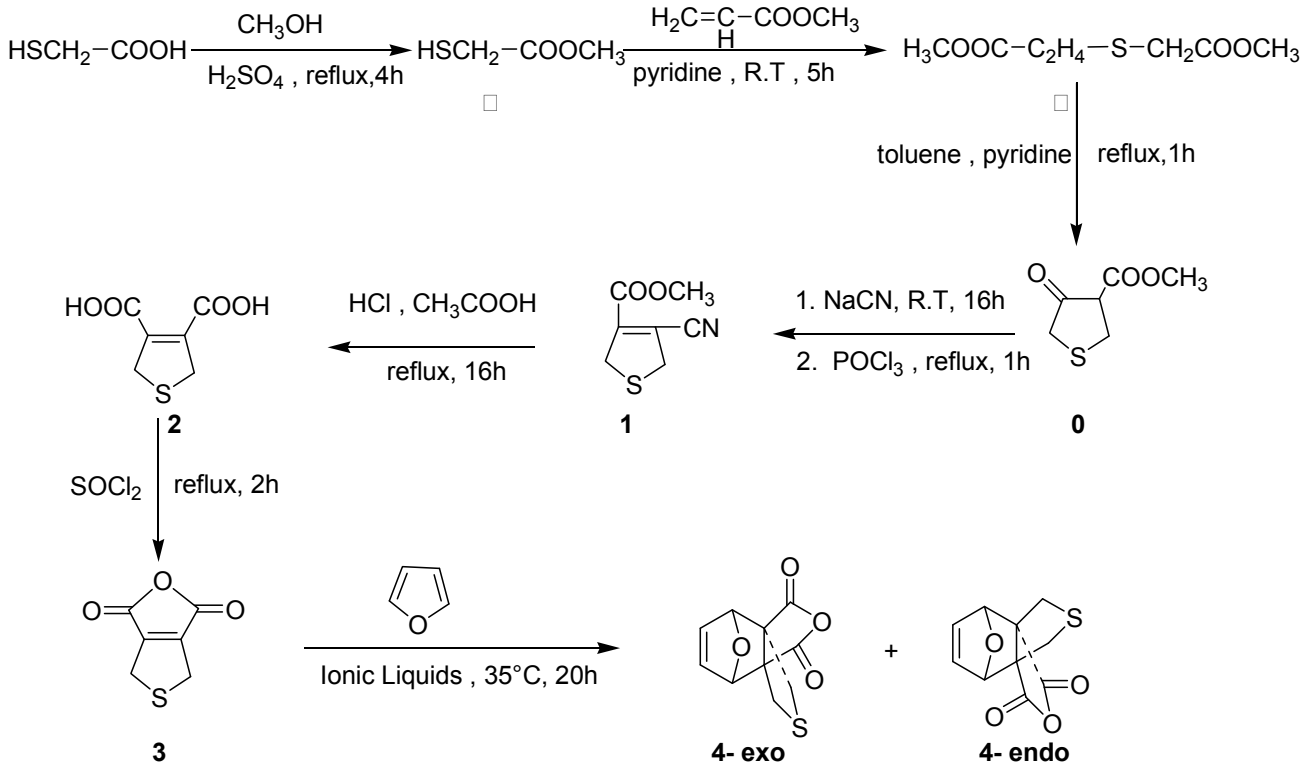

Fig. 2 – The synthetic route to SD under ambient pressure.

Synthesis of 3-keto-4-carbomethoxy-2,5-tetrahydrothiophene (0)

Pyridine (0.2 mol) and toluene (500 mL) was stirred under 80°C for 10 min, then (II) (0.2 mol) was added to the solution and it was maintained at the temperature 80°C for 1h. In addition of (II) (0.2 mol) was added to the mixture in 20 min and the solution was refluxed for 1h. When cooled down to the room temperature, the samples adjusted to pH 7 with 0.5M hydrochloric acid in the ice-sodium chloride cold bath, and the toluene layer were separated. Residual solution was extracted based on ether(3*50mL),and combined with toluene layer. The mixture was evaporated to dryness in a vacuum, recrystallization from petroleum ether-anhydrous ethanol gave 38.4g of yellowish-white crystals (compound **0**),Yield:80%; mp : 36~38 ºC; ^{1}H NMR($CDCl_3$) δ:3.75 (t, 2H, J=3 Hz), 3.78 (s, 3H), 3.81 (t, 2H, J=3 H z), 10.93 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 31.40, 38.03, 51.63, 99.21, 169.55, 172.43 ppm; ESI-MS: m/z183.01 ($M+Na^+$, 100%).

Synthesis of 3-Cyano-4-carbomethoxy-2,5-dihydrothiophene (1)

$NaHSO_3$ (113 g) and 600 mL purified water were added to an NaCN aqueous solution (176 g, 23%), and the solution was cooled in an ice bath while stirred. Additionally, 80 g 3-keto-4-carbomethoxy-2,5-tetra-hydrothiophene was added to 25 mL of methanol and the solution was maintained at room temperature. After 16 h, the mixture was extracted three times with 200 mL CH_2Cl_2 and the solvent was evaporated in a vacuum to yield the yellow crude cyanohydrin (90 g, 96%).

Without further purification, 123 g phosphoryl chloride ($POCl_3$) was added to 40 g of 3-hydroxyl-4-carbometh-oxy-2,5- tetrahydrothiophene, 50 mL benzene, and 70 g pyridine and the solution was cooled in an ice bath while stirred. The temperature was maintained between 50°C and 60°C, and 2 h later, the mixture was evaporated to dryness in a vacuum. Recrystallization from hep-tane-methylene chloride gave 28.8 g (80%) of white crystals (compound **1**). ^{1}H NMR ($CDCl_3$, TMS) δ: 3.86 (s, 3H), 4.04~4.0 (m, 2H), 4.08~4.10 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 38.3, 40.3, 52.9, 113.3, 120.5, 146.6, 161.4 ppm; ESI-MS: m/z 170.02 [$M+H^+$, 100%].

Synthesis of 2,5-dihydrothiophene- 3,4-dicarboxylic Acid (2)

A mixture of 36 g compound 1, 110 mL acetic acid, and 180 mL concentrated hydrochloric acid was refluxed for 16 h and then the mixture was evaporated to dryness in a vacuum. The residue was extracted with hot acetone, filtered, and evaporated to dryness to obtain 35.2 g (95%) of white crystals (compound **2**). ^{1}H NMR (DMSO) δ: 3.96 (s, 4H); ^{13}C NMR (DMSO) δ: 40.0, 138.5, 165.7ppm; ESI-MS: m/z 175.0 [$M+H^+$, 100%].

Synthesis of 2,5-dihydrothiophene-3,4-dicarboxylic Acid Anhydride (3)

A mixture of 16 g compound 2 and 64 mL $SOCl_2$ was refluxed for 2 h and the mixture was evaporated to dryness in a vacuum. Recrystallization first from the CH_2Cl_2 and then from the CH_3CN produced 6 g (33%) of white needle crystals (compound 3). ^{1}H NMR ($CDCl_3$, TMS) δ: 4.0 (s, 4H); ^{13}C NMR ($CDCl_3$) δ: 31.5, 153.9, 158.5; ESI-MS: m/z178.98 ($M+Na^+$, 100%).

Synthesis of Sulfur-containing dehydrocantharidin (4,SD)

A mixture of 0.5 g compound **3** and 0.26 mL 1-butyl-3-methylimidazolium tetrafluoro -borate was re-acted with 5 mL of freshly dried furan at 35°C. After 20 h, the mixture was extracted with CH_3CN (3*1 mL) to obtain 0.6 g of a white solid, and 1-butyl-3-methylimidazoliumtetrafluoroborate remained in the CH_3CN. We performed additional studies by changing the types of ionic liquids from 1-butyl-3-methylimid- azoliumtetrafluoroborate to other ionic liquids in this reaction. ^{1}H NMR ($CDCl_3$, TMS) δ: 3.1 (d, 2H), 3.5 (d, 2H), 5.0 (s, 2H), 6.7 (s, 2H); ESI-MS: m/z223.00 ($M-H^-$, 100%).

RESULTS AND DISCUSSION

Advantages and disadvantages of the two different methods to prepare 3-cyano-4-carbomethoxy-2,5-dihydrothiophene

For the synthesis of 3-cyano-4-car- bometh-oxy-2,5-dihydrothiophene (compound 1), the use of liquid hydrogen cyanide (HCN) for 15 h at a low temperature around 10~20°C was reported.[9,13] In the past, however, processing to remove excess HCN by bubbling nitrogen (N_2) proved to be diffi-cult, and the HCN safe limit point could not be reached even after several hours of N_2 bubbling. When the liquid HCN was replaced by sodium cyanide (NaCN), the reaction time could be short-ened to 10 h at room temperature. When NaCN is used, after the reaction is finished, the reaction mixture can be extracted with dichloromethane and then can be evaporated in a vacuum to obtain the crude cyanohydrins, which can be used directly for the preparation of compound 1. The excess NaCN can be treated with sodium thiosulfate, and it is easier to safely control its use in the lab.

Change benzene to dichloromethane to give 2,5-dithydrothioph-ene-3,4-dicarboxylic acid anhydride

In refs. 11 and 17, benzene was used to recrys-tallize 2,5-dithydrothiophene-3,4-dicarbo- xylic acid anhydride (compound 3). As is well known, benzene is toxic and not environmentally friendly. When dichloromethane is used to replaced benzene, similar results have been achieved for the product (compound 3). Thus, dichloromethane was deemed

to be a more suitable solvent to treat this crude reaction.

Factors influencing the synthesis of Cantharidin intermediates

The high-performance liquid chromatography (HPLC) data in Table 1 show the results of the D-A reaction by comparing several types of ionic liquids at different reaction temperatures and reaction times[18]. It is evident that this reaction depends on both the reaction conditions and the types of ionic liquids. For example, in entry A, the time to consume compound 3 was about 10 h at 45°C; when the temperature was decreased to 25°C, the time increased to 30 h. This means that higher temperatures contribute more quickly to reaction rates and shorten the reaction times. But the yield of compound 4 also was reduced (from 90% down to 80%), so the suitable reaction temperature and reaction time depend on the intent of the experiments.

Table 1 also shows that the D-A reaction proceeds to completion in ionic-liquids possessing of the imidazole ring at 35°C for 20 h. This is a better result than a pyrrolidine ring or pyridine ring containing ionic liquids, in which case the reaction cannot be completed even at 45°C for 20 h, to obtain Synthesis of Sulfur-containing dehydrocantharidin(SD). The factors that affect this maybe are the different ectric dipole moment which the order is imidazole ring (1.58) > pyrrolidine ring (0.29) > pyridine ring(-0.79)[19].These results confirm that the imidazole ring containing ionic liquids is the best choice for this D-A reaction.

As seen in Table 1, the length of the substituent chain on the imidazole ring can affect the D-A reaction. First, from butyl to hexyl, the longer the chain of the substituent in position 1 of the imidazole ring, the lower the yield of compound 4, and compound 3 cannot be fully consumed. This may explain why the yield of compound 4 in entry A is 86%, which is much higher than the yield in entry C, which is 57% under the same reaction conditions. Additionally, the yield of D is lower than the yield of F. As the chain length increases from hexyl to octyl, however, the yield also increases and approaches the yield of the butyl group, which is 88% for entry B. The reason maybe is the substituent effect.[20,21]

The type of salts in the ionic liquids also affected the D-A reaction. When entry D and entry C both have a hexyl-substituted imidazole ring, but entry C has borate salt and entry D has phosphorus acid salt, the difference in the conversion ratio is significant. In entry D, only 5% of compound 3 was not reacted, whereas 35% of compound 3 still was not reacted in entry C. Phosphoric acid salt ionic liquid contributes a better conversion ratio than borates, and this trend was supported by the comparison between entry F and entry A. By comparing entry A (borate salt) with entry E (sulfonyl imide salt), it is evident that the conversion ratios of compound 3 were almost the same. This mean that a variety of salts can affect the conversion ratio from compound 3 to compound 4 and demonstrates that phosphoric salt–type ionic liquid is better than types of borate salt and sulfonyl imide salt. The reason maybe is what the ability of absorb electrons were different[22].

The ionic liquid with a single substituent in pyridine ring (G) delivered the lowest yield. When the disubstituent pyridine ring was used, from H to K, the yields were 22% for I, 35% for J, and 51% for K. This means that means chlorine (Cl) salt is better than bromine (Br) salt and 3-methyl is better than 4-methyl. The yield of H was increased up to 62%, and the yield of L was 51% when disubstituent pyrrolidine ring was used.

Table 1

The influence of different ionic liquids on the synthesis of SDs

Entry	Ionic Liquid	Temp/°C	Time/h	Compound 3	Compound 4-exo
A	1-butyl-3-methylimidazolium tetrafluoroborate	25	30	0	90%
		35	20	0	86%
		45	10	0	80%
B	1-octyl-3-methylimidazolium tetrafluoroborate	35	20	0	88%
C	1-hexyl-3-methylimidazolium tetrafluoroborate	35	20	38%	57%
D	1-hexyl-3-methylimidazolium hexafluorophosphate	35	20	5%	93%
E	1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide	35	20	0	90%

Table 1 (continued)

F	1-butyl-3-methylimidazolium hexafluorophosphate	35	20	0	96%
G	1-butylpyridinium tetrafluoroborate	45	20	90%	9%
H	1-ethyl-3-methylpyridinium ethyl sulfate	45	20	37%	62%
I	1-butyl-4-methylpyridinium bromide	45	20	77%	22%
J	1-butyl-4-methylpyridinium chloride	45	20	52%	35%
K	1-butyl-3-methylpyridinium chloride	45	20	48%	51%
L	1-ethyl-1-methylpyrrolidinium bromide	45	20	48%	51%

Table 2

The HPLC analysis data of different solvent extraction for ionic-liquid recycling

solvent / Percentage	Et_2O*	EtOH**	$CHCl_3$	EtOAc	CH_3CN
Compound 4 in the residues	29%	24%	69%	49%	67%
Ionic liquid in the extraction	30%	65%	36%	11%	83%

* Three layers were obtained and most of compound 4 was in the middle layer.
**Compound 4 was decomposed in aqueous EtOH.

Recycle the ionic liquids

Ionic liquids are expensive and recycling these liquids is key to their use in scaled-up and commercial-scale preparations. Following are the experimental details of the ionic-liquid recycling. When 1-butyl-3-methylimidazolium tetrafluoroborate was used as the ionic liquid, the D-A reaction mixture was divided into five portions and extracted with ether, ethanol, chloroform, ethyl acetate, and acetonitrile. Table 2 shows the HPLC analysis data for each extraction and the residues.

As shown in Table 2, we used HPLC to analyze the ratio of compound 4 in the residues and the recycle ratio of the ionic liquid in the extractions, after we treated the mixture with one of the five organic solvents and separated the extractions from the residues. The ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate) remaining in the residue was the highest for the extraction with diethyl ether (Et_2O), lower for ethanol (EtOH), and the lowest for acetonitrile (CH_3CN), because the ionic liquid could be dissolved easily in EtOH and CH_3CN.[17,23] Moreover, the solubility of compound 4 in EtOH or CH_3CN was poor. After the mixture was extracted with CH_3CN or EtOH, most of compound 4 could be collected in the residues by filtering the extraction. After removing the organic solvents from the extraction, we recycled the ionic liquid. Additionally, compound 4 decomposes in aqueous EtOH, which further lowered the yield of compound 4 in the residue. Because compound 4 and the ionic liquid were well dissolved in ethyl acetate and chloroform, the isolation of the target compound 4 could not be achieved while recycling the ionic liquid. Therefore, we conclude that CH_3CN is the best organic solvent to recycle the ionic liquid without tampering with the separation of compound 4.

CONCLUSION

The SD was efficiently synthesized starting form methanol and mercapto acetic acid which were basic chemical raw materials. The structures of (I) ~ (II) and 0~4 were confirmed by ^1H NMR, ^{13}C NMR, HMBC, HMQC and HRMS. Effects of ionic liquid, reaction time and temperature on the D-A reaction were investigated as well. The optimum conditions conditions were as follows: 1-butyl-3-methylim- idazoliumtetrafluoroborate was as ionic liquid, reaction at 35 ºC for 20h. Because simplicity of operator and conveniently of the post-treatment on this process, which could be further developed at an industrial scale for the synthesis of CI and is destined to be an environmentally friendly way to solve the lack of cantharis as a natural resource.

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