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N-methylcantharidimide: Synthesis and purification

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ABSTRACT: N-methylcantharidimide (NMC), a derivative of cantharidin (C), shows antitumor properties. In this study, NMC was synthesized from C and methylamine with a yield of over 90%. We investigated the influences of different amounts of methylamine used in this reaction, and found that the optimal methylamine dosage was twice the theoretical amount. Furthermore, in the purification of NMC, distilled water was the most suitable solvent. This improved method is both simple and economical, and this process could be further developed at an industrial scale for the synthesis of NMC.

KEYWORDS: N-methylcantharidimide, cantharis, cantharidin, methylamine

INTRODUCTION

Cantharis, the dried body of the Chinese blister beet, has been used to treat tumors for over 2000 years in China, and it is still used as a folk medicine today in Asia [1, 2]. Cantharidin (C, Fig. 1), the active constituent of cantharis, has been reported to show activity in response to various cancers and causes leukocytosis, but with severe side effects [3]. To reduce the toxicity and side effects, derivatives of C have been identified as a research hot spot for antitumor drugs. A previous study showed that N-methylcantharidine (NMC), a derivative of C, caused relatively lower toxicity and fewer side effects than C [4]. In this study, NMC was synthesized (Fig. 2) according to the procedure described in the literature [5], but with some improvements to the process. The present process was then compared with the published method [5]. The process used in this study shows promise and could be further developed at an industrial scale for the synthesis of NMC.

MATERIALS AND METHODS

Bacteria, plasmids, and chemicals

Compound C was prepared following the method previously described [6, 7]. All of the reagents were purchased from Merck Chemical Technology (Shanghai) Co., Ltd, Sigma Aldrich (Shanghai) Trading Co., Ltd, and Sinopharm Chemical Reagent Co., Ltd, Fluka China general agent, all companies are in Shanghai, China. Nuclear magnetic resonance (NMR) data were recorded on an Agilent Technologies 600 MHz DD2 (Santa Clara, CA, USA). Electrospray ionization mass spectrometry (ESI-MS) data were recorded on a Waters Acquity® SQD (Milford, MA, USA).

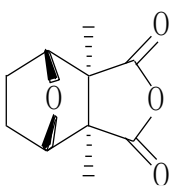

Fig. 1 The structure of cantharidin (C).

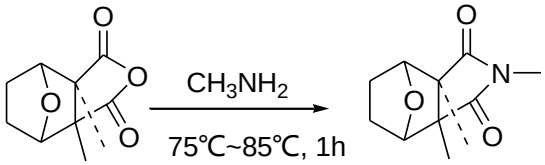

Fig. 2 Preparation of N-methylcantharidin (NMC).

General procedure for the preparation of NMC

A mixture of C (40 g) and 25% (v/v) methylamine solution (50 ml) was stirred in the reaction flask at 75 °C to 85 °C for 1 h. Then the solution was cooled to allow crystals to form and then recrystallized from hot water to afford white crystals of NMC. Yield: 94.7%; ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.13 (s, $-CH_3$, 6H), 1.68–1.79 (m, $-CH_2CH_2$, 4H), 2.98 (s, $-N-CH_3$, 3H), 4.55 (m, −O−CH−, 2H); ^{13}C-NMR ($CDCl_3$) δ: 12.59 ($-CH_3$), 23.65 ($-N-CH_3$), 25.21 ($-CH_2CH_2-$), 53.96 (−C−CO−), 83.48 (O−CH−), 181.48 (−CO−N) ppm; ESI-MS: m/z 210.11 (M^+, 100%).

RESULTS AND DISCUSSION

Effect of methylamine on the yield of NMC

Table 1 shows the yield of NMC with different dosages of methylamine. It is evident that the yield depends on the dosage of methylamine. For example, in entries 1 to 3, the reaction was not complete when the molar ratio of methylamine to C was less than 2, and when the molar ratio increased to 2, the yield of NMC was

Table 1 Influence of different dosages of methylamine on the synthesis of N-methylcantharidin (NMC).

Entry	C (g)	25% aqueous solution of methylamine (ml)	Molar ratio of methylamine to C	NMC (g, %)[†]
1	40.0	30	1.2	(25.5, 60.4)
2	40.0	40	1.6	(33.9, 80.3)
3	40.0	50	2.0	(40.0, 94.7)
4	52.7	70	2.1	(50.5, 90.8)
5	88.0	115	2.1	(88.9, 95.7)
6	91.5	120	2.1	(91.5, 94.7)
7	50.4	70	2.2	(49.5, 93.0)
8	115	950	11.6	(120.0,98.8)
9	30	250	13.0	(30.5, 96.3)
10	100	1000	15.6	(104.0, 98.5)
11	200	2000	15.6	(208.0, 98.5)

[†] The quality and yield of NMC. Taking '(25.5, 60.4)' as an example, it means that the quality of NMC was 25.5 g and the yield of NMC was 60.4%. C, cantharidin.

Table 2 Comparison of the synthesis process of NMC.

	Methylamine concentration (%)	Reaction time (h)	Reaction temperature (°C)	Recrystallization solvent	Yield (%)
Previous process [5]	40	(2+2)[†]	220	Ethanol	93.0
Present study	25	1	75–85	Water	94.7

[†] The solution was refluxed for 2 h at 220 °C, after which the solvent was refluxed off and the dried mass was refluxed for an additional of 2 h.

increased to 94.7%. The amount of methylamine continued to increase in entries 3 and 5, and the yield slightly increased to 95.7%. When the molar ratio of methylamine to C increased to 15.6 in entries 10 and 11, the yield of NMC was 98.5%.

Purification of NMC

The solubility of NMC in different solvents was tested. We found that NMC dissolved well in chloroform, acetone, ethyl acetate, ethanol, ether, and hot water, and it was slightly soluble in water and petroleum ether. The water was a green recrystallization solvent.

A mixture of the crude product of NMC (40 g) in 120 ml water was heated to 80 °C to complete the dissolution of the compound. Then the mixture solution underwent immediate filtration to remove the impurities. The filtrate was naturally cooled to 20 °C, and 3 h later the white crystals of NMC were obtained by filtration. One-third of the filtered mother liquor was evaporated and the remaining solution was transferred to another flask. The next day, the white crystals of NMC were obtained by filtration. All of the white crystal samples were combined and dried for 24 h at 50 °C.

The recrystallization of NMC with distilled water is a simple and cheap method. C does not dissolve in water, which prevents C from mixing with the NMC. NMC is easy to refine. NMC is well dissolved in hot water, so it is necessary to concentrate the mother liquor and recover the products.

Comparison with the previous process

In this study, NMC was synthesized according to the procedure described in the literature [5, 8–10]. However, as shown in Table 2, the modifications made in the present study improved the process and the yield of NMC did not decrease. For example, the reaction time was reduced to an hour and the reaction temperature dropped to within 100 °C. Moreover, the recrystallization solvent was changed from ethanol to water. In short, the process used in the current study has advantages.

CONCLUSION

NMC, which was confirmed by ^{1}H NMR, ^{13}C NMR, and ESI-MS, was efficiently synthesized. The effects of the dosage of methylamine and different recrystallization solvents on the yield of NMC were investigated as well. The optimum conditions were as follows: the molar ratio of methylamine to C was 2:1, the reaction proceeded at 75–85 °C for 1 h, and water was used as the recrystallization solvent. Because of the simplicity of operation and the convenience of the post-treatment method used in this process, it could be further developed at an industrial scale for the synthesis of NMC.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2022.060.

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Appendix A. Supplementary data

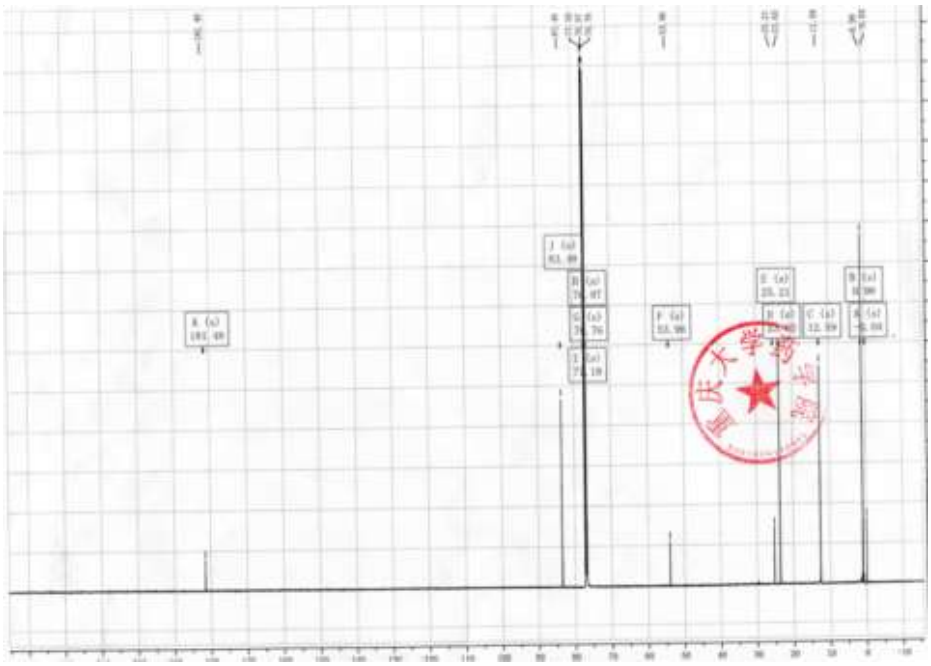

Fig. S1 ^{13}C NMR of NMC.

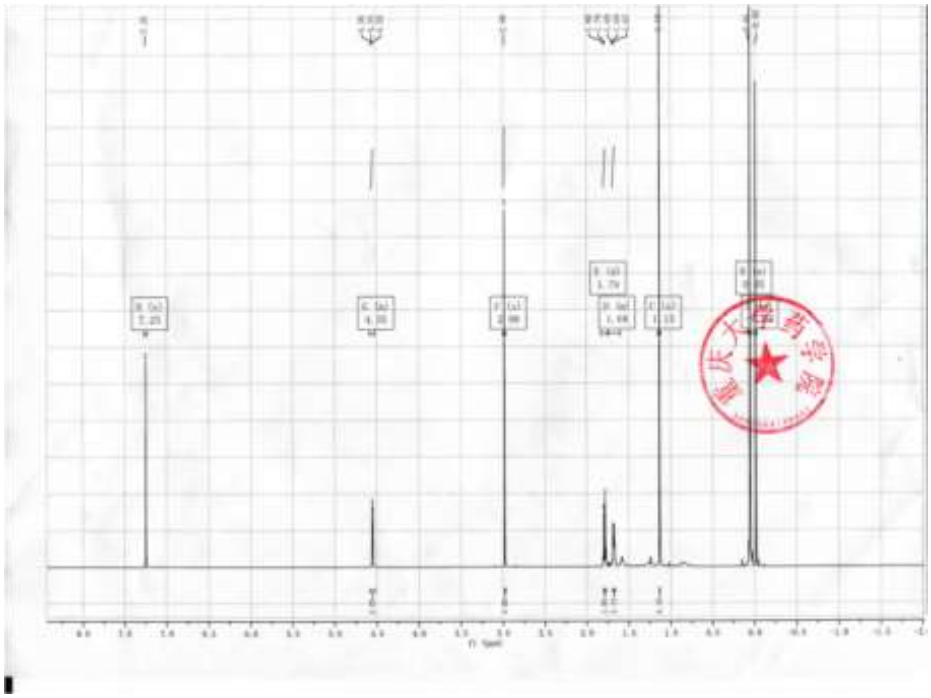

Fig. S2 ^{1}H NMR of NMC.