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Synthesis and Antibacterial Activity of 4-(4-Methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxamide

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In this paper, 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxamide was synthesized by β-myrcene through Diels-Alder reaction and ammonolysis reaction. The synthetic compound was proved to be the target product with the help of NMR, FT-IR and GC-MS. Diels-Alder reaction has been optimized under the following conditions: 150 ºC, reacting for 3 h, no catalyst and the production rate of 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxamide is 88.12 %, the selectivity is 97.49 %. Then get down to ammonolysis reaction under 0.4 MPa ammonia pressure for 18 h with methanol as the solvent and the production rate turned up to 88.7 %. At last, the test of the antibacterial activity of the target product showed optimistic to *Staphylococcus aureus* but passimistic to *Escherichia coli*.

Key Words: β-Myrcene, Diels-Alder reaction, Ammonolysis reaction, Antibacterial activity.

INTRODUCTION

In China, rosin resources are very rich, especially in Guangdong, Guangxi and Yunnan provinces[1] and the content of β-pinene in rosin can reach to 30.3 %. β-Myrcene can be obtained by β-pinene through thermal isomerization in industry, whose purity is 80 %. β-Myrcene is also refered as geraniolene and the chemical name is 7-methyl-3-methylene-1,6-octadiene and there are three unsaturated carbon-carbon double bonds, which are easy to generate chemical reactions, such as Diels-Alder reaction, oxidizing reaction and reduction reaction. Goldblatt and Palkin[2], Yin *et al.*[3-6] and Luo *et al.*[7] have investigated the Diels-Alder reaction of β-myrcene, however, none of them had investigated the further application of the Diels-Alder product in industry.

Diamide compounds have significant applications in chemical industry, which are widely used as chemical materials, agricultural materials, pharmaceuticals, fuel and intermediates of pigment productions. Synthesis of diamide compounds have been investigated for years[8-11] and in this paper, we firstly synthesized 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxamide by β-myrcene through Diels-Alder reaction and ammonolysis reaction and the antibacterial activity of the product was studied as well.

EXPERIMENTAL

β-Myrcene, (Jiangxi Kaiyuan spices Co. Ltd., China), NH_3 (Nanjing special gas Co. Ltd., China), Dimethyl maleate (AR), CH_3OH (AR), $CaCl_2$ (AR), HCl (AR).

500 MHz NMR (Bruker, Germany), 7890N/5975N GC-MS (Agilent, USA), 7890 GC (Agilent, USA), IS10 FT-IR (Nicolet, USA), WRS-1B Melting Point Apparatus (Shanghai exact science instrument Co. Ltd., China), BS110S electronic balance (Bejing Saiduoli Inc., China), SHZ-C vacuum pump (Yingyu Electronic Instrument Factory, China), temperature control system, JJ-1 electric mixer (Guohua Inc., China), HH-4 digital electronic constant temperature water-bath (Aorui Equipment Factory, China).

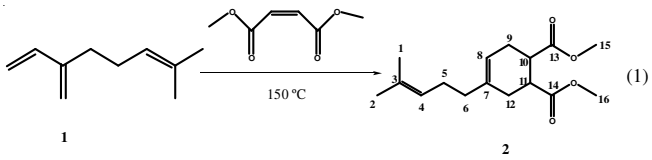
Antibacterial activity: The antibacterial activity of chemicals was estimated by a disc paper method. The test chemicals were dissolved in DMSO to 25 mg/mL. As test species, *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria) were cultivated in beef extract-peptone for 1 week. After cultivation, a small amount of (1 scratch *ca.* 2 scratch) fresh bacteria from the culture medium were added into the culture solution. The culture solution was diluted 10 fold to a concentration of $(5.0\sim10.0) \times 10^6$ (CFU) mL^{-1}. 1 mL of the bacterial solution

described above was then evenly coated on 90 mm plate of beef extract peptone medium. Six millimeter diameter sterile filter paper was dipped in the solution of antibacterial test chemicals for 10 min and then the paper was taken out and placed on the plate. The inhibition zones were measured by calipers in millimeters at the end of a 24 h incubation period. All experiments were conducted in triplicate to ensure reproducibility at a given concentration.

$$\text{Diameter of inhibition zone (mm)} = A_1 - A_0 \quad (1)$$

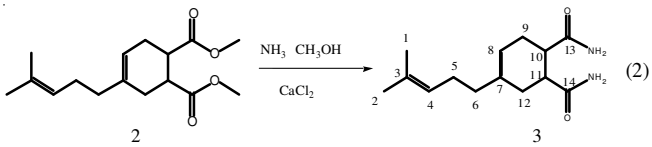
where A_0 is the diameter of the control (blank, ethanol and sterile water without an antibacterial test chemical) and A_1 is the diameter in the presence of test chemical.

Synthesis of dimethyl 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxylate: 72 g dimethyl maleate was added into 250 mL flask which was connected with water bath, stir device, thermograph, dropping funnel and reflux condenser. Turning on the stirring device and adding β-myrcene dropwisely, then heat up to 150 ºC for 3 h. When reaction finishes, draw out fraction of 205 ºC/1.05 kPa through vacuum distillation method and the left is 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxylate, which is light yellow liquid and its weight is 119.7 g. The yield is 88.1 %. IR (ν/cm^{-1}): 2951[s, -CH_3, ν_{as}], 2920[s, -CH_2, ν_{as}], 2852[s, -CH_2, ν_s], 1736[s, ν(C=O)], 1672[w, ν(C=C)], 1436[m, -C-CH_3, δ_{as}], 1376[m, -C-CH_3, δ_s], 1354(m), 1279(m), 1203[s, ν(C-O-C)], 1170(s), 1105(w, C-O-C, ν_s), 1074(w), 1024(m), 926(w), 848(w), 792(w); ^{1}H NMR (500 MHz, CD_3OD) δ/ppm: 5.37 (s, 1H, C_8-H), 5.08 (t, J = 6.8 Hz, 1H, C_4-H), 3.65 (2s, 6H, C_{15}-H_3 and C_{16}-H_3), 3.08-3.00 (m, 2H, C_{10}-H and C_{11}-H), 2.51-2.27 (m, 4H, C_9-H_2 and C_{12}-H_2), 2.08-1.96 (m, 4H, C_5-H_2 and C_6-H_2), 1.67 (2s, 6H, C_1-H_3 and C_2-H_3); ^{13}C NMR (125 MHz, CD_3OD) δ/ppm: 175.3 (C_{13} and C_{14}), 137.2 (C_7), 132.3 (C_3), 125.1 (C_4), 120.0 (C_8), 52.2 (C_{15} and C_{16}), 49.5 (C_{10}), 41.4 (C_{11}), 38.5 (C_6), 29.7 (C_{12}), 27.3 (C_5), 26.8 (C_9), 25.9 (C_2), 17.8 (C_1); GC-MS (*m/z*): 280.1 [M-e]$^+$, 248.1 [M-CH_3OH-e]$^+$, 220.1 [M-CH_3COOH-e]$^+$. The reaction eqn. (1) shows as follows:



1 2 (1)

Synthesis of 4-(4-methylpent-3-enyl)cyclohex-4-ene-1, 2-dicarboxamide: Add 11.8 g of 4-(4-methylpent-3-enyl)-cyclohex-4-ene-1, 2-dicarboxylate into 150 mL stainless steel reaction kettle, then add 9.3 g anhydrous $CaCl_2$ and 60 mL anhydrous CH_3OH. Put them into ice water bath airtightly and turn on the magnetic stir device, at the same time, enter NH_3 till the pressure of stainless steel reaction kettle ups to 0.4 MPa. When the temperature downs to 8-10 ºC, stop adding of NH_3, warm up to 80 ºC slowly and keep it for 18 h. When reaction finishes, adjust pH to 5-6 with diluted HCl and wash the product till there is no Cl^-, then after filtering and vacuum drying, 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxamide is obtained, which is tan solid. The yield is 88.7 %. m.p.: 209-211 ºC; IR (ν/cm^{-1}): 3417(w), 3330[m, -NH_2, ν_{as}], 3202[m, -NH_2, ν_s], 2967[m, -CH_3, ν_{as}], 2919[m, -CH_2, ν_{as}], 2849[m, -CH_2, ν_s], 1711(w), 1657(s, peak of primary amide), 1610(m, peak of primary amide), 1371[m, -C-CH_3, δ_s], 1252(w), 1190(w), 1114(w), 787(w); ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm: 7.18, 6.65 (m, 4H, 2*NH_2), 5.37 (d, J = 4.1 Hz, 1H, C_8-H), 5.09-5.06 (m, 1H, C_4-H), 2.53-2.41 (m, 2H, C_{10}-H and C_{11}-H), 2.19-1.92 (m, 8H, C_9-H_2, C_{12}-H_2, C_5-H_2 and C_6-H_2), 1.64 (2s, 6H, C_1-H_3 and C_2-H_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm: 180.5 (C_{13} and C_{14}), 137.4(C_7), 132.5(C_3), 125.1(C_4), 121.2(C_8), 49.5(C_{10}), 44.2(C_{11}), 38.4(C_6), 30.4(C_{12}), 28.3(C_5), 26.8(C_9), 25.8(C_2), 17.8(C_1); GC-MS (*m/z*): 250.1 [M]$^+$, 233.1 [M-NH_3]$^+$, 205.1 [M-$HCONH_2$]$^+$. The reaction eqn. (2) shows as follows:



2 3 (2)

RESULTS AND DISCUSSION

Effects on different conditions towards Diels-Alder reaction between β-myrcene and dimethyl maleate: Effects on different conditions towards productivity and selectivity of Diels-Alder reaction between β-myrcene and dimethyl maleate are given in Table-1. Entry 10-14 indicate that with the extension of the reaction time, the productivity rises at first and then fluctuates in a level, which indicates Diels-Alder reaction between β-myrcene and dimethyl maleate is reversible and it will get to balance in a certain extent and the productivities are different under different temperatures (entrys 9, 12). The contrast of data in the table (entry 1, 4, 5, 8, 12, 21) shows that within the same reaction time, the productivity continues rising with the increase of reaction temperature until the temperature rises up to 150 ºC. The data of Table-1 (entry 1-3,

TABLE-1
EFFECTS OF DIFFERENT FACTORS ON THE DIELS-ALDER REACTION OF β-MYRCENE AND DIMETHYL MALEATE

Entry	Temp (ºC)	Time (h)	Catalyst	Yield[a] (%)	Selectivity[b] (%)
1	75	3	-	12.08	93.43
2	75	3	$LiClO_4$	81.86	94.91
3	75	5	$LiClO_4$	80.52	94.96
4	95	3	-	18.25	95.19
5	110	3	-	41.45	95.11
6	135	1	-	72.18	93.10
7	135	2	-	81.05	94.51
8	135	3	-	80.79	93.58
9	135	4	-	84.19	95.52
10	150	1	-	78.26	94.82
11	150	2	-	84.61	96.13
12	150	3	-	88.12	97.49
13	150	4	-	86.19	94.35
14	150	5	-	86.98	94.89
15	150	1	$LiClO_4$	86.22	95.74
16	150	2	$LiClO_4$	88.70	96.50
17	150	3	$LiClO_4$	85.99	94.38
18	150	4	$LiClO_4$	87.01	94.89
19	160	1	-	87.35	95.31
20	160	2	-	88.72	93.95
21	160	3	-	84.54	93.68

[a]Mass fraction of product in the reaction system whose retention time is 19.0-21.0 min; [b]Mass fraction of product retention time at 20.38 min in the yield

10-18) shows that the catalyst $LiClO_4$ can shorten the reaction time and reduce the temperature to the same balance, but at the same time reduce the product selectivity and increases the difficulty of the product post-processing, which complicates the experiment operation and increase the cost.

The contrast of data in Table-1 (entry1-21) shows that the balance forms under 150 ºC after reacting for 3 h has both high productivity and good selectivity. Considering the cost of catalyst and post-processing of the product, the reaction conditions are made as follows: no catalyst, reaction temperature of 150 ºC and reaction time of 3 h.

Effects on productivity of ammonolysis reaction of dimethyl 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxylate: Effects on productivity of ammonolysis reaction of dimethyl 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxylate are given in Table-2. Data from entry 1-4 indicates that the demand of solvent in this reaction is strict, because when acetone, formamide and DMF are used as solvents, there is none of 4-(4-methylpent-3-enyl)cyclohex-4-ene-1, 2-dicarboxamide. The reason may be that the reaction needs large concentration of NH_3 and the hydrogen bond between H-N or O-H from -OH in methanol and NH_3 increases the solubility of NH_3 in the reaction system which promotes the reaction to a great extent.

TABLE-2
OPTIMIZATION OF DIFFERENT FACTORS ON THE AMMONOLYSIS REACTION OF DIMETHYL 4-(4-METHYLPENT-3-ENYL)CYCLOHEX-4-ENE-1,2-DICARBOXYLATE

Entry	Solvent	Temp (ºC)	Pressure (MPa)	Time (h)	Yield [a] (%)
1	Acetone	80	0.5	24	-
2	Formamide	80	0.5	24	-
3	DMF	80	0.5	24	-
4	Methanol	80	0.5	24	88.9
5	Methanol	60	0.5	24	40.7
6	Methanol	70	0.5	24	67.4
7	Methanol	90	0.5	24	86.5
8	Methanol	80	0.2	24	49.6
9	Methanol	80	0.3	24	75.8
10	Methanol	80	0.4	24	88.7
11	Methanol	80	0.4	6	74.8
12	Methanol	80	0.4	12	85.6
13	Methanol	80	0.4	18	88.7
14	Methanol	80	0.4	24	88.7

Data of entry 2 show that the reaction temperature should be 80 ºC. With the rise of temperature, 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxamide will turn to 5-(4-methylpent-3-enyl)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione (structure can be proved through GC-MS method). The contrast of productivity of 4-(4-methylpent-3-enyl)cyclohex-4-ene-1, 2-dicarboxamide showed in entry 4 and entry 8-10 indicates that productivity will rise with the increase of the NH_3 pressure till the pressure reach to 0.4 MPa. Considering the cost of NH_3, 0.4 MPa is chosen to be the reaction pressure. Entry 11-14 show the effect of reaction time towards productivity of 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxamide. The test shows big rate at the beginning of the reaction and the productivity will not increase when it gets to balance after reacting for 18 h. Therefore, the best reaction time is 18 h.

Antibacterial activity of the 4-(4-methylpent-3-enyl)-cyclohex-4-ene-1,2-dicarboxamide: Table-3 shows that 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxamide has antibacterial effect to *Staphylococcus aureus* but not to *Escherichia coli*, which indicates the antibacterial activity of 4-(4-methylpent-3-enyl)cyclohex-4-ene-1, 2-dicarboxamide is specific.

TABLE-3
ANTIBACTERIAL ACTIVITIES OF THE 4-(4-METHYLPENT-3-ENYL)CYCLOHEX-4-ENE-1,2-DICARBOXAMIDE

Bacterias	Diameter of inhibition zone (mm)
Escherichia coli	0.40
Staphylococcus aureus	2.66

Conclusion

The optimized β-myrcene and dimethyl maleate Diels-Alder reaction condition is 150 ºC, react for 3 h and no catalyst. Productivity of dimethyl 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxylate is 88.12 % and the selectivity is 97.49 %.

Target compound 4-(4-methylpent-3-enyl)cyclohex-4-ene-1,2-dicarboxamide was synthesized by β-myrcene through Diels-Alder reaction and ammonolysis reaction. The optimized ammonolysis reaction condition is methanol as solvent, reacting under 0.4 MPa ammonia pressure for 18 h and the yield is 88.7 %. The antibacterial activity of 4-(4-methylpent-3-enyl)-cyclohex-4-ene-1,2-dicarboxamide have been studied, which shows an efficient antibacterial effect towards *Staphylococcus aureus*.

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