Heterocyclic Letters
Vol. 9| No.2|141-148|Feb-April |2019
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

OPTIMIZATION OF N-BUTHYLPHTHALIDE SYNTHESIS PROCESS

ElnazYaghoobi[a], Saeed Shojaee[a], FarzinHadizadeh*[b],Seyed Ahmad Mohajeri*[c] and MahmoudSeifi[b]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS).*
[b]*Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.*
[c]*Targeted Drug Delivery Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.*
**Corresponding Authors: FarzinHadizadeh and Seyed Ahmad Mohajeri*
Mail: HadizadehF@mums.ac.ir and MohajeriA@mums.ac.ir

Abstract
In this research, one of the active chemical compounds with various medicinal applications in celery, which is NBP (n-Buthylphthalide), was selected to be synthesized through two distinct ways. Ortho-bromo benzaldehyde and naphthalene were opted as starting materials. Compared to previous proposed methods, Reaction times were short, the procedure and work-up were simple. Using inexpensive, readily available starting materials and the yield of reaction were considered as the next highlighted points According to gained data from HPLC and NMR, the way in which Ortho-bromo benzaldehyde was used was noticeable in terms of efficiency and purity, being 89.74% and 72% respectively. On the other hand, naphthalene used in this synthesis was not at all comparable to Ortho-bromo benzaldehyde regarding efficiency and purity, with 37.15% and 23% respectively.

Keywords: Celery, Ortho-bromo benzaldehyde, medicinal applications, naphthalene and NBP (n-Buthylphthalide)

Introduction

Celery (Apiumgraveolens) is a vegetable that has been commonly eaten in the local diet. It has been also recommended in traditional Chinese medicine for treatment of hypertension[i-ii].According to previous studies, it was reported that celery was able to decrease the blood pressure of renovascular occlusive hypertensive dogs and rats[i, iii]. Apart from the role in rheumatism, celery seeds proved its applicationsincluding asthma, bronchitis and inflammatory conditions[iv-vi],Celery has an anticoagulant activities[vii]. Essential oil of celery has antibacterial effects. Moreover, this plant has cooperation in the molecular mechanisms and cellular targets that have a significant effect on the treatment of human cancers[viii].The derived compounds were also included containedselenine (10-15%), limonene (60%), β-

pinene, camphene, cymene, limonene, α-thuyene, α-pinene, β-phellendrene, p-cymene, γ-terpinene, sabineneterpinolene, myristicic, myristic, linoleic, petroselinic, palmitoleic, palmitic, oleic, myristoleic, stearic acid, santalol, β-eudesmol, α-eudesmol and sedanenolide[ix].It is said that the NBP was a major fragrance component of the celery oil[x]. NBP, a chemical unique in celery, is a small lipophilic molecule with a molecular weight of 190.242$\frac{gr}{mol}$. It contributes to the characteristic flavor of celery[xi]. It is also reported that NBP decreased the systolic blood pressure (SBP) of normotensive Sprague Dawley rats significantly after daily injection for a fortnight[xii]. Previous studies in rats have shown that NBP has neuroprotective effects that are beneficial for stroke treatment, including improving microcirculation dysfunction during insomnia[xiii], decreasing the area of cerebral infarct in focal cerebral ischaemicrats[xiv, xv] and improving energy metabolism in mice with complete brain ischaemia[xvi, xvii].

To synthesize NBP from natural sources (celery), NBP has been extracted and purified. Moreover, there are some chemical methods proposed by different researchers. Although, some various methods have already been mentioned, none of them had been fast and high yielding.

NBP was synthesized by perkin condensation reaction between phthalic anhydride and alkylcarboxylic anhydride (figure 1)[xviii, xix]. Its reported yield was less than 30%.

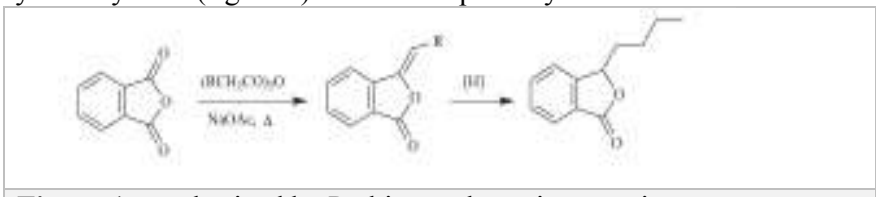


Figure 1: synthesized by Perkin condensation reaction.

The Japanese chemist,Nakai, developed a new method for synthesis of NBP via nucleophilic addition of a Grignard reagent to o-formylbenzoic acid under mild reaction conditions, its yield was 38% (figure 2).

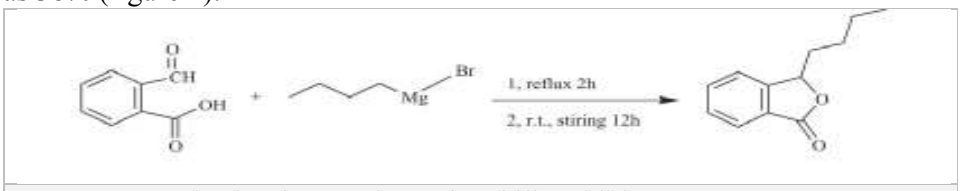


Figure 2: synthesis of NBP via nucleophilic addition**.**

Ogawa and Yorozu proposed new method as a patent that a protected carboxyl group was considered to make the yield more than 50% [xix](figure 3).

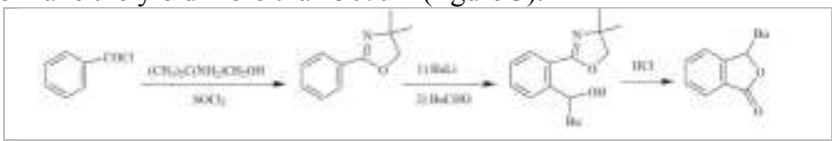


Figure 3*:* using a protected carboxyl group**.**

2. Result and discussion

All intermediate and final products were analyzed by ^{1}H NMR (NMR (300 Hz), BRUKER) and HPLC (YOUNGLIN). Then, both efficiency and yield of each method were recorded in following paragraphs.

2.1. Result of first synthesized method

2.1.1. Intermediate compound (O-formylbenzoic acid)

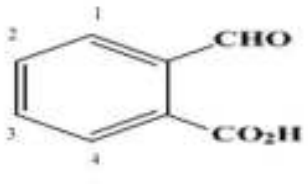


Figure 4: Intermediate compound (O-formylbenzoic acid)

HNMR spectra (300 Hz, DMSO): 7.31 – 7.39 (2H, m, Ar-H) H-1, H-3 and 7.45- 7.55 (2H, m, Ar-H) H-4, H-2

In Phthalaldehydic acid (O-formylbenzoic acid), both acidic and aldehyde groups create a five-membered hydroxyl lactone. Therefore, there is a balance between acid and aldehyde, and an average peak in ^{1}HNMR spectra has been appeared.

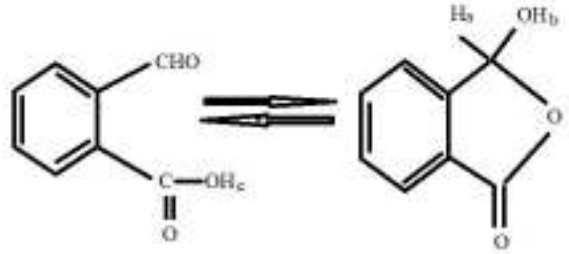


Figure 5:equivalence form of phthalaldehydic acid

^{1}HNMR spectra (300 Hz, DMSO): 6.68 (1H, brs, Ha), 10.42 (1H, brs, OHb) and 13.48(1H, brs, OHc)

2.1.2. N-Buthylphthalide

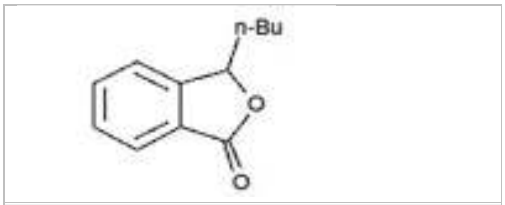


Figure 6:n-Buthylphthalide

^{1}HNMR spectra (300 Hz, MeOD): 0.779-0.824(t, 3H, CH_3), 1.124-1.363(m, 4H, 2CH_2 (n-Bu)), 1.944- 1.991(m, 2H, - CH_2 (n-Bu)), 5.367 - 5.406(q, 1H, phthalyl CH) and 7.258 – 7.786(m, 4H, Ar-H*)*

2.2. the second synthesized method

2.2.1.*Intermediate compound (1-(2-bromophenyl) pentan-1-ol)*

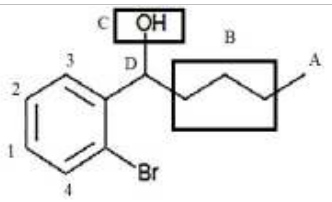


Figure 7:intermediate compound synthesized from Ortho-bromobenzaldehyde

^{1}HNMR spectra (300 Hz, CDCl_3): 0.919 (3H, t, J=7.1 Hz, CH_3 (A)), 1.305 – 1.761 (m, 6H, 3CH_2's of n-buthty (B)), 1.97 (1H, brs(C)), 5.07 (1H, dd, J=8 Hz and J=4Hz, D), 7.12 (1H, td, J=7.5 Hz and J=1.5 Hz, Ar-H(H-1)),), 7.33 (1H, td, J=7.5 Hz and J=1 Hz, Ar-H(H-2)), 7.50 (1H, dd, J=8 Hz and J=1 Hz, Ar-H(H-3)) and 7.54 (1H, dd, J=7.5 Hz and J=1 Hz, Ar-H(H-4)).

2.2.2.Synthesizedn-Buthylphthalide

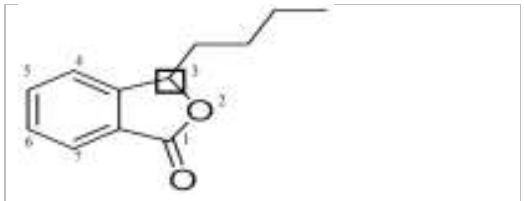
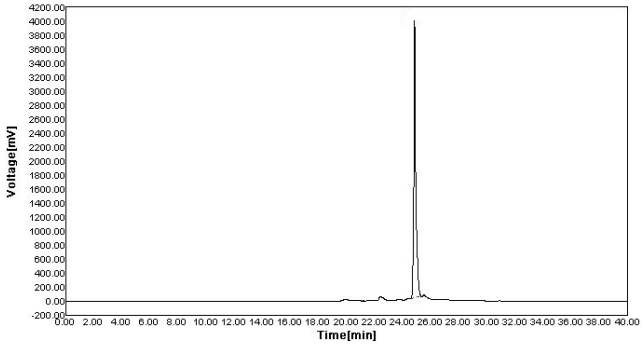


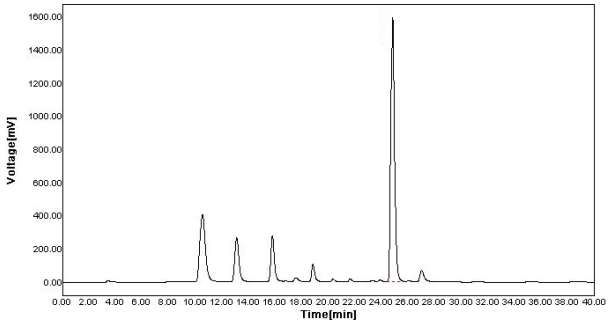
Figure 8: n-Buthylphthalide

^{1}HNMR spectra (300 Hz, CDCl_3): 0.835 (3H, t, J=7Hz, CH_3 (n-Bu)), 1.215 – 1.431 (4H, m, 2CH_2 (n-Bu)), 1.609-1.731 (1H, m, CH_2(n-Bu)), 1.917-2.015 (1H, m, CH_2(n-Bu)), 5.418 (1H, dd, J=7.8 Hz and J=4 Hz, phthalyl CH), 7.3 (1H , d , J=7.6 Hz , Ar-H(H-4)), 7.4 (1H, t, J=7.4 Hz, Ar-H(H-6)), 7.6 (1H, t, J=7.4 Hz, Ar-H(H-5)) and 7.8 (1H, d, J=7.6 Hz, Ar-H(H-7)).

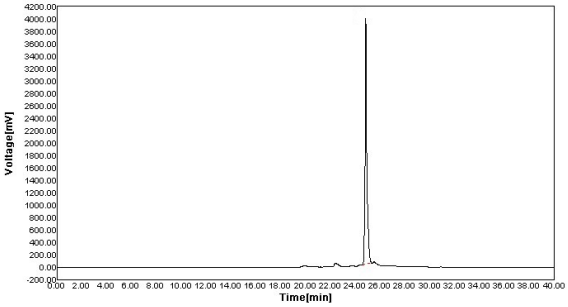
To calculate and monitor each method's purity and accuracy, three different solutions with specific concentration (50µg/ml) from synthesized NBP and purchased NBP (Sigma-Aldrich) were prepared to be injected into HPLC (YOUNGLIN), and HPLC was run with following condition including flow rate (0.5 ml/min), column temperature (30ºC), detector (UV detection at 230nm) and gradient mobile phase (a mixture of methanol (HPLC grade) and deionized water).



Chromatogram 1: standard solution of NBP



Chromatogram 2:sample solution of first method (starting material was naphthalene)



Chromatogram 3: Sample solution of second method (starting material was o-bromobanzealdehyde)

According to gained data from Chromatograms, it is shown that NBP was successfully synthesized.

2.3. Evaluation of purity and the yield

In order to calculate the purity of two methods, standard diagram was drawn in which three distinct concentration of standard solutions were prepared and injected into HPLC.According to obtained line equation, the purity of first and second method were calculated, being 23% and 73% respectively. The yield of two synthesized methods were obtained, being 37.15% for the first method and 89.74 for the second one.

3. Experimental

3.1. Materials

In this study, All used materials were purchased from Merck company located Germany, including bromobenzene, magnesium turnings, diethyl ether, sodium hydroxide, hydro chloric acid, anhydrous ether, buthyl bromide, benzene,trimethylamine, naphthalene,

potassium permanganate, sodium bisulfite, methanol, Acetone, chloroform, sodium, sodium thiosulfate, decolorizing carbon, sulfate calcium anhydrous, chloride ammonium and sulfate sodium anhydrous.

3.2. Methods

All chemicals were available commercially and used without additional purification.

3.2.1. First method

In general, naphthalene, as a starting material, was selected to react with NaOH, $KMnO_4$and $NaHSO_3$ under heated condition.Then, the production of previous step was O-formylbenzoic acid (compound 1), as an intermediate compound. It then reacted with organometallic compound, named n-buthylmagnesium bromide. Compound (2), being under heated and acidic condition, was turned into NBP (compound 3).

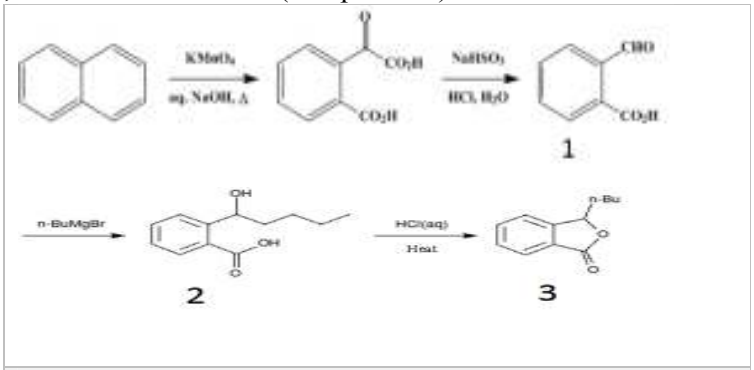


Figure 9 :the first method

In detail, First of all, NaOH (83.3 cc, 0,5N) and naphthalene (5.3 gr) were added and stirred until it boiled. Then, $KMnO_4$ (35.3 gr) was dissolved into 250cc of boiled water, and this mixture was gradually added to previous mixture. Here, the mixture was left until oxidation was completed. To remove left materials, it was filtered. Filtered mixture was then acidified (pH=3) by adding HCl. Its solvent was evaporated by rotary evaporator. After that, cooled mixture was then neutralized by NaOH. Sodium bisulfite (8.3gr) was added, and then the whole mixture left for 24 hours. Here, soxhlet extractor was used with benzene as a solvent. This process took 24 hours. Accuracy of synthesis was monitored by TLC, and then its solvent was evaporated. After being dissolved (left solid compound) into hot water, it was filtered. To make crystal, it was put into ice bath. Melt point was also monitored to be in range of 94 to 95ºC. Crystalized compound was dissolved into hot water. At the same time, activated carbon was added into boiled water, and then it was filtered. Subsequently, filtered compound was put into ice bath to appear crystal. Therefore, intermediate compound was finally synthesized named O-formylbenzoic acid. After synthesis this intermediate compound, Grignard reagent was prepared[20]. Intermediate compound was then added to anhydrous diethyl ether (15cc), and this mixture was gradually added to synthesize Grignard reagent, which was in mixture of salt and ice. To complete the reaction. It was left approximately 5 hours. Saturated chloride ammonium (10cc) was then added. This mixture was acidified (pH=2) by HCl, and it was left at room temperature for an hour. To separate organic phase from aqueous phase, diethyl ether (25cc) was added (three times). Following that, Sulfate sodium anhydrous was added to organic phase. Finally, the mixture was filtered, and its solvent was evaporated. Consequently, something was left named NBP.

3.2.2. The second method

In general, The Grignard heavy plan is to alkylate o-bromobenzaldehyde (1)(figure 5) to form secondary alcohol (2). This secondary alcohol is then converted to the corresponding organomagnesium compound and treated with carbon dioxide to yield the carboxylic acid (3).

Acid quench and further refluxing will hopefully drive lactonization to form a racemic mixture 3-butylphthalide.

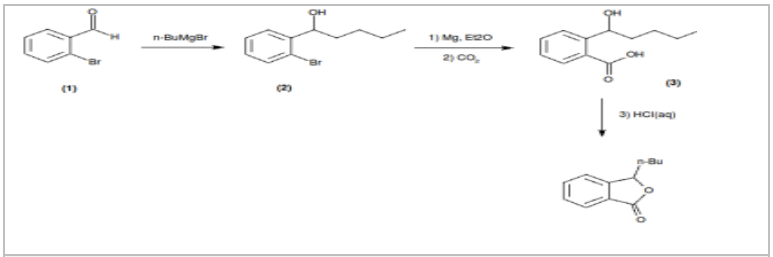


Figure 10:the second method

In detail, First of all, Grignard reagent was synthesized according to the literature[XX]. Ortho-bromo benzaldehyde (0.56 cc) was poured into anhydrous diethyl ether (15cc), and this mixture was gradually poured into Grignard reagent. The whole mixture left for 5 hours. After neutralizing the reaction by saturated chloride ammonium (10cc), it left at room temperature for an hour. Subsequently, both organic and aqueous phases were separated by adding diethyl ether (25cc). Sulfate sodium anhydrous was added to make it anhydrous. After filtering and evaporating its solvent, the secondary alcohol was completely synthesized. Then, Grignard reagent was synthesized again with following structure. Magnesium (0.3 gr) was located in Erlenmeyer flask, whichwas in warm bath (40ºC). Both anhydrous diethyl ether (5cc) and synthesized intermediate compound (1-(2-bromophenyl) pentan-1-ol, 0.3gr) were added to Erlenmeyer flask. Appearing small bubbles on the surface of Magnesium was the sign of starting reaction. After opening refluxing system, the mixture was added to Becher including dry ice (10gr). Anhydrous ether (5-10cc) was then poured to reduce its viscosity. After disappearing all dry ice, the whole mixture was acidified (pH=4) by HCl. Following that, this mixture was transferred to separatory funnel in order to separate its ethereal phase from aqueous phase. Both distilled water (12.5cc) and saturated sodium thiosulfate (2.5cc) were then poured into ethereal phase. Finally, its solvent was evaporated, and the NBP was successfully synthesized.

4. Conclusion

According to acquired results from HPLC and NMR as well as investigating the purity and yield of two methods, the way in which Ortho-bromo benzaldehyde was selected as a starting material are proposed. The purity obtained from the synthesis of the second method is equal 73% and the purity obtained from the first method is 23%. Due to the fact that the NBP synthesized is oily, its purification is not possible by recrystallization. Therefore, achieving a purity of 70-80% is acceptable.

References

i. Tsi, D., et al., *Cardiovascular pharmacology of 3-n-butylphthalide in spontaneously hypertensive rats.* 1997. **11**(8): p. 576-582.
ii. Lu, H.C., *Chinese system of food cures: Prevention and remedies*. 1986: Sterling Pub. Co.
iii. Mohajeri, S., et al., *antihypertensive Effect Of Celery Seed On Rat Blood Pressure In Acute And Chronic Administration: 26.* 2014. **115**: p. 9.
iv. Momin, R. and M.J.P. Nair, *Antioxidant, cyclooxygenase and topoisomerase inhibitory compounds from Apium graveolensLinn. seeds.* 2002. **9**(4): p. 312-318.

v. Bjeldanes, L.F. and I.-S.J.T.J.o.O.C. Kim, *Phthalide components of celery essential oil.* 1977. **42**(13): p. 2333-2335.
vi. Al-Hindawi, M.K., et al., *Anti-inflammatory activity of some Iraqi plants using intact rats.* 1989. **26**(2): p. 163-168.
vii. Shirzad, H., F. Taji, and M.J.J.o.m.f. Rafieian-Kopaei, *Correlation between antioxidant activity of garlic extracts and WEHI-164 fibrosarcoma tumor growth in BALB/c mice.* 2011. **14**(9): p. 969-974.
viii. Atta, A. and A.J.J.o.e. Alkofahi, *Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts.* 1998. **60**(2): p. 117-124.
ix. Kooti, W., et al., *A review on medicinal plant of Apium graveolens.* 2015. **1**(1): p. 48-59.
x. Barton, D. and J.X.J.J.o.t.C.S. de Vries, *356. The constitution of sedanolide.* 1963: p. 1916-1919.
xi. Uhlig, J.W., A. Chang, and J.J.J.J.o.f.s. Jen, *Effect of phthalides on celery flavor.* 1987. **52**(3): p. 658-660.
xii. LE, O. and W. Elliott. *HYPOTENSIVE AND HYPOCHOLESTEROLEMIC EFFECTS OF CELERY OIL IN THE RAT MAY BE DUE TO 3-BUTYL PHTHALIDE*. in *Clinical Research*. 1991. SLACK INC 6900 GROVE RD, THOROFARE, NJ 08086 USA.
xiii. Chang, Q. and X.L.J.A.P.S. Wang, *Effects of chiral 3-n-butylphthalide on apoptosis induced by transient focal cerebral ischemia in rats.* 2003. **24**(8): p. 796-804.
xiv. Liu, X. and Y.J.Y.x.x.b.A.p.S. Feng, *Protective effect of dl-3-n-butylphthalide on ischemic neurological damage and abnormal behavior in rats subjected to focal ischemia.* 1995. **30**(12): p. 896-903.
xv. Peng, Y., et al., *L-3-n-butylphthalide improves cognitive impairment and reduces amyloid-β in a transgenic model of Alzheimer's disease.* 2010. **30**(24): p. 8180-8189.
xvi. Feng, Y., D. Hu, and L.J.Y.x.x.b.A.p.S. Zhang, *Effect of DL-butylphthalide (NBP) on mouse brain energy metabolism in complete brain ischemia induced by decapitation.* 1995. **30**(10): p. 741-744.
xvii. Wei, W., et al., *The therapeutic effect of DL-3-n-butylphthalide in rats with chronic cerebral hypoperfusion through downregulation of amyloid precursor protein and matrix metalloproteinase-2.* 2012. **40**(3): p. 967-975.
xviii. Himes, C.L., *Relationships among health behaviors, health, and mortality*, in *International handbook of adult mortality*. 2011, Springer. p. 289-310.
xix. Xiong, M.J. and Z.H.J.C.O.C. Li, *Progress in syntheses of 3-n-butylphthalide and its analogues.* 2007. **11**(9): p. 833-844.
xx. Baker, K.V., et al., *Mechanical activation of magnesium turnings for the preparation of reactive Grignard reagents.* 1991. **56**(2): p. 698-703.

Received on February12, 2019.