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Research Article

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Application of superbase catalyst γ-Al_2O_3/NaOH/Na for prenylation reaction of resveratrol (3,5,4'-trihydroxystilbene)

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ABSTRACT

Prenylated polyphenols have been known to exhibit significant bioactivities such as antioxidant, antibacterial, antitumor and anticancer activities. Generally, the synthesis of prenylated polyphenols was carried out by using homogeneous catalyst. Utilization of heterogeneous catalyst have several advantages compared to homogeneous catalyst. The aim of this research is to obtain the prenylated resveratrol.In this research, the heterogenous catalyst was applied in prenylation reaction of polyphenol compounds resveratrol (3,5,4'-trihydroxystilbene). The heterogeneous catalyst are solids that was made through mixing process of γ-Al_2O_3, NaOH and sodium metal at a temperature of 400^{0}C. The results of XRD analysis showed the difference in character between solids superbaseγ-Al_2O_3/NaOH/Na and γ-Al_2O_3. Prenylation of resveratrol with prenyl bromide (3,3-dimethyl allyl bromide) and solids superbase catalyst γ-Al_2O_3/NaOH/Na was conducted in methanol solvents. The results of LC-MS analysis showed the presence of prenylated resveratrol with molecular weight 364 g/mol. This prenylated resveratrol was sustituted with 2 prenyl groups.

Keywords : Heterogeneous catalyst, prenylation, prenylated resveratrol, resveratrol, superbase catalyst

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INTRODUCTION

Polyphenols compounds such as flavonoid and stilbenes groups have been known to containis o prenoid group[7, 9, 11, 16]. Some research indicates that these prenylated polyphenols have significant bioactivities such as antioxidant [2,12], antibacterial[14,2], antitumor[3] and anticancer [10,20] activities. The addition of isoprenoid substituents at various skeleton of polyphenols significantly increased bioactivity compared to similar compounds that are not prenylated phenols[3, 10, 14, 20].

A wide range of important bioactivities of these prenylated polyphenols encourage research to obtain these compounds.Castanheiro et al., 2009 reported that they had synthesized theprenylated xanthon with a 6% yield by reaction between1-hydroxyxanthone and prenyl bromide using homogeneous basic catalyst K_2CO_3.This compoundexhibited inhibitory activity against human tumor cell lines MCF-7 (breast adenocarcinoma) [3]. Koolaji *et al*., 2013 had synthesized prenylated stilbenes (*E-1-[5-hydroxy-3-methoxy-2-(3-methyl-2-butenyl)phenyl]-2-[4-hydroxy-3-methoxyphenyl]ethene*)by using homogeneous basic catalyst NaH. This prenylated stilbenes exhibited inhibitory activity against K562 leukemic cancer cell line with IC_{50}=0,10µM [10].

The use of homogeneous catalysts in synthesis of natural products have some weakness, such as requiring further separation process in obtaining a pure product, the price is expensive, and cause environmental pollution because metals are not degraded in nature but rather accumulates.These condition can be overcome by using heterogeneous catalyst, because these catalysts havea different phase with the substrate so that it can be easily separated, environmentally friendly, and has activity and higher selectivity. Solids γ-Al_2O_3/NaOH/Na is heterogeneous superbase catalysts that had been used in synthesis of natural products [1, 4, 17, 19, 21].

Resveratrol (3,5,4'-trihydroxystilbene) as a polyphenols is classified into stilbenes group. The synthesis of prenylated resveratrol compound will be conducted by reaction between resveratrol (3,5,4'-trihydroxystilbene) and prenyl bromide (3,3-dimethyl allyl bromide) using heterogeneous superbase catalystsγ-Al_2O_3/NaOH/Na. This researchis expected to be an alternative method of synthesis of prenylated resveratrol compound that has a wide range of important bioactivities such as anti-bacterial and anti-cancer activities.

EXPERIMENTAL SECTION

Materials

Resveratrol (3,5,4'-*trihydroxystilbene*)(sigma), prenyl bromide (3,3-dimethyl allyl bromide), γ-Al_2O_3 (Merck), aquades, methanol, ethanol,sodium metal, NaOH (Merck) dan Nitrogen gas (99.9*% extra pure*).

Preparation ofSuperbase Catalystγ-Al_2O_3/NaOH/Na

Solidsgammaalumina(γ-Al_2O_3) wascalcined attemperatures550ºC.A total of10g ofγ-$Al_2O_3$was heatedandstirred at400°C and nitrogen atmosphere for 2hours. Furthermore, 1.75g of NaOHwas added and stirred with γ-Al_2O_3 until NaOHhave melted and formed homogeneous mixture.After the formation of white solids 0.5 g of sodium metal was added to the mixtures and stirring process continued for 1 hours.

XRD (X-Ray Diffraction) Analysis

Diffractometer Type: PANalytical PW 3040/60, Goniometer Radius [mm]240.00, Dist. Focus-Diverg. Slit [mm]91.00, Start Position [°2Th.]2.9047, End Position [°2Th.]69.9607, Step Size [°2Th.]0.0330, Scan Step Time [s]54.2693, Measurement Temperature [°C]22

Prenylation Reaction of Resveratrol by using Superbase Catalystγ-Al_2O_3/NaOH/Na

Prenylation Reaction was conducted at room temperature (28^0C) in methanol solvents. 2 mg resveratrol was reacted with 0.01 ml prenyl bromide (d = 1,29 g/ml; Mr = 149,029 g/mol) in 3 ml methanol solvents.

LC-MS Analysis

LC-MS analysis was performed using an Mariner Biospectrometry. LC: Hitachi L 6200, System ESI (Electrospray Ionisation), Positive Ion Mode, Kolom C18 (RP 18) Supelco, Column length: 250 mm, Vol injection 20 ul, Flow rate 1 ml/min,Eluent: Methanol +Water =95+ 5.

RESULTS AND DISCUSSION

Superbase Catalyst γ-Al_2O_3/NaOH/Na

The materials for preparation of superbase catalyst γ-Al_2O_3/NaOH/Na are gamma-alumina (γ-Al_2O_3), NaOH and sodium metal solids. Preparation of superbase catalyst was conducted by mixing sodium metals, hidroxide of alkali metals and gamma alumina solids at temperature that higher than melting points of alkali metals [1, 4, 21].

The mixing process of γ-$Al_2O_3$and NaOH at 400^0C will form β-sodium aluminate that has cationic vacancies. The sodium metal that was subsequently added to β-sodium aluminate would occupy the cationic vacancies and be ionized, introducing electrons to the oxygen atoms adjacent to the vacancies.The oxygen atoms with increased negative chargespossesthe electron-donating ability. The more oxygen atoms with increased negative charges will generate superbase sites onγ-Al_2O_3[4, 21].

Characteristics of the obtainsuperbase catalyst γ-Al_2O_3/NaOH/Na were determined by XRD (*X-Ray Diffraction*) analysis. The XRD analysis is a method of analysis for determining of the crystal structure of solid. The measurement by X-Ray diffractometerwasobtained thed-value (the distance of crystals plane) from the angle of 2θ and its intensity.

Difractogram of γ-Al_2O_3/NaOH/Na shows the appearance of several peaks that are not on difractogram of γ-Al_2O_3, i.e 2θ = 20,3362, 2θ = 30,2888, 2θ = 33,5951, 2θ = 34,3083, and 2θ = 34,9357.The structure of γ-$Al_2O_3$was no longer occured on superbase catalystγ-Al_2O_3/NaOH/Na that was caused by the addition of NaOH and sodium metal. The addition of sodium metal had caused the formation of superbase sites on γ-Al_2O_3.

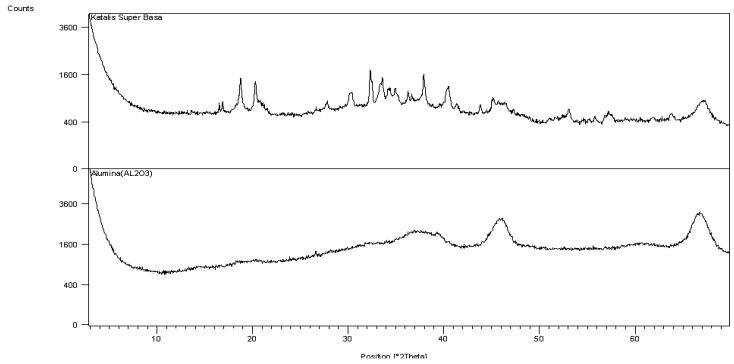


Figure1.Difractogramof Superbase Catalyst γ-Al_2O_3/NaOH/Naand γ-Al_2O_3

For determining whether the obtained catalyst is the superbase catalyst γ-Al_2O_3/NaOH/Na, the difractogram of the obtained superbase catalyst was compared to the difractogram of superbase catalyst that was prepared by Nagase et al., 1974 [1]. These dataare presentedin the following table:

Table1.The Data of XRD analysis of Superbase Catalystγ-Al_2O_3/NaOH/Na

Peak	Nagase et al., 1974			The obtained superbase catalyst		
	2θ (deg)	d (Å)	Intensity (%)	2θ (deg)	d (Å)	Intensity (%)
1	21,0	4,159	28,57	20.3362	4.36701	78.22
2	30,0	2,918	85,71	30.2888	2.95093	35.99
3	33,2	2,683	71,43	33.5951	2.66768	74.73
4	34,3	2,613	100,0	34.3083	2.61385	39.74
5	35,0	2,574	100,0	34.9357	2.56833	42.86
6	38,4	2,350	21,43	37.9307	2.37214	86.80
7	47,0	1,979	21,43	46.2957	1.96115	27.20
8	56,9	1,584	28,57	57.3577	1.60646	14.84
9	61,7	1,498	21,43	63.8683	1.45750	12.03
10	68,5	1,359	92,86	67.2032	1.39190	41.62

The obtained superbase catalyst had 2θ positions and absorbance values that were almost the same with the superbase catalyst that was syhthesized by Nagase et al.,1974, so it can be concluded that the obtained catalyst is a superbase catalystγ -Al_2O_3/NaOH/Na [1].

Prenylation Reaction of Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenol compound that classified into stilbenes group. There are two geometric isomers *cis* and *trans*-resveratrol. The biologically active compound is *trans*-resveratrol[9, 13, 15].

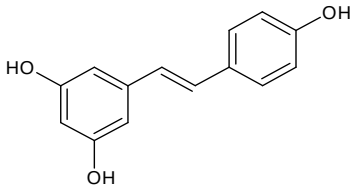


Figure 2. Structure Of *trans*-Resveratrol (3,5,4'-*trans-trihidroxystilbene*)

Prenylation reaction is the addition of prenylgroup (dimethyl allyl) on C- and or O- atoms in an organic compound. Substitution of prenyl group was occured on C- and O- atoms in synthesis of prenylated phenolic compounds [3, 20]. Koolaji et al.,2013 reported that they had synthesized prenylated C- and O-piceatannol((E)-3,3',4',5-tetrahydroxystilbene) by using homogeneous base catalyst NaH [10].

Prenylation could be occured on C- (aromatic ring) or O- atoms (-OH group) of resveratrol. Prenylation reaction was conducted on polyphenol compounds by using homogeneous base catalyst [3, 10, 20]. In this research, prenylation reaction was conducted by using heterogeneous superbase catalystγ-Al_2O_3/NaOH/Na and 3,3-dimethyl allyl bromide (prenyl bromide) as a source of prenyl group.

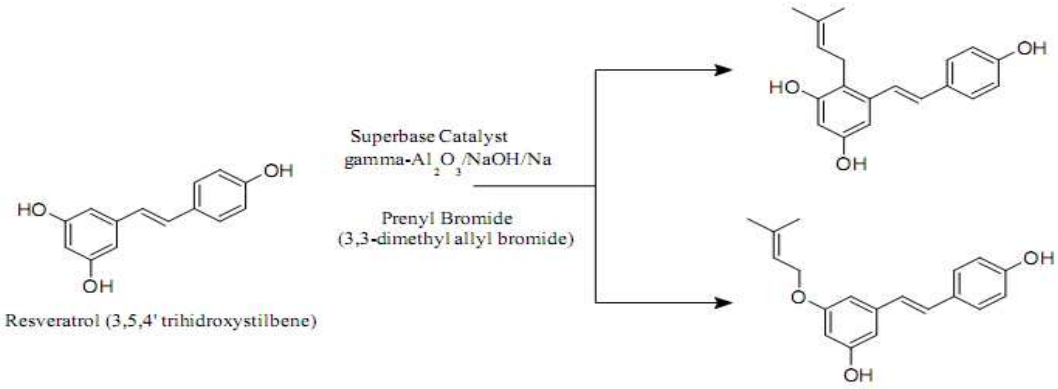
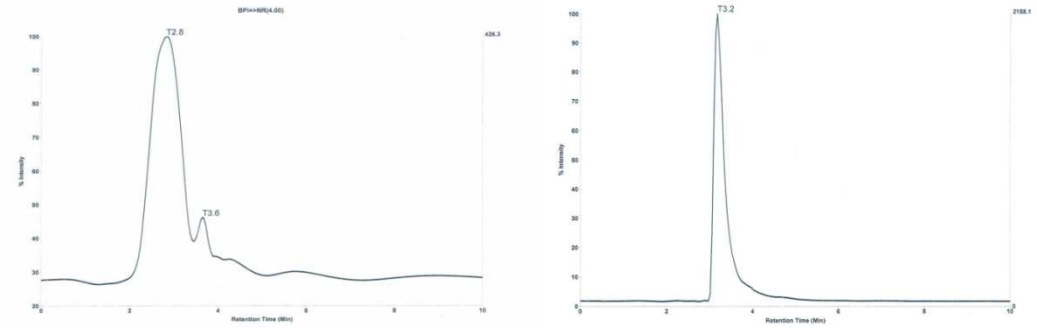


Figure 3. Prenylation Reaction Of Resveratrol

Prenylation reaction mechanism of resveratrol was occured through proton abstraction on C- atom (aromatic ring) by superbase sites of heterogeneous catalyst γ-Al_2O_3/NaOH/Na to form carbanion which act as a nucleofile for prenyl bromide in SN2 reaction. Prenylation reaction could be also occured through abstraction process on -OH phenolic that has acidic character and then substitution of prenyl group on resveratrol structure to form the prenylated resveratrol. Prenylation Reaction of resveratrol is shown by the figure 3.

Figure 4 shows separation of the product reaction and resveratrol in liquid chromatography coloumn (LC) based on retention time.



(a) **(b)**

Figure4.LC-Chromatogram Of The Product Reaction (a) And Resveratrol (b)

Based on theLCchromatogramin Figure4a, it appears there aretwodominantpeaks, namely theretention timeof 2.8, and3.6. Infigure 4athere is no longervisiblepeakwitha retention timeof 3.2whichis the peakofresveratrol. Its shows that resveratrol as a starting material in prenylation reaction has totally changed into product reaction.

Peak with a retention time of 3.6 with an explanation by electron spray mass spectra as shown in Figure5is known to hasa molecular weight of364g/mol.

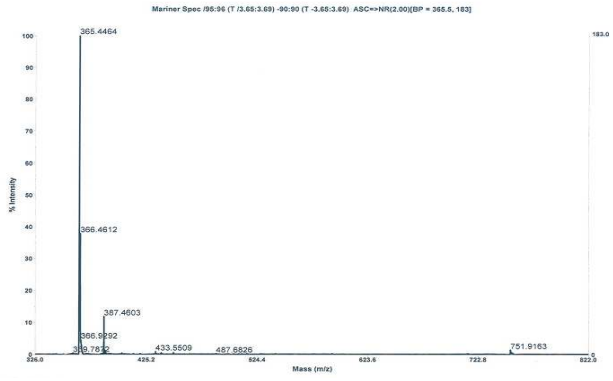


Figure5. ESI Mass Spectrum for Peak With Retention Time 3.6

According to LC-MS analysis using standard resveratrol as shown in Figures 4 and 5, it can be concluded that resveratrol which was used as starting material had completely reacted and the product reaction with the retention time of 3.6 and molecular weight of 364 g/molpredicted to be the product reaction prenylated resveratrol. Based on the molecular weight of the obtained prenylated resveratrol, could be predicted that the substitution of prenyl group had occured on C- (aromatic ring) or O- (-OH phenolic) atoms on resveratrol. Prenyl group has a molecular relative weight of 69.125 g/mol, if the prenylated resveratrol was formed therefore its molecular relative weight would be added by prenyl's group. The obtained product reaction showed that there were 2 prenyl groups on resveratrol structure.

CONCLUSION

The obtained superbase catalyst γ-Al_2O_3/NaOH/Na had different character with γ-Al_2O_3 and resembled character of the superbase catalyst of Nagase et al 1974. The superbase catalyst γ-Al_2O_3/NaOH/Na had been succesfully used for synthesis of prenylated resveratrol. The results of LC-MS analysis showed the presence of prenylated resveratrol with molecular weight 364 g/mol. The prenylated resveratrol is resveratrolswhich is sustituted with 2 prenyl groups.

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