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Synthesis and Antioxidant Assay of C-2-Ethoxyphenylcalix[4]resorcinarene

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Abstract : C-2-ethoxyphenylcalix[4]resorcinarene (CEFKR) can be synthesized in 2 steps; i.e. ethylation of 2-hydroxybenzaldehyde with diethyl sulphate (DES) and condensation of 2-ethoxybenzaldehyde and resorcinol with acid catalyst. The product 2-ethoxybenzaldehyde was analyzed with infra red (FT-IR) spectrophotometer and GC-MS. The product 2-ethoxy-phenylcalix[4]resorcinarene was analyzed with FT-IR, ^{1}H NMR and ^{13}C NMR. The product of condensation was subjected to antioxidant assays using DPPH(1-1-diphenyl-2-picrylhydrazyl) method.
Reaction of 2-hidroxybenzaldehyde, DES, and NaOH was performed by refluxing the mixture for 2.5 hours and gave 2-ethoxycalix[4]resorcinarene in viscous dark yellow 84.68 % yield. The aromatic electrophilic substitution-cyclization of ethylation product and resorcinol in presence of HCl gave C-2-ethoxyphenylcalix[4]resorcinarena as yellow solid in 99.26 % yield with m.p > 380 ^{o}C. It has strong antioxidant activity in DPPH methods with ES_{50} 83.62 ppm.
Keywords : synthesis, antioxidant assay, 2-ethoxyphenylcalix[4]resorcinarene.

Introduction

Excessive sun exposure may cause the process of aging. This will causing formation of free radicals that will cause autoxidation reactions in the body, especially in the lipid layer of the cell membrane[1]. Free radicals are known to have high activities to trigger a chain reaction in the cell. Autooxidation process will continue to produce hydroperoxides which may react further to form aldehydes, ketones, alcohols and hydrocarbons[2,3]. The free radical activity can be inhibited by the action of antioxidant[4,5].

Antioxidants or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc[6-8]. Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders[9].

Studies of antioxidant activity increased because has the potential to prevent various chronic diseases[10]. Many studies showed a relation between increasing of oxidative cell damage due to an imbalance between free

radicals and natural antioxidants in the body, so that it becomes a major factor of many diseases such as cardiovascular, cancer, aging, and others[11].

The antioxidants might be prepare from compounds having phenolic group as active materials. Calixarenes, groups of synthetic oligomer compounds containing an aromatic ring in a cyclic series connected by methylene or methyne bridges are suitable for this purpose. The structure of calix[4] resorcinarene allows to be modified to have properties as antiradical, antioxidant as well as sunscreen agents. Hasbullah[12] reported *p*-ethoxyphenylcalix[4]resorcinarene as an antioxidant with free radicals reduction of 67.30%. Antioxidants and antiradical activities of resorcinarene tetranitroxide are found 100 times more effective than resorcinol in reducing free radicals[13]. Synthesis of 2-ethoxyphenylcalix[4]resorcinarene (CEFKR) and its antioxidant activities will be reported in this study. The aim of the research was to synthesis and to evaluate the antioxidant activity of CEFKR.

Research Methods

Materials

Chemicals were salicylaldehyde, diethyl sulphate (DES), resorcinol, hydrochloric acid (HCl 37%), sulphuric acid (H_2SO_4), sodium hydroxide (NaOH), acetone, dichloromethane, n-hexane, ethanol, aquadest, methanol, DMSO, and DPPH ((1,1-diphenyl-2-picrilhidrazyl). All chemicals except aquadest were purchased from E. Merck. Aquadest and aquabidest were obtained from Laboratory of General Chemistry, UGM.

Equipments

Equipments were laboratory glassware, Büchner funnel, Buchi evaporator R-124, melting-point apparatus (Electrothermal 9100), Camac UV-Cabinet II,analytical mass balance (Mettler AT200), infra red spectrophotometer (IR, Shimadzu-Prestige 21), proton nuclear magnetic resonance spectrometer (^{1}H-NMR, JEOL JNM-MY60 and JEOL MY-500 MHz), carbon nuclear magnetic resonance spectrometer (^{13}C-NMR, JEOL MY-500 MHz), gas chromatography-mass spectrometer (GC-MS, Shimadzu QP-20105), and UV-Vis spectrophotometer (Type 722).

Experiments

Synthesis of 2-ethoxybenzaldehyde

Diethyl sulphate (10.7 mL, 0.08 mol) was added dropwise into 250 mL of three-necked-flask containing mixture of 5.0 g (0.04 mol) of salicylaldehyde, 3.2 g (0.08 mol) of sodium hydroxide and 50 mL of aquadest. The mixture was then refluxed at 93^o C for 2.5 hours and monitored by TLC (dichloromethane : n-hexane = 2:3) and the cool reaction mixture was extracted with dichloromethane 50 mL for three times. The combined organic layer was washed with NaOH 10 % (3 X 50 mL) continued washed with aquadest, dried with Na_2SO_4 anhidrous, and evaporated. The product was obtained as a viscous dark yellow. IR (KBr pellet) wave number in cm^{-1}: 1689 (C=O), 2862 and 2931 (Csp^2-H aldehida), 1242 and 1041 (C-O-C eter), and 1597 and 1458 (C=C aromatic). GC-MS in purity 97.34%.

Synthesis of C-2-ethoxyphenylcalix[4]resorcinarene

Into 100 mL three-necked flask equipped with water condenser, 1.1 g (10 mmol) of resorcinol and 1.50 g (10 mmol) of 2-ethoxybenzaldehyde were dissolved in 100 mL of ethanol. Then, concentrated hydrochloric acid (1 mL) was added into the solution. The mixture was refluxed for 24 hours and monitored by TLC (acetone:hexane = 7:1) and allowed to cool to room temperature. The solid was collected by vacuum filtration with Büchner funnel, washed using the mixture of water and ethanol (1:1) and dried. The C-ethoxyphenylcalix[4]resorcinarene was obtained orange solid in 99.26 % yield with m.p. > 380 ^{o}C. IR (KBr pellet) wave number in cm^{-1}: 3448.72 (-OH), 1620.21 and1489.05 (C=C aromatic), 1435.04 (C-H bridge and methylen), 2931.80 (Csp^3-H), 1426 and 1080 (C-OC). ^{1}H NMR (DMSO) δ in ppm from TMS: 0.9632 ($C\underline{H}_3$-CH_2-O-), 3.6715 (CH_3-$C\underline{H}_2$-O-), 5.8688 and 5.9453 (H bridge), 6.0310 – 6.9143 (Ar-H), and 8.1689 – 8.3126 (-OH). ^{13}C NMR (DMSO) δ in ppm from TMS: 14.7085 and 14.7371 ($\underline{C}H_3$), 34.4909 and 35.1109 (-C-H bridge), 63.5923 and 63.6782 (-O$\underline{C}H_2$), and 101.3641 ppm – 156.0473 (Ar-C).

Antioxidant test

A stock solution of DPPH was prepared by dissolving 0.4 mg of DPPH in 1L methanol and the solution was kept in the dark at 4 ^{o}C[14]. A stock solution of the C-2-methoxyphenylcalix[4]resorcinarene was prepared at 12.5, 25, 50, 100, and 200 ppm in DMSO respectively. Five hundred µL from the stock solution of the compound was added to the 2 mL of DPPH 0,05 mM. The mixture was shaken well and kept in dark at room temperature for 2 hour. The absorbance of the mixture was measured at 517 nm by using spectrophotometer. The percent inhibition of radical scavenging ability was calculated as[15-20]:

$$\% \text{ inhibition} = \frac{(A\ DPPH - A\ sample)}{A\ DPPH} \times 100\ \% \qquad \text{Equation 1}$$

Linear regresion regresion y = ax + b made concentration as absis (x axis) and % inhibition as ordinat y. The 50% inhibition (ES_{50}) of antioxidant activity was calculated as the concentrations of samples that inhibited 50% of scavenging activity of DPPH radicals activity under these conditions[21].

Nihlati et al.[22] suggested a standard activities antioxidant of degree a group of compound based activites values of that: - ES_{50}< 50 µg/mL = very strong

- ES_{50} 50- 100 µg/mL = strong
- ES_{50} 101- 150 µg/mL = moderate
- ES_{50}> 150 µg/mL = weak

Results and Discussion

1. Ethylation of 2-hidroxybenzaldehyde

Ethylation of 2-hydroxybenzaldehyde can reacted salicylaldehyde and diethyl sulphate (DES) with in presence of base of the NaOH in aquadest. Phenol compound reacted strong base to produce the phenoxide ions. Acidity phenol caused stability of phenoxide ion with presence resonance. Phenoxyde ion is characteristic as strong nucleophile and attack the alkylating agent such as reaction with DES in heat via nucleophile substitution reaction. Reaction was conducted in water solvent under reflux condition 2.5 hours. The product was obtained as viscous dark yellow in weigth 5.35 g (84.68% yielded). The IR spectrum showed strong absorption at 1242 and 1041 cm^{-1} as asymetric and symetric stretching vibration C-O-C and displayed absorption O-H at 3400 cm^{-1} .

Analysis with GC-MS yielded chromatogram and mass spectra which were presented in Figure 1 and Figure 2. Chromatogram on Figure 2 showed one maximum peak at retention time (T_r) of 15.8 minute with 97.34 % purity. The mass spectrum (Fig. 1) gave molecular ion at m/z 150 indicating the molecular mass of 2-ethoxybenzaldehyde. Based on FT IR, and GC-MS analyses, it can be concluded that ethylation of 2-hydroxybenzaldehyde to produce 2-ethoxybenzaldehyde in 84.68% yield and the product is viscous dark yellow. Mechanism reaction of 2-ethoxy benzaldehida shown in Figure 3.

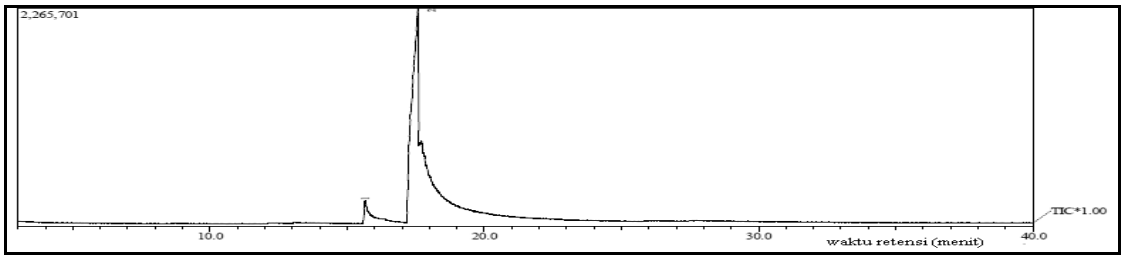


Figure 1. GC-Chromatogram of the ethylation product

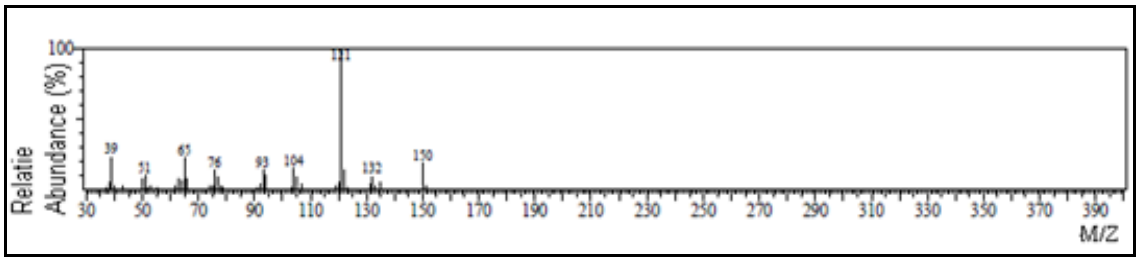


Figure 2. Mass spectrum of 2-ethoxybenzaldehyde

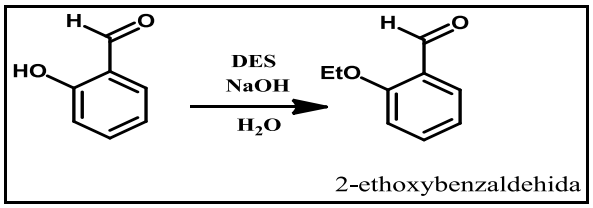


Figure 3. Mechanism reaction of 2-ethoxylbenzaldehida

2. Synthesis of C-2-ethoxyphenylcalix[4]resorcinarene

The 2-ethoxybenzaldehyde is a benzaldehyde derivative and can be condens with resorcinol as raw materials in the synthesized of calix[4]resorcinarene. In order to obtain it, 2-ethoxybenzaldehyde and resorcinol (1:1) was refluxed in ethanol in the presence of hydrochloric acid catalyst. The reaction was carried out for 24 hours. The reaction gave orange solid in 99.26 % yield with m.p > 380 ^{o}C. The compound is insoluble in water, ethanol and good soluble in DMSO. The solubility of limited in organic solvent and high of melting point are caused the more hydroxyl groups and phenyl group in C-phenylcalix[4]recorcinarene molecule.

The FT IR spectrum of calix showed characteristics absorption at 3425 cm^{-1} indicating the presence of hydroxyl (-OH) group. The most important evidence indicated that the reaction had taken place was the disappearance of strong aldehyde carbonyl absorption of the reactant.

^{1}H NMR spectrum of the synthesized product (Figure 4) showed that there were 4 proton groups with different chemical environment. They were ethoxy, methyne, aromatic and hydroxyl groups. First group (A) consisted of signal 0.9632 ppm (triplet, 12 H) expressed proton methyl ($C\underline{H_3}$-CH_2-O) and δ 3,6715 ppm (*kuartet*, 8 H) proton CH3-$C\underline{H_2}$-O expressed the protons resonance of ethoxy group Second group (B) consisted of signal singlet and doublet at 5.8688 and 5.9453 ppm with total integration of 4 protons belonged to proton of methyne bridge. The product condensation reaction shown a mixture more conformer calyx[4]resorcinarene in cone, partial cone or the other[23]. Signal B with ratio of integration δ 5.9453 ppm and 5.8688 ppm 1.38 : 1.654 are shown conformer crown (C_{2v}) and partial cone (C_{4v})with comparation:1:1. Moreover, signal multiplet (C, 24 H, δ 6.0310 ppm – 6.9143 ppm) came from the resonance of aryl protons with the detail 16 protons from residue of 2-ethoxybenzaldehyde and 8 protons residue of resorcinol. The last signals (D, 8 H, 8.1689 ppm, 8.1984 ppm, 8.2542 ppm and 8.3126 ppm, quartet singlet) represented 8 hydroxyl protons [3].

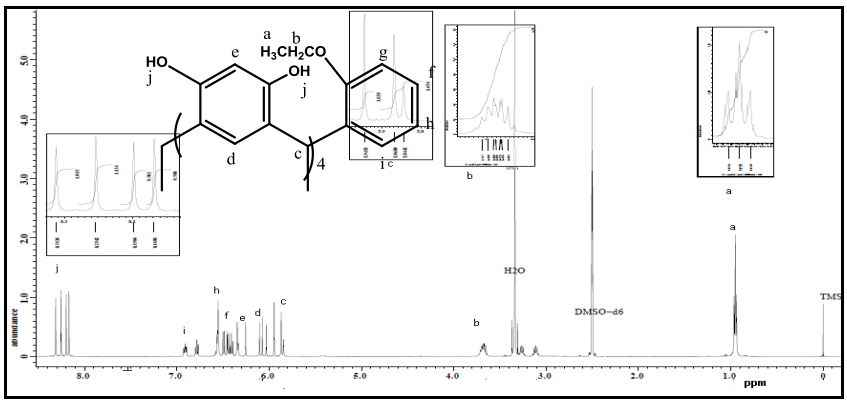


Figure 4.^{1}H-NMR spectrum of the condensation between 2-ethoxybenzaldehyde and resorcinol

Several peaks of the ^{13}C NMR spectrum (Figure 5) is represented the carbons consist of the product. Spectrum ^{13}C NMR analysis was also conducted to support the previous analyses. Peaks at 14.7085 ppm dan 14.7371 ppm indicated to carbon CH_3 with 2 different characteristic of carbon. Peaks at 34.4909 ppm and 35.1109 ppm indicated methylen bridge carbons with 2 different characteristic of conformer in compound. Peaks at63,5923 ppm dan 63.6782 shown carbon in $-CH_2-O$. Peaks at region 101,3641 ppm - 156,0473 ppm indicated the presence of aromatic carbons (12 C). The reaction condensation and cyclization between 2-ethoxybenzaldehyde and resorcinol yielded 2-ethoxyphenylcalix[4]resorcinarene. The mechanism reaction is presented in Figure 6 [3].

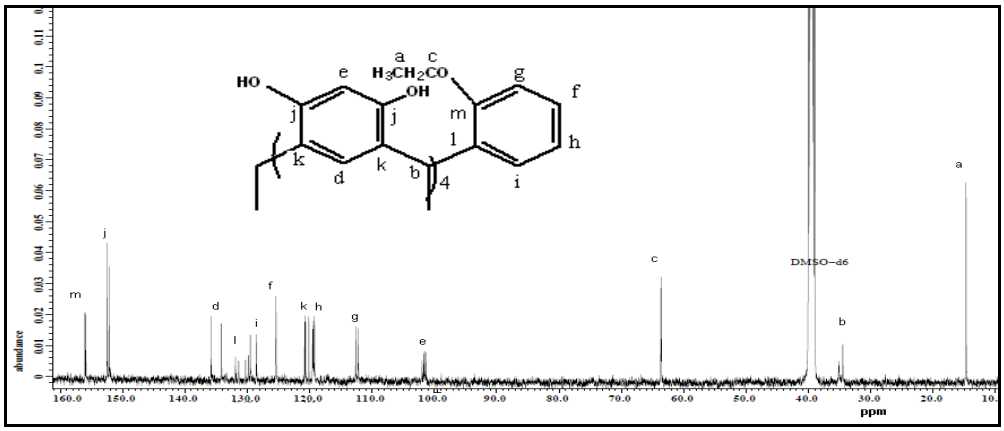
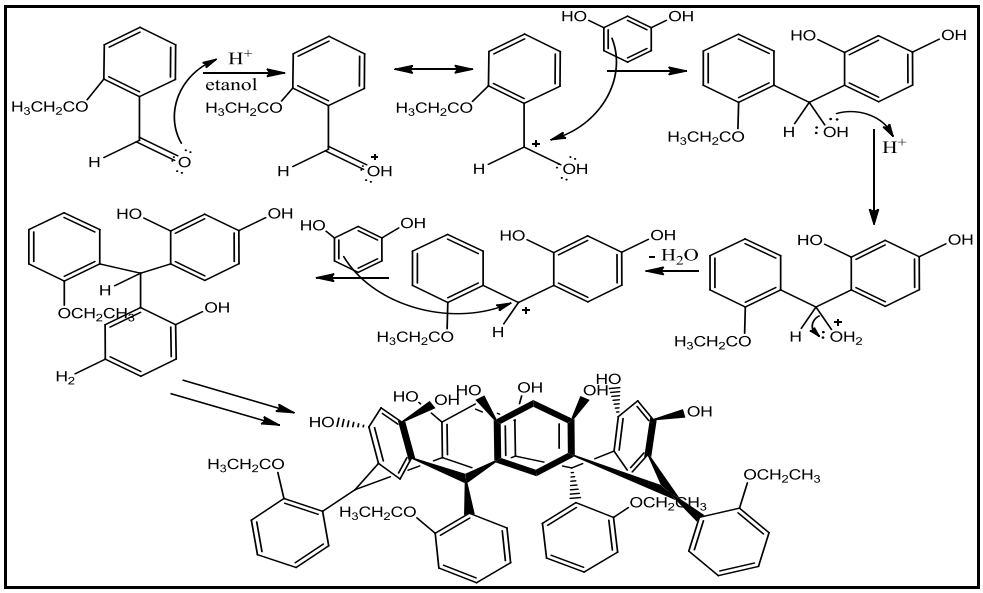


Figure 5.^{13}C NMR spectrum product of cyclisation 2-ethoxyphenylcalix[4]resorcinarene



2-ethoxyphenylcaliks[4]resorcinarene
$C_{2v} : C_{4v} = 1 : 1$

Figure 6. Mechanism reaction of cyclisation 2-ethoxyphenylcalix[4]resorcinarene

3. Antioxidant assays of product cyclisation with DPPH methods

Antioxidant property of C-2-ethoxyphenylcalix[4]resorcinarene was calculated with decrease in absorbance 1,1-diphenyl-2-picrylhydrazyl (DPPH). Calix[4]resorcinarene can donated proton to the non radical from DPPH is highly antioxidant agent. Percent inhibition can calculated as Equation 1.

The electron scavenging 50 (ES_{50}) was calculated with intrapolation concentration (axis x) versus % inhibition (axis y) curve (Figure 7) represent 83.62 ppm. The 2-ethoxyphenylcalix[4]resorcinarene has a strong antioxidant property, because it can stabilize the radical DPPH to reaction with the hydroxy phenol groups produce in molecule stabilization.

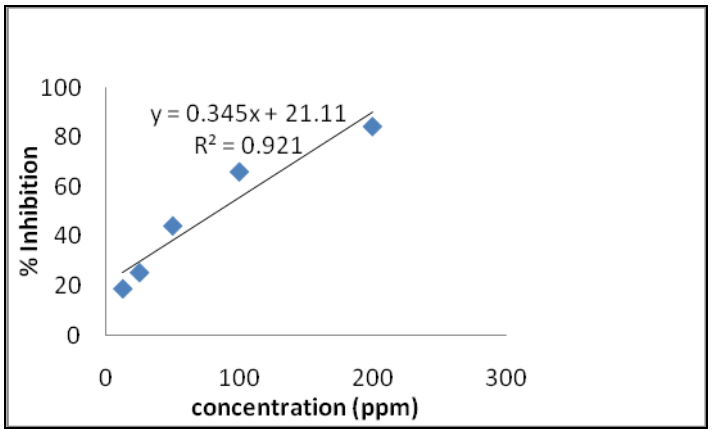


Figure 7. Curve concentration of 2-ethoxyphenylcalix[4]resorcinarene versus % inhibition

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