International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.08 pp 594-599, 2016

Synthesis and antioxidant properties of C–4–allyloxy–phenylcalix[4]resorcinarene

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Abstract : The synthesis of C–4–allyloxy–phenylcalix[4]resorcinarene (AOPC) hasbeen conducted through the following steps, i.e. 1) allylation reaction of 4–hydroxy–benzaldehyde to give 4–allyloxy–benzaldehyde, and 2) synthesis of AOPC via condensation of 4–allyloxy–benzaldehyde with an acid catalyst. The synthesized products were characterized using FTIR, ^{1}H–NMR, and LC–MS spectrometer. The product of 4–allyloxy–benzaldehyde compound was obtained in light yellow liquid with 85% in yield. Meanwhile, the AOPC was attained in dark red solid with 67% in yield and m.p. 237–238 °C (decomposed). The antioxidant activity assays of AOCP was conducted by 1,1–diphenyl–2–picrylhydrazil (DPPH) methods with quercetin as a control. Antioxidantassay of AOPC and quercetin showed ES_{50} 12.46 and 34.90 respectively. This result showed that AOPC compound has higher antioxidant activity than quercetin and categorized as a strong antioxidant.
Keywords:C–4–allyloxyphenylcalix[4]resorcinarene; synthesis; antioxidant; DPPH; quercetin.

Introduction

Free radicals are molecular species with unpaired electrons[1] and highly reactive to the cells of the body[2]. Free radicals have the ability to oxidize of macromolecules such as DNA, proteins, and lipids[3]. In normal cells, there is a balance amount of free radicals and antioxidants[4]. The excess of free radical could trigger oxidative stress in the body, which can lead to various diseases such as cancer, arthritis, ageing,cardiovascular, inflammatory, diabetes and Alzheimer[5–6]. The free radical activity could be inhibited by addition of antioxidants that has the ability to capture free radicals to produce stable species or neutral molecules.

Phenolic compounds, such as resorcinol derivatives, are reported as an antioxidant agent which acts as a free radical scavenger. Calix[n]resorcinarene is one of the calix[n]arenederivative from resorcinol which have antioxidant properties. Calix[n]resorcinarene with 4 resorcinol group is called calix[4]resorcinarene which could be synthesized by condensation reaction of resorcinol and aldehyde (aliphatic or aromatic) with acid catalyst[7–9]. Some studies showed the synthesis of calix[4]resorcinarene derivatives, such as C–methoxyphenylcalix[4]resorcinarene, C–4–hydroxy–3–methoxy–phenylcalix[4]–resorcinarene and also tetrakis–thiomethyl–C–4–methoxyphenyl–calix[4]resorcinarene[10–12].

Synthesis of calix[4]arene based on 1,3,4–oxadiazole and thiadiazole derivatives have been done, which1,3,4–oxadiazole and 1,3,4–thiadiazole derivatives have been coupled with 5,11,17,23–tetra–tert–butyl–25,27–bis(chlorocarbonyl–methoxy)–26,28–dihydroxy–calix[4]arene. The results showed that all of the final scaffolds have been subjected to have antioxidant activity[13]. Furthermore, C–2–hydroxyphenyl–calix[4] resorcinarene and C–*p*–methoxy–phenylcalix[4]resorcinarene compounds have been synthesized and also showed to have antioxidant activity[14–15]. Meanwhile, other studies mentioned the used of calix[4]resorcinarene other than antioxidant i.e. an adsorbent of heavy metal, membrane, and also applied in electrophoresis, extraction, and chemical sensing process[10,16].

This study aims to synthesized of C–4–allyloxy–phenylcalix[4]resorcinarene (AOPC) compound and determined the antioxidant activity. The synthesis of AOPC was initiated with 1) allylation reaction of 4–hydroxybenzaldehyde to gave 4–allyloxybenzaldehyde, and followed with 2) condensation reaction of 4–allyloxybenzaldehyde and resorcinol with an acid catalyst to obtained AOPC [see Fig.1]. The antioxidant activity was tested by DPPH methods.

1. Experimental

2.1 Materials

All Chemical used were purchased from *Sigma–Aldrich and Merck*, i.e. 4–hydroxybenzaldehyde, sodium metals, resorcinol, ethanol, dichloromethane, methanol, dimethyl sulfoxide, chloride acid, sodium sulfate anhydrous, DPPH (1,1–diphenyl–2–picrylhidrazyl), and quercetin.

2.2 Instrumentation

Equipment used in this research were, Buchi evaporator R–124, melting point apparatus (Electrothermal 9100), analytical mass balance (Mettler AT200) and Camac UV–Cabinet II. Characterization of the synthesizedcompoundswas using IR spectrometer (Shimadzu Prestige–21 FTIR spectrometer), ^{1}HNMR spectra were recorded on a JEOL JNM ECA 500 MHz spectrometer, LC–MS spectrometer (Mariner Biospectrometry, ESI). The antioxidants activity assay was investigated using UV–Vis (Shimadzu UV–1800 UV–spectrophotometer).

2.3 Synthesisof 4–allyloxybenzaldehyde

A sodium metal (0.38 g; 16.5 mmol) and 10 mL of ethanol were added into a three–necked flask equipped with a reflux condenser. The mixture was stirred until homogeneous. The amount of 4–hidroxybenzaldehyde(1.00 g; 8.2 mmol) were added to the mixture and stirred at± 40 °C for 30 minutes. Allylbromide (2.98 g; 24.6 mmol) were added slowly into the mixture then refluxed for 24 h. The resulting mixture was allowed to cool down and ethanol was evaporated under vacuum. A residue was diluted with aquadest then added NaOH 0.1 M andextracted with dichloromethane. The organic layer was separated and rinsed with aquadest then dried with added sodium sulfateanhydrous. A sodium sulfatewas removedfrom the solvent and the filtrate was evaporated.The resulting product was characterized using FTIR, ^{1}HNMR, and GC–MS.

2.4 Synthesis of AOPC

Resorcinol (0.50 g; 4.54 mmol), 4–allyloxybenzaldehyde (0.74 g; 4.54 mmol), ethanol (10 mL) and concentrated hydrochloric acid (0.5 mL) were added into a three–necked flask equipped with a reflux condenser. The mixture was refluxed for 24 h. The precipitate formed was filtered, neutralized with ethanol–aquadest(ratio 1:1)and then driedfor further analysis. The product was characterized using of FTIR, ^{1}HNMR, and LC–MS.

2.5 Antioxidant test

A solution of 0.05 mM of DPPH was prepared by dissolving 1.97 mg of DPPH in 100 mL methanol and stored in the dark place at 4 °C[17]. A solution of AOPC was prepared at various concentration i.e. 6.25; 12.5; 25; 50; 100 ppm in DMSO. AOPC solution(500 µL) was added into 2 ml of DPPH 0.05 mM respectively. The mixture was shaken well and stored in a darkplace at room temperature. After 45 minutes, the absorbance (A)

of the mixture was measured at 515.8 nm using UV spectrophotometer and compared with the corresponding absorbance of quercetinstandard.Formula **(1)**was used for measuring the radical scavenging activity(RSA) or electron scavenging (ES) of the sample against the stable radical of DPPH in percent (%).

$$ES = \frac{(A_{DPPH} - A_{sampel})}{A_{DPPH}} \times 100\% \qquad (1)$$

Linear regression equation, y = mx + b, was determinedfrom ES data, where variable x is described as concentration and ordinate as ES. ES_{50}value indicates the concentration of the compounds with 50% of radical scavenging activity. ES_{50} is inversely related to the antioxidant activity, which the smaller of ES_{50}value would give a greater/better antioxidant activity.

2. Result and Discussion

3.1 Synthesis of 4–allyloxybenzaldehyde

Synthesis of4–allyloxybenzaldehyde is involved allylation reaction of benzaldehyde which called Williamson synthesis from 4–hydroxybenzaldehyde [see Fig.1]. Synthesis of 4–allyloxybenzaldehyde was carried out by reaction of 4–hydroxybenzaldehyde (1 equivalent) withallyl bromide (3 equivalent) in ethanol and sodium metal at 78 °C for 24 hours. According to the work conducted, 4–allyloxybenzaldehyde compound was obtained in light yellow liquid with 89% in yield.

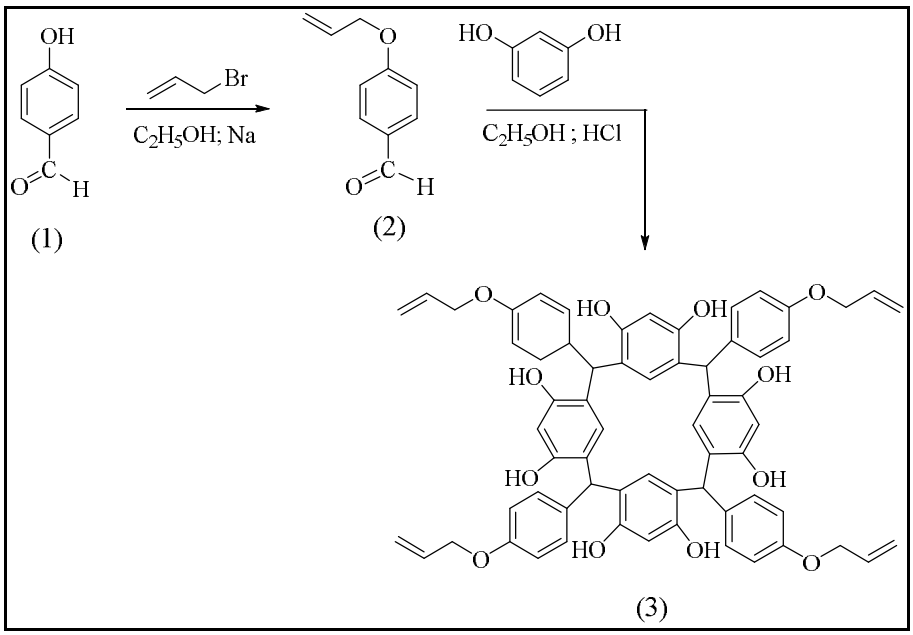

Fig. 1 Synthesis of AOPC 4–hydroxybenzaldehyde; (2) 4–allyloxybenzaldehyde; (3) AOPC

Based on the FTIR spectrum (KBr), wavenumber(cm^{-1}) at 1512–1669 indicated the existence of an allyloxy group (C=C aliphatic). Meanwhile, at 3076 cm^{-1} showed as C_{sp}^{2}–H group (vibration), 2831–2924 cm^{-1} as C_{sp}^{3}–H (vibration); 1425 cm^{-1}as $=CH_2$(vibration). Therefore, an asymm. C–O–C stretch showed at 1226 and 1002 cm^{-1} respectively.

The ^{1}HNMR spectrum of 4–allyloxybenzaldehyde (in DMSO) with TMS as an internal standard showed that signals at chemical shift (δ) 5.3 ppm refers to the terminal proton resonance of allyloxy ($=CH_2$) group. Meanwhile, the signals at δ = 9.8 ppm exhibited as proton resonance of carbonyl group (HC=O) and the existence of the benzene proton (–CH) is shown at δ = 7.0 ppm. The MS spectrum showed the appearance of a molecular ion peak at m/z = 162 [M^{+}] which is equal to the molecular mass of 4–allyloxybenzaldehyde.

3.2 Synthesis of AOPC

Synthesis of AOPC was carried out by refluxing a mixture of 4–allyloxybenzaldehyde with resorcinol (ratio 1:1) in ethanol and chloride acid as a catalyst for 24 hours as seen in Fig.1. AOPC compound was obtained as dark red solid with m.p. 237–238 °C (decomposed) in 67% yield.

FTIR spectrum (KBr, cm^{-1}) of AOPC showed absorption band at 3425 cm^{-1} from hydroxyl (–OH) group, 1612 as aromatic C=C, 1427 as C–H bridge and methylene. Meanwhile, the absorption band at 2924 cm^{-1} indicated the C_{sp}^3–H and 1086 cm^{-1} from C–OC of allyloxy group. The ^{1}HNMR spectrum (in DMSO) showed that signals at chemical shift (δ) 8.5 ppm belong to a hydroxyl group (O–H), 6.1–6.6 ppm as Ar–H, 5.2–5.6 ppm was from proton of –CH, and 4.4 ppm from CH_2proton. The LC–MS spectrum showed a fragmentationon molecular ion (m/z) 1016at retention time 3.26 minutes which is similar to the AOPC molecular weight.

3.3 AOPC Antioxidant Activity Assays

Antioxidant activity of AOPC compound was tested by DPPH method with quercetin as the positive control. The antioxidant properties of AOPC were calculated from the decreasing absorbance of DPPH from UV spectrometer measurement. In this method, DPPH is a radical nitrogen purple source. Rationally, the presence of antioxidantscould cause a diminishingof the intensity of the purple color to yellow[6,18].

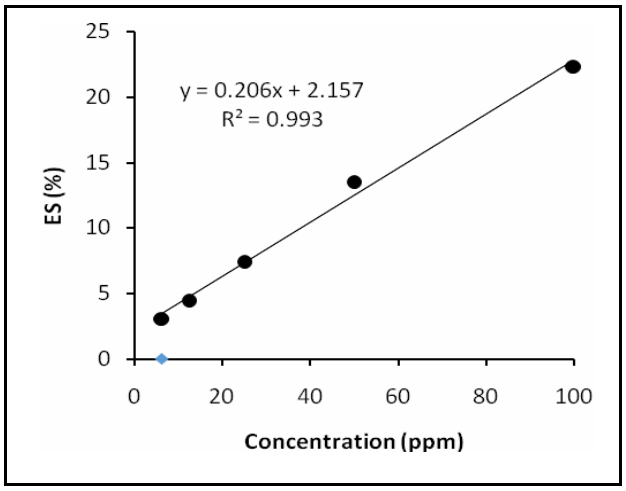

Fig. 2.Regression line for Percent Electron Scavenging (ES(%)) vs concentration of AOPC

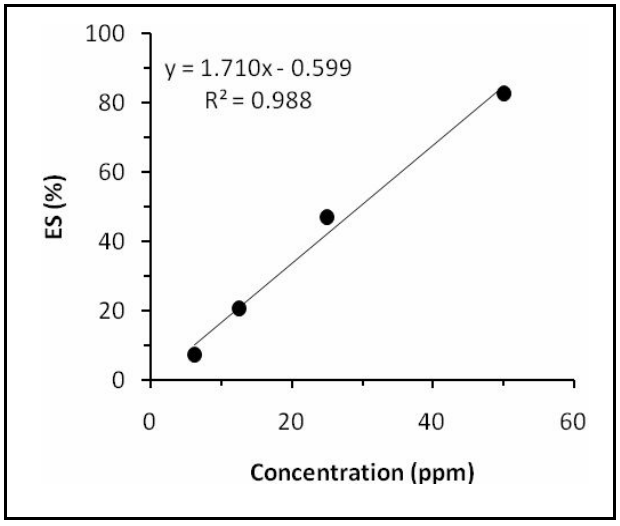

Fig. 3 Regression line for Percent Electron Scavenging (ES(%)) vs concentration of quercetin

Table 1. Concentration of AOPC versus ES

Concentration (ppm)	Absorbance	ES	Y = mx + c	ES_{50} (ppm)
6.25	0.399	3.07	Y = 0.206x + 2.157	12.46
12.5	0.394	4.45	R^2 = 0.993	
25	0.381	7.44		
50	0.356	13.51		
100	0.320	22.33		

Table 2. Concentration of quercetin versus ES

Concentration (ppm)	Absorbance	ES	Y = mx + c	ES_{50} (ppm)
6.25	0.262	7.30	Y = 1.710x – 0.599	34.90
12.5	0.224	20.73	R^2 = 0.988	
25	0.150	47.11		
50	0.048	82.80		

Comparison of the absorbance measurement of AOPC and quercetin listed in Table 1 and Table 2. From these data, we could calculate the ES value using ***formula1)*** to generate the curves between concentrations versus ES and determine the linear regression equation to calculate the ES_{50} values. Antioxidant assay of AOPC showed ES_{50}value 12.46 and quercetin as controls showed ES_{50}34.90. Jun et al.[19]suggested a standard antioxidant activity of compound based of ES_{50}[19]. If $ES_{50} < 50$ then the compounds is categorized as a very strong antioxidant. This study revealed that AOPCcompound has a strong antioxidant activity based on the ES_{50}value which is better than quercetin. This result has a meaning that the ability AOPC compound to transfer hydrogen radical is greater than quercetin.

3. Conclusion

The synthesis of AOPC hasbeen successfully done through two stages of reaction i.e. allylation reaction of 4–hydroxybenzaldehyde to afford4–allyloxybenzaldehyde and continued with condensation reaction of 4–allyloxybenzaldehyde with resorcinol using anacid catalyst. The synthesized 4–allyloxy–benzaldehydewas afforded in light yellow liquid with 85% yield. Meanwhile, AOPC was obtained in dark red solid with m.p. 237–238 °C (decomposed) and 67% yield. Antioxidant assay ofAOPC and quercetin as a controlshowed ES_{50}value 12.46 and 34.90 respectively. Therefore, AOPCcompound had a higher antioxidant activity than quercetin and categorized as a strong antioxidant.

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