

Synthesise and 2-Deoxyribose Degradation Inhibition of Two Benzalacetone Derivatives

S. Handayani¹., S. Matsjeh²., C. Anwar², S. Atun¹.

¹Department of Chemistry Education, Yogyakarta State University

²Department of Chemistry, Gadjah Mada University

Abstract—Two benzalacetones derivatives – i.e. 3-hydroxybenzalacetone and 4-hydroxy-3-methoxybenzalacetone – have been synthesized and its antioxidant activity have also been tested. Synthesise were done through cross aldol condensation reaction under basic condition using ethanol as solvent. Re-crystallization using methanol was used to obtain pure material, which were yielding 50.62% of 3-hydroxybenzalacetone and 52.38% of 4-hydroxy-3-methoxybenzalacetone. Resulting materials have been characterized by TLC, FTIR, and NMR spectrometer. The activity tested performed with Deoxyribose assay. 3-hydroxybenzalacetone has higher antioxidant activity than 4-hydroxy-3-methoxybenzalacetone, which 3-hydroxybenzalacetone more active inhibited 2-Deoxyribose degradation than 4-hydroxy-3-methoxybenzalacetone.

Keywords—antioxidant activity, benzalacetone, deoxyribose assay

I. INTRODUCTION

Currently, cancer is being one of the diseases that caused most human died in the world. Cancer is disease which relating to uncontrolled cell growth, including premature aging [1]. Premature aging is one of physiological functional reduction process that caused by oxidative damage of cellular organ especially mitochondria. Mitochondria oxidative damage could be a major trigger of aging mechanism on aerobic organism, because about 90% of cellular oxygen has been consumed by mitochondria and 3 % of oxygen molecules were reduced by mitochondria. Therefore, mitochondria could be most contributor of oxidative stress.

To overcome premature aging and body functional damage that caused by free radical oxidation, it needs antioxidant i.e. a substantive with ability to delay, prevent or omit the damage causing by oxidative reaction [2]. Antioxidant can also defined as substantive that in small amount can prevent oxidation of cellular organ with minimize damage effect of *reactive oxygen species* (ROS) and/or oxidative stress [3].

One of ROS important species which is a byproduct of normal cellular metabolism is hydroxyl radical. Cellular organ damage caused by hydroxyl radical or other species of ROS can be measured from malondialdehyde (MDA) levels [4]. Malondialdehyde is a compound that resulted from reaction of 2-deoxyribose with hydroxyl radical. Thus, antioxidant activity of a compound could be simulated using deoxyribose assay.

Natural compound that generally used as antioxidant is vitamin C, curcumine, β -caroten and tocoferol [5], which could trap free radical and ROS, Usually, antioxidant compound have active site that easily oxidized as phenolic compound. One of common synthetic antioxidant is benzalacetone derivatives [6]. It were reporting that benzalacetone has low antioxidant activity, whereas 4-methoxybenzalacetone and 3,4-dimethoxydibenzalacetone have antioxidant activity with IC₅₀ of 662.44 and 354.38 μ g/ml. The result showed that substituent addition at benzene ring will influence the antioxidant activity of a compound.

This article will report synthesise of benzalacetone derivatives, one of phenolic compound that produced through cross aldol condensation reaction between acetone (1) and benzaldehyde derivatives i.e. 3-hydroxybenzaldehyde (2) and 4-hydroxy-3-methoxybenzaldehyde (3). The influence of substituent to antioxidant activity will measured by its ability to inhibit 2-deoxyribose degradation.

II. METHODOLOGY

General. All materials used from Merck, among other acetone, 3-hydroxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, ethanol, methanol, kloroform, hexane. The ¹H, ¹³C-NMR, HMQC and HMBC Spectra were recorded on 500 MHz Jeol spectrophotometer. IR analysis were conducted using a Shimadzu 8300 FTIR spectrometer.

Synthesise of 3-hydroxybenzalacetone (4). Into a solution of sodium hydroxide (0.025 mol, 1 g) in aquades that was prepared at ice bath environment, acetone (0.01 mol, 0.58 g) and 3-hydroxybenzaldehyde (0.01 mol, 1.22 g) were added drop wise alternately. After additional stirring for 3 hours, 3 mL HCl 37% were added. The mixture was kept under 10⁰C for 24 hours. Then, the mixture was filtered and followed by purification by recrystallization with ethanol-water as solvent. The yield was identified by thin layer chromatography, FTIR and NMR spectrophotometer.

Synthesise of 4-hydroxy-3-methoxybenzalacetone (5). Into a solution of sodium hydroxide (0.025 mol, 1 g) in 12,5 mL aquades, 4-hydroxy-3-methoxybenzaldehyde (0.005 mol, 0.76 g) and excess acetone were added dropwise. The mixture was stirred for 3 hours in icebath and followed by standing over 6 days under 10⁰C. Ethanol-water solvent was used to purification of filtered

mixture. Then the yield was identified by TLC, FTIR and NMR spectrophotometer.

Deoxyribose assay.

The method was performed by modification of some deoxyribose assay [7,8,9] All solutions were freshly prepared. Into a solution of 0.2 ml 5 mM 2-deoxyribose was added 0.2 ml 5 mM ascorbic acid; 0.2 ml buffer phosphate (pH 7.2); 0.2 ml 0.5 mM H₂O₂; 0.2 ml 0.5 mM ferrosulphate and 0.2 ml of various concentration sample (62,5; 125, 250, 500 and 1000 µg/ml) followed by stirring for a few minutes. After an incubation period of 30 minutes at 37°C, the reaction was stopped by adding 1 ml of TBA 1% and 1 ml of TCA 25% followed by heated at 100°C for 15 minutes. After the mixture is being cooled, absorbance of the red complex TBA-MDA solution's was measured at 532 nm against a blank (only contain buffer and 2-deoxyribose). The inhibition percentage was calculated following the formula:

$$I(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

The IC₅₀ value represents the concentration of the compound that caused 50% inhibition. BHT was used as a positive control.

III. RESULTS

A. Synthesis of compound 4 and 5.

Improved synthesis of 3-hydroxybenzalacetone (4).

The preparation of compound 4 was initiated by the mixing of acetone (1) and 3-hydroxybenzaldehyde (2) with sodium hydroxide as catalyst (Figure 1). After stirring for 3 hours followed by filtration, 50.62% (purity : 76.01%) yellow crystalline solid was obtained. The structure of 4 was determined by chromatographic and spectroscopic data. Rf=0.3 (TLC; methanol : chloroform=1:9); FTIR (KBr), NMR spectrometer datas and HMBC for compound 4 are given in Figure 1, TABLE 1 and Figure 2 respectively

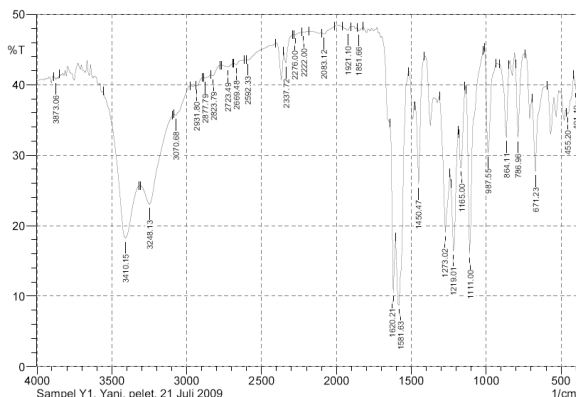


Fig. 1. FTIR of 3-hydroxybenzalacetone (4)

TABLE I
¹H AND ¹³C-NMR DATA OF COMPOUND 4 (CD₃OD)

C no	δH(Σ, m, J Hz) ppm	δC
1	3.3 (3; s; -)	49
2	-	191
3	7.16 (1; d; 15.85)	126
4	7.7 (1; d; 15.85)	145
1'	-	137
2'	7.10 (1; s; -)	115
3'	-	159
4'	6.86 (1; dd; 8)	119
5'	7.2 (1; t; 8)	131
6'	7.17 (1; d; 8)	121

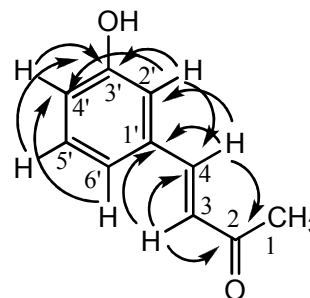


Fig. 2. HMBC of 3-hydroxybenzalacetone (4)

Improved synthesis of 4-hydroxy-3-methoxybenzalacetone (5).

Into a solution of sodium hydroxide (0.025 mol, 1 g) in 12,5 mL aquades, 4-hydroxy-3-methoxybenzaldehyde (0.005 mol, 0.76 g) and excess acetone were added dropwise. The mixture was stirred for 3 hours in icebath and followed by standing over 6 days under 10°C. After purification, 52.38% (purity :98.03%) a yellow crystalline was produced. Structure elucidation of compound 5 was determined by chromatographic and spectroscopic datas. Rf=0.47 (TLC; methanol : chloroform=1:9); FTIR (KBr), NMR spectrometer datas and HMBC for compound 5 are given in Figure 3, TABLE 2 and Figure 4 respectively

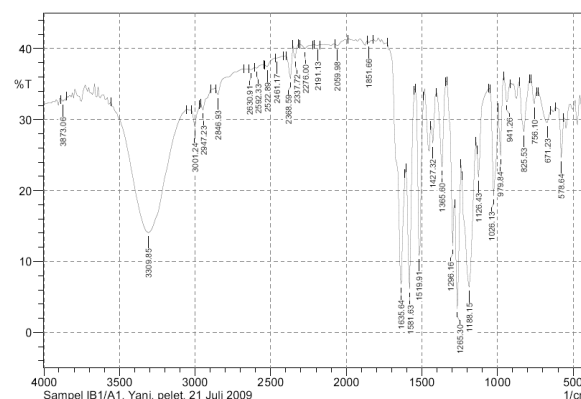


Fig 3. FTIR of 4-hydroxy-3-methoxybenzalacetone (5)

TABLE 2
¹H AND ¹³C-NMR DATA OF COMPOUND 5 (CD₃OD)

C no	δH(Σ, m, J Hz) ppm	δC
1	2.4 (3; s; -)	27.5
2	-	198
3	6.57 (1; d; 16.5)	125
4	7.45 (1; d; 16.5)	143
1'	-	127
2'	7.05 (1; d; 1.85)	109.5
3'	-	148
4'	-	147
5'	6.93 (1; d; 8.55)	114.9
6'	7.08 (1; dd; 8.55)	123

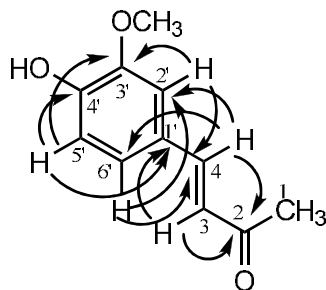


Fig 4. HMBC of 4-hydroxy-3-methoxybenzalacetone (5)

B. Activity test as 2-deoxyribose degradation inhibition of compound 4 and 5.

The result of antioxidant activity test using deoxyribose assay have been showed in Tabel 3.

TABLE 3
 Antioxidant activity of compound 4 and 5

No of Compound	IC ₅₀ (µg/mL)
4	2290.28
5	10391

IV. DISCUSSION

Synthesize of 4 and 5 compounds have been done through cross aldol condensation reaction following the scheme as shown in Figure 5.

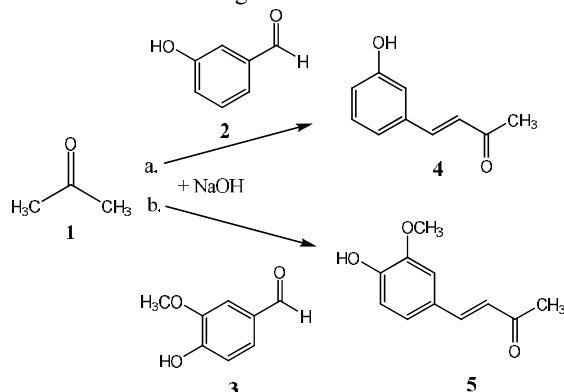


Fig 5. Scheme of cross aldol condensation to synthesized of compound 4 and 5

FTIR data of compound 4 indicates the existence of OH (3410.15 cm⁻¹), C=O (1620.21 cm⁻¹), aromatic groups (3070.68 and 1581.53 cm⁻¹) and CH (2931.80 and 2877.79 cm⁻¹). From NMR data, 2 singlet peaks appear at 3.3 ppm and 7.10 ppm. The first singlet peak indicates methyl proton and the second singlet peak shows the existence of 1 aromatic proton that is not neighbor. H_α dan H_β showed by two doublet peak with J coupling of 15.85 Hz at 7.16 and 7.7 ppm. Another H NMR signal showed the existence of aromatic proton with J coupling of 8 Hz. The FTIR data completed by HMQC and HMBC data showed that compound 4 is 3-hydroxibenzalacetone.

Support spectra data for 5 provided by the IR (KBr), which indicates the existence of C=O (1635.64 cm⁻¹), aromatic groups (3001.24 and 1581.63 cm⁻¹), OH stretching (3309.85), C-O-C (1126.43 and 1026.13 cm⁻¹) and CH stretching (2947.23 and 2846.93 cm⁻¹). The multiple bond correlation of HMBC supported the structure. In the ¹H-NMR spectrum (500 MHz, CD₃OD), appear 3 equivalent proton singlet that indicates methyl group at δ 2.4 ppm. H_α and H_β (H3 and H4) appeared at δ 6.57 and 7.45 ppm reinforced by J coupling data of 16.5 Hz. Aromatic proton, i.e. H2', H5' and H6' appear at δ 7.05; 6.93 and 7.08 ppm. NMR data completed by HMBC and FTIR data proofed that compound 5 is 4-hydroxy-3-methoxybenzalacetone.

The activity test as a hydroxyl radical scavenger was conducted by modification fenton method. The reaction started by adding ferrosulphate and H₂O₂ to produce radical that will react with the deoxyribose. The reaction was stopped by adding TBA reagent that will give red color if the malondialdehyde has been formed as the result of the reaction between the radical and the deoxyribose. The absorbance of the red color was measured by using UV Spectrometer at optimum wave number. The %activity as antioxidant was calculated as the percentage of the absorbance decrease of the yield of the synthesis compounds that can prevent the degradation of the 2-deoxyribose compared to the blank. When the sample of the synthesis works well as the hydroxyl radical scavenger, then it will decrease the deoxyribose degradation so that the malondialdehyde-TBA complex will only give small intensity of red color. Thus, the more intense the red color, the less active the sample is. The % inhibiton graphic of compound 4 and 5 is presented in Figure 6.

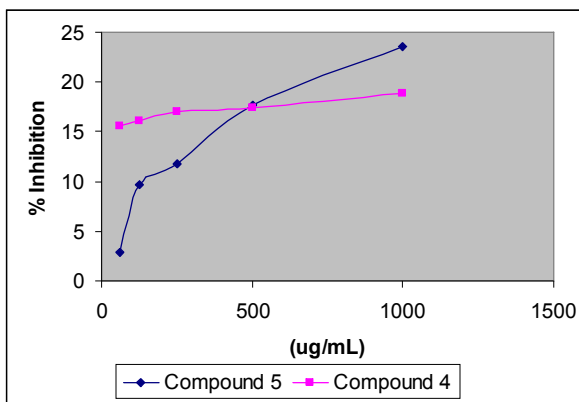


Fig 6. Graphic antioxidant activity of compound 4 and 5

From the graphic in Fig 6 between % 2-deoxyribose degradation inhibition and concentration ($\mu\text{g/mL}$), the IC_{50} value of compound 4 and 5 are 2290.28 and 10.391 $\mu\text{g/mL}$ respectively. It could be concluded that compound 4 have low antioxidant activity, whereas compound 5 have no antioxidant activity.

Relating to the theory, compound 5 should be more active since it's have methoxy group at benzene ring. The existence methoxy group at phenolic compound should improve antioxidant activity which measured by DPPH method because this method is more sensitive at small concentration so it will easier to detected [10]. Therefore, it's necessary to test the antioxidant activity using other method as comparative data.

V. CONCLUSION

Synthesize of compound 4 and 5 could be done through cross aldol condensation reaction under basic condition. Compound 4 has been found more active in inhibiting 2-deoxyribose degradation than compound 5.

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