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Synthesis and activity test as antioxidant of two hydroxydibenzalacetones

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Abstract: Synthesis and antioxidant activity test of two hydroxydibenzalacetones have been conducted. 2,2'-Dihydroxydibenzalacetones and 3,3'-dihydroxydibenza--lacetones were synthesized by crossed aldol condensation in base condition with water-ethanol solvent from 2-hydroxybenzaldehyde and 3-hydroxybenzaldehyde as raw material respectively. The synthesize reaction was conducted in ice bath environment under stirring and followed by precipitation and purification by recrystalization with methanol-water as solvent. After filtration, 60.15% blackish green crystalline of 2,2'-dihydroxydibenzalacetone and 78.94% yellow crystalline of 3,3'dihydroxydibenzalacetone were obtained. The IC_{50} of two compounds were 791.62 and 196.56 µg/mL respectively.

Introduction

As curcumine analogue, dibenzalacetone is an attractive compound to be developed. Curcumine and dibenzalacetone structure was distinguished by a carbonyl and a methylene group so they have been predicted having similar activity. Itokawa et.al. [1] reported the relationship between structure and activity of curcuminoid compounds as antioxidant, anti-inflammatory, chemoprefentive and anti prostate cancer. Antiinflammatory and antioxidant activity was found not only in curcuminoid compounds, but also in dibenzalacetone [2]. Previous research was resulting that symmetric [3] and asymmetric dibenzalacetone also have antioxidant activity [4].

Dibenzalacetone could be synthesized by crossed aldol condensation easily.. Aldol Condensation is occured by a nucleophilic addition of the enolate ion to a carbonyl. Acetone also undergoes aldol condensation, but the equilibrium concentration of the product is generally small. Analogue benzalacetones still have a potential development based on previous reports of its biological activity. Some of researchers had reported dibenzalacetone synthesis by different method. Sardjiman had synthesized some of analogue hydroxydibenzalacetones using hydrochloric acid as acid catalyst while Pudjono [5] used sulphuric acid. Affandi [6] had synthesized 4-hydroxydibenzalacetone in base condition. Solvent also has a significant influence in benzalacetone synthesis [7].

2,2'-dihydroxydibenzalacetone and 3,3'-dihydroxydibenzalacetone will be synthesized in this research. Dihydroxydibenzalacetone compound has phenolic group that performing very reactive oxidative reaction and could produced free radical, so it was predicted that dihydroxydibenzalacetone has potency of an antioxidant activity.

Materials and Methods

General . All materials used was supplied from Merck, some of these are acetone, 2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, ethanol, chloroform, hexane, and ethyl acetate. The ^{1}H, ^{13}C-NMR, HMQC and HMBC Spectra were recorded on 500 MHz Jeol spectrophotometer. IR spectra were conducted using a Shimadzu 8300 FTIR spectrometer.

2,2'-dihydroxydibenzalacetones(4). Into a solution of acetone (0.01 mol, 0.58 g) in 5 mL ethanol that was prepared at ice bath environment, 2-hydroxybenzaldehyde (0.02 mol, 2.44 g) and NaOH (0.025 mol, 1 g) were added drop wise alternately. After stirring for 3 hours, the mixture was kept under 10^0C for 24 hours before filtering and purification by recrystalization using methanol as solvent. The obtained product was identified by thin layer chromatography, FTIR and NMR spectrophotometer.

3,3'-dihydroxydibenzalacetone (5). Into a solution of acetone (0.01 mol, 0.58 g) in aquades that was prepared at ice bath environment, NaOH (0.025 mol, 1 g) and 3-hydroxybenzaldehyde (0.02 mol, 2.44 g) were added drop wise alternately. After additional stirring for 3 hours, 3 mL HCl 37% and 5 mL aquades were added. The mixture was kept under 10^0C for 24 hours. Then, the mixture was filtered and followed by purification by recrystalization with ethanol-water as solvent. The yield was identified by thin layer chromatography, FTIR and NMR spectrophotometer.

Deoxyribose assay

The assay was performed by the following method as described by Halliwell [8]. All solutions were freshly prepared. Into a solution of 6 mM 2-deoxyribose (0.2 mL), 0.01 mM ascorbic acid (0.2 mL); buffer phosphate pH 7.4 (0.2 mL); 0.01 mM H_2O_2 (0.2 mL); 0.1 mM ferrosulphate (0.2 mL) and 0.02 mL of sample in various concentration (62.5; 125; 250; 500; 1000 µg/mL) were added. After an incubation period of 30 minutes at 310°K, the extent of deoxyribose degradation was measured by the TBA reaction. 3 ml of TBA and 3 ml of TCA were added to the reaction mixture and heated for 15 minutes at

353°K. After cooling, the absorbance of mixture was measured at 532 nm against a blank solution (the same solution but without sample). The percentage inhibition was calculated by the formula:

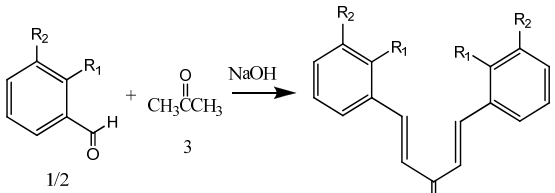
$$I(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} x100\%$$

The IC_{50} value represent the concentration of the compounds that caused 50% inhibition. BHT was used as a positive control.

Results and Discussion

The Synthesis of 2,2'-dihydroxydibenzalacetone (4). The preparation of compound **4** was initiated by the mixing of **1** and **3** with sodium hydroxide as catalyst in ethanol solvent (Figure 1). After stirring for 3 hours followed by filtration, 60.15% blackish green crystalline solid was obtained. The structure of **4** was determined by chromatographic and spectroscopic data: Rf (TLC; methanol: chloroform=1:9) 0.36; FTIR (KBr) cm^{-1}: 3425.58; 2939.52; 1589.34; 1543; 1458 and 1118.71.

The multiple bond correlation of HMBC supported the structure (Table 1, Figure 2). In the ^{1}H-NMR spectrum (500 MHz, CD_3OD), four equivalent protons with multiplicity as doublet and two equivalent protons with the multiplicity as triplet were observed. The doublet signal at δ = 8.4 ppm was assigned to H1 and H5, δ = 7.6 ppm to H6'and H6", δ = 7.1 ppm to H2 and H4 and δ = 6.69 ppm to H2' and H2". The triplet signal at δ = 6.47 ppm was assigned to H4' and H4" meanwhile δ = 7.06 ppm to H5' and H5". Support spectra data provided by the IR (KBr), which indicates the existence of C=O (1589.34 cm^{-1}), aromatic C=C (1543-1458 cm^{-1}) and CO (1118.71 cm^{-1}). Therefore, the structure of **4** was 2,2'-dihydroxydibenzalacetone.



1/2 3 4/5

1. R_1=OH; R_2=H; 2-hydroxybenzaldehyde
2. R_1=H; R_2=OH; 3-hydroxybenzaldehyde
3. acetone
4. R_1=OH; R_2=H; 2,2'-dihydroxydibenzalacetones
5. R_1=H; R_2=OH; 3,3'-dihydroxydibenzalacetones

Figure 1. Synthesis reaction of **4** and **5** by crossed aldol condensation

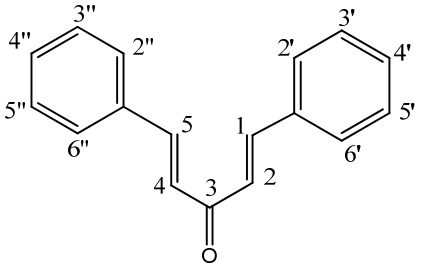


Figure 2. Numbering of dibenzalacetone structure for NMR data

Table 1: ^{1}H and ^{13}C-NMR data of compound **5** (CD_3OD)

C no.	δH (∑H; m;J Hz) ppm	δC ppm	HMBC (500 MHz)
1	8.42 (1 H; d; 15,9)	143	C3, C2', C6'
2	7.15 (1H; d; 15,9)	122	C3, C1'
3	-	193	-
4	7.15 (1H; d; 15,9)	122	C1", C3
5	8.42 (1H; d; 15,9)	143	C3, C4,C6''
1'	-	124	-
2'	-	169	-
3'	6.68 (1H; d; 15,9)	121	C1', C4'
4'	6.47 (1H; t; 8)	115	C3', C6'
5'	7.06 (1H; t; 8)	133	C2', C6'
6'	7.55 (1H; d; 8)	128	C2', C1, C5'
1''	-	124	-
2''	-	169	-
3''	6.68 (1H; d; 8)	121	C1'', C4''
4''	6.47 (1H; t; 8)	115	C3'', C6''
5''	7.06 (1H; t; 8)	133	C2", C6''
6''	7.55 (1H; d; 8)	128	C2'', C5, C5"

The Synthesis of 3,3'-dihydroxydibenzalacetone (5). The preparation of compound **5** was initiated by the mixing of **3** with sodium hydroxide as catalyst in aquadest as solvent (Figure 1). To this reaction mixture was directly added **2,** followed by stirring at ice bath for 3 hours. After additional stirring, provided yellow crystalline **5** in 78.94% was resulted. The structure of **5** was determined by chromatographic and spectroscopic data: Rf (TLC;methanol:chloroform = 1:9) 0.34; FTIR (KBr) 1/cm: 3379.29; 3248.13; 1620.21; 1581.63; 1450; 1211.30 and 1103.28.

The multiple bond correlation of HMBC supported the structure (Table 2, Figure 1). In the ^{1}H-NMR spectrum (500 MHz, CD_3OD), one set protons singlet, four set protons doublet and one set protons triplet were observed. Support spectra data were provided by the IR (KBr), which indicate the existence of C=O (1620.21 cm^{-1}), aromatic C=C (1581.63-1450 cm^{-1}) and CO (1103.28 cm^{-1}). Therefore, what can be concluded for the structure of **5** was 3,3'-dihydroxydibenzalacetone.

Table 2: ^{1}H and ^{13}C-NMR data of compound 6 (CD_3OD)

C no.	δH (∑H; m) ppm	δC ppm	HMBC (500 MHz)
1	7.68 (1 H; d; 15,9)	145	C3, C2', C2, C2'
2	7.16 (1H; d; 15,9)	126	C3, C1, C1'
3	-	191	-
4	7.16 (1H; d; 15,9)	126	C3, C1", C5
5	7.68 (1H; d; 15,9)	145	C3, C4, C1",C2''
1'	-	137	-
2'	7.1 (1H; s)	115	C1, C3', C6'
3'	-	159	-
4'	6.86 (1H; d; 8)	119	C3', C2', C6'
5'	7.2 (1H; t; 8)	131	C4', C3', C1'
6'	7.15 (1H; d; 8)	121	C1, C1'
1''	-	137	-
2''	7.1 (1H; s)	115	C5, C3'', C6''
3''	-	159	-
4''	6.86 (1H; d; 8)	119	C3'', C2'', C6"
5''	7.2 (1H; t; 8)	131	C4'',C3'',C1''
6''	7.15 (1H; d; 8)	121	C5, C1''

Deoxyribosa assay

Activity test as deoxyribose degradation inhibitor was done by fenton reaction. The IC_{50} value represented the consentration of the compounds, that caused 50% inhibition. All experiment were repeated for five times. Data of IC_{50} values were showed in Table 3.

Table 3: IC_{50} datas for compounds **4** dan **5**

No	Compound	IC_{50} (µg/mL)	activity
1.	4	791.62	Low
2.	5	196.56	active

Conclusions

In conclusion, two dihydroxydibenzalacetones, **4** and **5** were succesfully synthesized in 60.15 and 78.94% respectively. Compund **4** and **5** exhibited significant antioxidant activity with the IC_{50} of 791.62 and 196.56 µg/mL respectively. Compound **5** is more potent than **4** to inhibit deoxyribose degradation.

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