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Synthesis of 3-*O*-Benzylstentorin Analog as Precursor for the Basic Skeleton of Blepharismin

(Sintesis Analog 3-*O*-Benzylstentorin sebagai Prekursor untuk Kerangka Dasar Blepharismin)

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Abstract: A Method for the synthesis of precursor for the basic skeleton of blepharismin and structural analog was described. Such compound is identified as potential drug for the treatment of antiviral and photodynamic therapy. Benzyl stentorin analog as the basic skeleton of blepharismin was effectively synthesized via reaction one of hydroxyl groups in bianthraquinone derivative with *p*-methoxybenzylchloride in the presence of potassium carbonate as base. Dimerization of bromo anthraquinone derivative produced dimmer in 55% while the formation of compounds analogous of 3-*O*-benzylstentorin resulted in 70% yield.

Keywords: blepharismin, 3-*O*-benzylstentorin, synthesis, dimerization, anthraquinone derivative.

Abstrak: Metode sintesis prekursor untuk kerangka dasar dan struktural analog *blepharismin* telah dijelaskan. Senyawa tersebut diidentifikasi sebagai obat yang potensial untuk pengobatan terapi antivirus dan *photodynamic*. Analog benzil stentorin sebagai kerangka dasar blepharismin secara efektif disintesis melalui reaksi antara salah satu gugus hidroksil senyawa biantrakuinon dengan *p*-metoksi benzyl klorida dengan adanya kalium karbonat sebagai basa. Dimerisasi senyawa turunan bromo antrakuinon menghasilkan produk dimmer sebesar 55% sedangkan untuk pembentukan senyawa analog 3-*O*-benzylstentorin diperoleh rendemen hasil 70%.

Kata kunci: *blepharismin*, 3-*O*-benzilstentorin, sintesis, dimerisasi, turunan antrakuinon.

INTRODUCTION

BLEPHARISMIN is a primary photosensor pigment in *Blepharisma japonicum*. The pigment of blepharismins has great variations from colorless to deep pink[1]. The pigment also absorb light and completely bleached by exposure to light[2]. These pigments are known to have three functions that is light perception, chemical defense against predators and protection against UV radiation[3]. It means that *B. japonicum* posses a photoreceptor system mediating the step up photophobic response and can act as a photosensitizer[4]. It also represent in the development of potential treatment as photodynamic therapy (PDT). PDT is one of method for cancer which is a light-activated chemotherapy that allows selective treatment of localized cancer[5]. This is an approach for destroying tumors that uses light and a light-sensitive chemical to rapidly kill cells[6]. When photosensitizers are exposed to a specific wavelength of light, they produce a form of oxygen that kills nearby cells[7]. Each photosensitizer is activated by light of a specific wavelength[8]. This wavelength determines how far the light can travel into the tissues[9]. Thus, doctors use specific photosensitizers and wavelengths of light to treat different areas of the body with PDT.

Blepharismin was found to inhibit the growth of Gram-positive bacteria such as MRSA (methicilin-resistant *Staphylococcus aureus*) and also inhibit the activity of protein kinase C. Blepharismin was also reported as highly toxic to *Dileptus margaritifer* (LD_{50}, 0.46 µg/mL) than of Climocostol (LD_{50}, 0.70 µg/mL)

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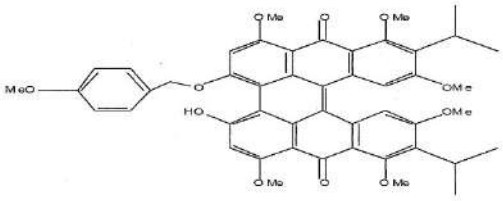
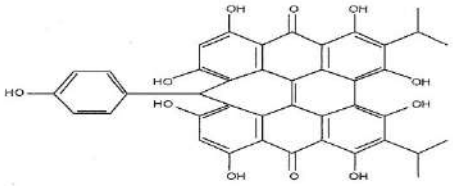
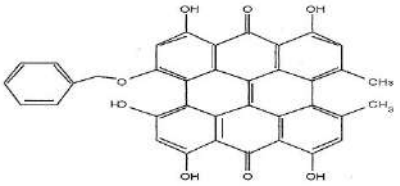


3-*O*-benzylstentorin analog



Blepharismin-3



3-*O*-benzylhypericin

Figure 1. Structures of 3-*O*-benzylstentorin analog, blepharismin and 3-*O*-benzylhypericin.

and Stentorin (LD_{50}, 0.46 μg/mL)[10].

The chemical structure of blepharismin is similar to that of stentorin, hypericin and 3-*O*-benzylhypericin but not identical. It also has biologically significant parts in its structure which is phenanthroperylenequinones. Blepharismin have a seven membered ring structurally specific featured (Figure 1).

The biosynthetic pathway of blepharismin has been investigated in our laboratory. Dibenzoperylenequinone moiety of blepharismin was biosynthesized via polyketide pathway by assignment of the ^{13}C NMR spectrum of blepharismin. Analyses were based on HMQC, HMBC and INDEQUATE spectra of ^{13}C-enriched samples obtained by feeding experiment of ^{13}C-labeled acetate[11]. Another biosynthetic pathway of blepharismin uses starter units isovaleryl-CoA and butyryl CoA, which was derived from L-leucine and sodium butyrate. The analyses of the pathway were based on HPLC, LC/ESI-MS, 1H NMR and ^{13}C NMR data[12].

In this paper we describe the synthesis of precursor for the basic skeleton fo blepharismin i.e. 3-*O*-benzylstentorin analog. The same synthetic of 3-*O*-benzylstentorin was used approach previously synthesis of stentorin[13].

MATERIALS AND METHODS

MATERIALS. All reactions were carried out under a static argon atmosphere. All solvents were dried and distilled according to standard procedure. Analytical thin layer chromatography (TLC) was performed on Merck silica gel plates (Kiesel gel $60F_{254}$ 0.25 mm) and preparative TLC was carried out on Merck silica gel plates (Kiesel gel $60F_{254}$ 0.5 mm). Silica gel column chromatography was carried out on Daisogel IR-60. 5-Bromo-2-isopropyl-1,3,8-trimethoxy -6-methoxymethoxy anthraquinone was used as starting material for the synthesis of bianthraquinone. Cu powder was used as catalyst, naphthalene and chloroform was used as solvent.

Instruments. ^1H and ^{13}C NMR spectra were recorded on JEOL 1NM–LA for 400 MHz or JEOL 1NM-LA 300 for 300 MHz in deuterio chloroform unless otherwise specified. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (δ 0.00) or $CHCl_3$ (δ 7.26) for ^1H NMR and δ 77.0 for ^{13}C NMR as internal standard, and coupling constant are reported in Hertz. EI Mass Spectra were recorded on JEOL JMS-700T and FAB Mass Spectra were recorded on JEOL JMS-700T. IR spectra were obtained on a JUSCO A-100.

METHODS. Synthesis of 2,2'-Didydroxy-6,6'-diisopropyl-4,5,7,4',5',7'-hexamethoxy- [1,1'] bianthranyl -9,10,9',10'tetraone ($C_{42}H_{42}O_{10}$). Activation of cu powder: commercially available Cu powder was treated with iodine at 100°C for 1 hour (h). The mixture was dried in vacuum at room temperature and stored under an atmosphere of argon. To a solution of 5-Bromo-2-isopropyl-1,3,8-trimethoxy -6-methoxymethoxy anthraquinone (32 mg, 0.067 mmol) was added activated the Cu powder (42 mg, 0.67 mmol) in naphthalene (0.5 gr, 3.9 mmol) with stirring. The reaction mixture was heated at 240°C for 2 hours and then cooled to room temperature and the mixture was extracted with $CHCl_3$. The crude product was purified by column chromatography (hex:AcOEt: 2:1) to obtain product (26.7 mg, 55%).

Synthesis of 3,4-dihydroxy-9,14-diisopropyl-1,6,8,13,15-hexamethoxydibenzo [*a,o*] perylene-7, 16(3aH,3a^{1}H)-dione. To a solution of anthraquinone dimmer in acetic acid was added cu powder and starting material was stirred for 1 h. After that time to a solution of reaction mixture was added hydrochloric acid then stirred for 1 h. The reaction mixture was filtered via celite then the organic solvent washed with sodium bicarbonate and extracted with ethylacetate. The crude was purified with CH_2Cl_2: AcOEt = 2:1.

Synthesis of 3-dihydroxy-9,12-diisopropyl-1,6,8,10,11,13-hexamethoxy-4-(4-methoxy benzoloxy) phenentro[1,10,9,8-*opqra*]perylene-7,14(3aH,3a^{1}H)-dione. To a solution of anthraquiononein dimmer in DMF solvent was added K_2CO_3 and p-methoxybenzylchloride. The reaction mixture was stirred at 80°C for 16 h. After that time the reaction mixture was washed with brine then was added 1 N HCl and extracted with petroleum ether[14]. The extract then was purified by silica gel column chromatography to obtain product in 70% yield.

RESULTS AND DISCUSSIONS

The precursor for the basic skeleton of blepharismin should be synthesized from simple and readily available chemicals using applying synthetic approach for stentorin. The bromo anthraquinone derivates (obtained from benzaldehyde and benzamide)[15] were synthesized via Ullman coupling reaction. The Ullman reaction is the synthesis of biaryls compound in which two molecules of aryl halide in the presence of activated copper to form a new aryl-aryl bond with the elimination of copper halide (Figure 2). This reaction has been used to prepare symmetrical and unsymmetrical biaryls. Ullman synthesis is related to a whole family of reactions in that copper appears as a catalyst or as an organocopper compound. Dimerization of anthraquinones is the key point in the synthesis of 3-*O*-benzylstentorin analog. Herein, we have chosen Ullman coupling reaction for dimerization of bromo anthraquinone derivate because the Ullman coupling is more effective than Suzuki coupling. In this condition, Cu was activated by iodine at 100°C for 30 minutes then Cu was added to bromoanthraquinone as the starting material in naphthalene and excess toluene. The reaction was stirred at high temperature of about 240°C for two hours. The reaction produce anthraquinone dimmer of 55% yield and the anthraquinone about 30 % yield.

The anthraquinone dimmer was dissolved in acetic acid and followed by an addition of Cu powder and the reaction mixture was stirred for one hour. To the reaction mixture was then added concentrated hydrochloric acid solution and was stirred for one hour. In this reaction, was carried out deoxygenolysis in acidic condition. One of hydroxyl groups in bianthraquinone derivative was introduced with p-methoxybenzylchloride in the presence of potassium carbonate as base. This coupling is selectively of phenolic acceptor to afford 3-*O*-benzylstentorin analog.

Mass Spectrometry, ^{1}H NMR and ^{13}C NMR methods have been applied to elucidate the chemical structure of 3-*O*-benzylstentorin analog (Figure 3) for precursor the basic skeleton of blepharismin. The Molecular weight of 3-*O*-benzylstentorin analog was determined to be 798 for $C_{48}H_{46}O_{11}$ by mass spectroscopy. The high-resolution FAB value for the positive ion MS (FAB) $[M+H]^+$ was 799.3010. The theoretical value for the 3-*O*-benzylstentorin positive ion for $C_{48}H_{47}O_{11}$ is 799.3074.

The ^{1}H NMR and ^{13}C NMR data are presented in Table 1 below. In the 1H NMR spectra data of

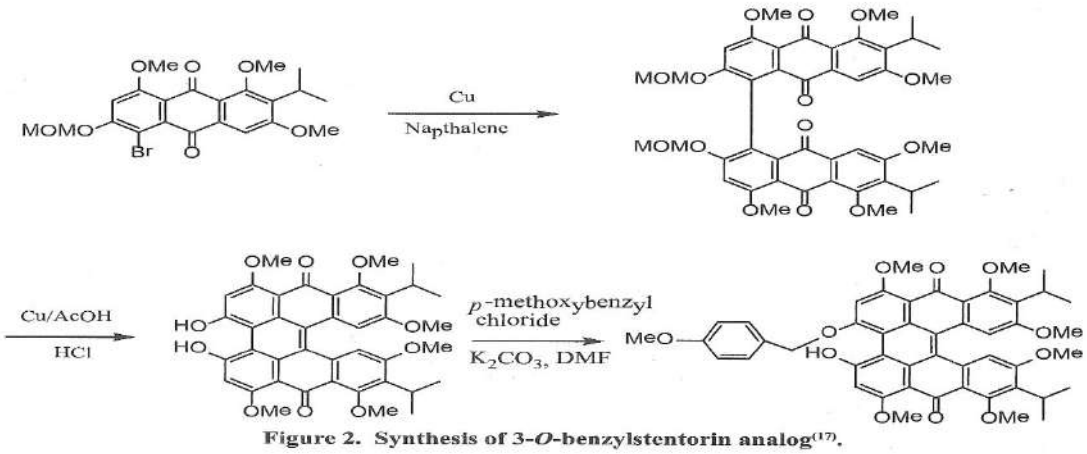


Figure 2. Synthesis of 3-*O*-benzylstentorin analog[17].

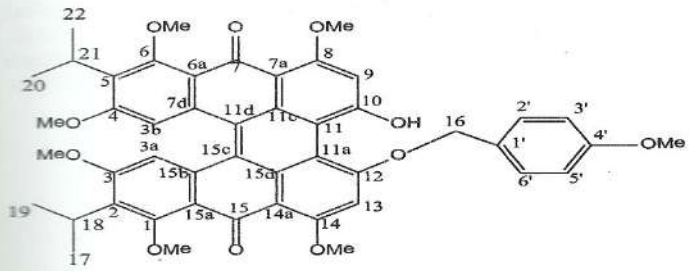


Figure 3. Structure of 3-*O*-benzylstentorin analog.

Table 1. The ^{1}H NMR (400 MHz) and ^{13}C NMR (400 MHz) Data for 3-*O*-benzylstentorin analog in $CDCl_3$.

Position	δC	δH
1	162.3	
2	122.2	
3	160.4	
3a	120.8	7.11
3b	120.8	7.11
4	160.4	
5	122.2	
6	162.3	
6a	103.7	
7	184.4	
7a	104.2	
7d	103.7	
8	159.1	
9	103.7	7.16
10	182.4	
11	132.9	
11a	94.4	
11c	104.2	
11d	132.9	
12	158.5	
13	103.7	7.16
14	159.1	
14a	94.4	
15	184.4	
15a	104.2	
15b	132.9	
15c	119.5	
15d	122.2	
1'	132.9	
2' 6'	134.4	7.16
3' 5'	119.5	7.11
4'	158.5	
1-OMe 6-OMe	62.7	4.05
3-OMe 4-OMe	55.9	3.78
8-OMe 14-OMe	55.6	3.92
4'-OMe	56.5	3.92
16	62.7	5.04 ; 5.08
17	20.5	1.3
18	25.1	3.65
19	20.5	1.3
20	20.5	1.28
21	25.1	3.65
22	20.5	1.28

3-*O*-benzylstentorin analog, six methoxy signals (δH 3.78, 3.92, and 4.05) and two protons (δH 5.04, 5.08) were detected along with two isopropyl groups (δH 1.28, 1.30) and aromatic proton (δH 7.1, 7.16).

CONCLUSION

Synthesis of 3-*O*-benzylstentorin was achieved using reaction one of hydroxyl groups in bianthraquinone derivative with *p*-methoxybenzylchloride in the presence of potassium carbonate as base. Dimerization of bromo anthraquinone derivative produced dimmer in 55% while the formation of compounds analogous of 3-*O*-benzylstentorin resulted in 70% yield.

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