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by Nanik Siti Aminah

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Carbazomarin: A New Potential of α-Glucosidase Inhibitor From *Clausena excavata* Roots

Nanik S. Aminah[1], Tin M. Thant[1,2], Alfinda N. Kristanti[1], Rico Ramadhan[1], Hnin T. Aung[3], and Yoshiaki Takaya[4]

Abstract

Continuing our exploration for dual functions antidiabetic and antioxidant agents from Myanmar medicinal plant, a new carbazole-pyranocoumarin conjugate, carbazomarin-C (**1**) along with a known carbazole alkaloid, mukonine (**2**) and a pyranocoumarin, xanthoxyletin (**3**), was isolated from the roots of *Clausena excavata*. The chemical structures of these compounds were identified using a combination of spectroscopic methods. Among isolates, there was a strong inhibition of compounds (**1**) and (**3**) on yeast α-glucosidase in a dose-dependent manner. It was shown when *p*-nitrophenyl-α-D-glucopyranoside was used as a substrate in vitro with IC_{50} values 0.22 and 4.81 mM, respectively. However, all isolated compounds displayed no inhibition against DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals.

Keywords

Clausena excavata, coumarin, carbazole alkaloid, carbazomarin-C, α-glycosidase, DPPH

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Clausena excavata Burm. f. is commonly found in the tropical and subtropical regions such as India, China, and Southeast Asia countries. The plants are a member of *Rutaceae* family and they are in a form of wild shrubs. They are known to have medicinal properties since its leaves, twigs, and roots are widely used for the traditional treatment of cold, fever, wound, abdominal pain, snake-bite, a preliminary stage of AIDS, and skin diseases.[1] Previous phytochemical analyses found that *C. excavata* possesses an abundant amount of coumarins,[2-4] carbazole alkaloids,[5] and a few limonoids.[6] The coumarins isolated from this plant raised the writers' attention due to its bioactive properties. For instance, clauslactones A to J which were isolated from the leaves exhibited tumor promotion inhibitory effects. Nordentatin showed antibacterial and antioxidant properties, while pyranocoumarin and clausenidin which were isolated from roots displayed an anti-HIV-1 activity.[4,7]

Diabetes mellitus (DM) is one of the complex chronic illness which demands constant medical checkup. As a consequence, many strategies are already developed in order to reduce the multifactorial risk through glycemic control.[8] Elevated plasma glucose causes overproduction of free radicals and other reactive oxygen species that destroy cells through oxidative stress, which supports the goal of developing antidiabetic drugs with radical scavenging. Dual function agents which have both antidiabetic (α-glucosidase inhibitor) and radical scavenging capacities are particularly relevant for the treatment of T2DM (Type 2 Diabetes Mellitus) and its complications. In this study, we searched for an antidiabetic and antioxidant agent having a dual mechanism from a medicinal plant.[9] Here, we have been isolated 1 new carbazomarin-C (**1**) along with carbazole alkaloid, mukonine, and pyranocoumarin, xanthoxyletin. Carbazomarin-C (**1**) was obtained as a solid with yellowish color with a melting point of 251°C to 252°C. The absorption maxima shown by the UV spectrum were at 335, 278, and 227 nm due to 7-oxygenated coumarin. The ^{1}H NMR (Nuclear Magnetic Resonance) spectrum (Table 1; Supplemental Figure S1) displayed the presence of 2,7-dihydroxy-1,3,6-tri-substituted carbazole skeleton by 1 aldehydic proton δ_H 9.75 (1H, s, 3-CHO) and 3 aromatic singlet protons at δ_H 7.88 (1H, s, H-4), 7.41 (1H, s, H-5), and 6.97 (1H,

[1]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Komplek Kampus C UNAIR, Jl. Mulyorejo, Surabaya, Indonesia
[2]Department of Chemistry, Mandalar Degree College, Mandalay, Myanmar
[3]Department of Chemistry, Mandalay University, Myanmar
[4]Faculty of Pharmacy, Meijo University, Tempaku, Nagoya, Japan

Corresponding Author:
Nanik S. Aminah, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Komplek Kampus C UNAIR, Jl. Mulyorejo, Surabaya, Indonesia.
Email: nanik-s-a@fst.unair.ac.id

Table 1. ^{1}H (600 MHz), ^{13}C (151 MHz) NMR and HMBC Spectral Data of **1** in $CDCl_3$.

Position	δ_H multiplet/J values	δ_C (ppm)	HMBC
1a	-	145.3	
1	-	108.9	
2	-	156.7	
3	-	114.8	
4	7.88 (s, 1H)	123.7	C-1a, C-2, C-4a, -CHO
4a	-	116.8	
5a	-	116.2	
5	7.41 (s, 1H)	117.2	C-6", C-7, C-8a
6	-	124.1	
7	-	153.8	
8	6.97 (s, 1H)	97.2	C-5a, C-6, C-7, C-8a
8a	-	140.9	
1'	3.54 (d, J = 6.8 Hz, 2H)	21.6	C-1, C-1a, C-2, C-2', C-3'
2'	5.30 (d, J = 6.8 Hz, 1H)	121.2	
3'	-	132.0	
3a'	1.68 (s, 3H)	23.6	C-2', C-3', C-3b'
3b'	1.83 (s, 3H)	16.8	C-2', C-3', C-3a'
3-CHO	9.75 (s, 1H)	195.8	
2"	-	162.6	
3"	6.00 (d, J = 9.6 Hz, 1H)	107.2	C-2", C-4a"
4"	8.04 (d, J = 9.6 Hz, 1H)	140.8	C-2", C-5"
4a"	-	103.9	
5"	-	152.6	
5a"	-	109.8	
6"	4.76 (dd, J = 8.0, 10.0 Hz, 1H)	29.5	C-5, C-6, C-5a", C-7"
7"	2.05 (dd, J = 10.0, 13.6 Hz, 1H) 2.31 (dd, J = 8.0, 13.6 Hz, 1H)	40.8	C-5", C-8", C-8a", C-8b"
8"	-	76.4	
8a"	1.34 (s, 3H)	22.3	C-7", C-8", C-8b"
8b"	1.41 (s, 3H)	27.4	C-7', C-8', C-8a"
9a"	-	159.5	
10"	-	114.8	
10a"	-	158.6	
1'"	-	40.9	
1a'"	1.72 (s, 3H)	28.9	C-1'", C-1b'", C-2'", C-10"
1b'"	1.72 (s, 3H)	28.9	C-1'", C-1a'", C-2'", C-10"
2'"	6.35 (dd, J = 10.7, 17.4 Hz, 1H)	150.8	C-1'", C-3'"
3a'"	4.93 (dd, J = 1.2, 10.7 Hz, 1H)	106.6	C-1'", C-2'"
3b'"	4.85 (dd, J = 1.2, 17.4 Hz, 1H)		

s, H-8). The 3-isomethyl prenyl group attached to the ring A of carbazole alkaloid was signified by 2 peaks at δ_H 3.54 (2H, d, J = 6.8 Hz, H-1'), δ_H 5.30 (1H, d, J = 6.8 Hz, H-2') and 2 isomethyl groups signals at δ_H 1.68 (3H, s, H-3a'), δ_H 1.83 (3H, s, H-3b'), respectively. Moreover, the existence of pyranocoumarin unit was revealed by 2 pairs of doublet protons at δ_H 6.00 (1H, d, J = 9.6 Hz, H-3") and δ_H 8.04 (1H, d, J = 9.6 Hz, H-4"), pyran ring at δ_H 4.76 (1H, dd, J = 8.0, 10.0 Hz, H-6"), 2 anisotropic protons at δ_H 2.05 (1H, dd, J = 10.0, 13.6 Hz, H-7"), δ_H 2.31 (1H, dd, J = 8.0, 13.6 Hz, H-7"), and gemdimethyl at δ_H 1.34 (3H,s, H-8a"), δ_H 1.41 (3H,s, H-8b"). In addition, the characteristic of prenyl group attached to C-10 position of core coumarin has shown signals at δ_H 6.35 (1H, dd, J = 17.4, 10.7 Hz, H-2'"), 4.93 (1H, dd, J = 10.5, 1.2 Hz, H-3a'"), 4.85 (1H, dd, J = 17.4, 1.2 Hz, H-3b'"), and 2 methyl groups at δ_H 1.72 (6H, s, H-1a'" and -1b'"). The ^{13}C-NMR (Nuclear Magnetic resonance) and DEPT (Distortionless Enhancement by Polarization Transfer) (90, 135) spectra of **1** indicated the presence of 1 aldehyde carbon δ_c 195.8, 1 cyclic lactone carbonyl carbon δ_c 162.6, 16 sp^2 quaternary carbons (δ_c

159.5, 158.6, 156.7, 153.8, 152.6, 145.3, 140.9, 132.0, 124.1, 116.8, 116.2, 2 × 114.8, 109.8, 108.9, 103.9), 2 sp^3 quaternary carbons (δ_c 76.4, 40.9), 7 sp^2 methine carbons (δ_c 150.8, 140.8, 123.7, 121.2, 117.2, 97.2), sp^3 methine (δ_c 29.5), 1 exomethylene carbon (δ_c 106.6), 2 methylene carbons (δ_c 40.8, 21.6), and 6 methyl carbons (δ_c 2 × 28.9, 27.4, 23.6, 21.6, 16.8) (Table 1; Supplemental Figure S2). The data presented above, also DQF-COSY (Double Quantum Filtered- ^{1}H-^{1}H correlated spectroscopy), and HSQC (Heteronuclear Single Quantum Correlation) data indicate that **1** is the binary of carbazole-pyranocoumarin conjugate (Supplemental Figures S3 and S4). There are several correlations pointed out by the ^{1}H-^{13}C long range coupling of HMBC (Heteronuclear Multiple Bond Correlation) spectrum of **1,** which are between H-4/C-1a, C-2, C-4a, and -CHO. The location of H-4 proton and the group of aldehyde were confirmed to be attached to C-3 carbon of ring-A in carbazole unit. Another correlation was that a group of prenyl was attached to C-1 position of carbazole and it was revealed by H-1' to C-1, C-1a, C-2, C-2', and C-3'. Its pattern is also similar to heptaphylline. Moreover, the singlet proton, H-5 on ring-C of carbazole, gave correlation to C-6", C-7, C-8a, and again, H-6" of pyranocoumarin to C-5, C-6, C-5a", C-7" proved that 2 units are connected at C-6 of carbazole and C-6" of pyranocoumarin. The existence of typical pyranocoumarin lactone carbon was showing correlation H-2", H-3" to C-2", C-4a", and C-5". The attachment of prenyl group to C-10" of pyranocoumarin was confirmed by the cross peaks of H-1a"' to C-1"', C-1b"', C-2"', and C-10" by HMBC spectrum (Table 1; Figure 1 and Supplemental Figure S5). The spectrum of NOESY owned by **1** showed the cross-peaks of H-4 with H-5, 3-CHO, and another cross-peaks displayed H-5 to H-7", H-4 (Figure 1 and Supplemental Figure S6). This type of binary compounds previously has been reported from *C. excavata* as carbazomarin-A[10] and carbazomarin-B.[11] The spectral data of **1** in pyranocoumarin unit are the same as the previously reported compounds. However, the carbazole unit is different from **1**. The spectral data of **1** revealed that the carbazole unit is similar to 7-hydroxyheptaphylline. In addition, the previously reported binary compounds explained that the coumarin unit was substituted at ring A of carbazole, whereas **1** was substituted at ring C. Hence, the structure of **1** was assigned to be (*S*)-2,7-dihydroxy-6-(5-hydroxy-8,8-dimethyl-10-(2-methylbut-3-en-2-yl)-2-oxo-7,8-dihydro-2H,6H-pyrano[3,2-g]chromen-6-yl)-1-(3-methylbut-2-en-1-yl)-9H-carbazole-3-carbaldehyde (Figure 2). It was named as carbazomarin-C.

Careful analyses were carried out to determine the physicochemical properties and spectroscopic data. Later on, a comparison step was carried out with the previously reported literatures where 2 known compounds were identified as mukonine[2,6] and xanthoxyletin[3,12] as shown in Figure 2. All of the isolated compounds were then undergone examination to measure antidiabetic activity. The examination was done by using yeast α-glucosidase inhibitory assay. Meanwhile, in order to measure the antioxidant activity, DPPH assay (Table 2) was carried out. There was a potent inhibition demonstrated by the isolated compounds **1** and **3** against yeast α-glucosidase with IC_{50} values 0.22 and 4.81 mM. Both **1** and **3** have stronger inhibition activity than standard acarbose (IC_{50} value 4.89 mM). Especially the new compound, carbazomarin-C (**1**), which has inhibitory effect exhibited the highest values. Unfortunately, there were no inhibition on antioxidant activity presented by all of the isolated compounds. Referring to the newest studies, the isolated components from the root of *C. excavata* possess the potentials to act as natural α-glucosidase inhibitors. More

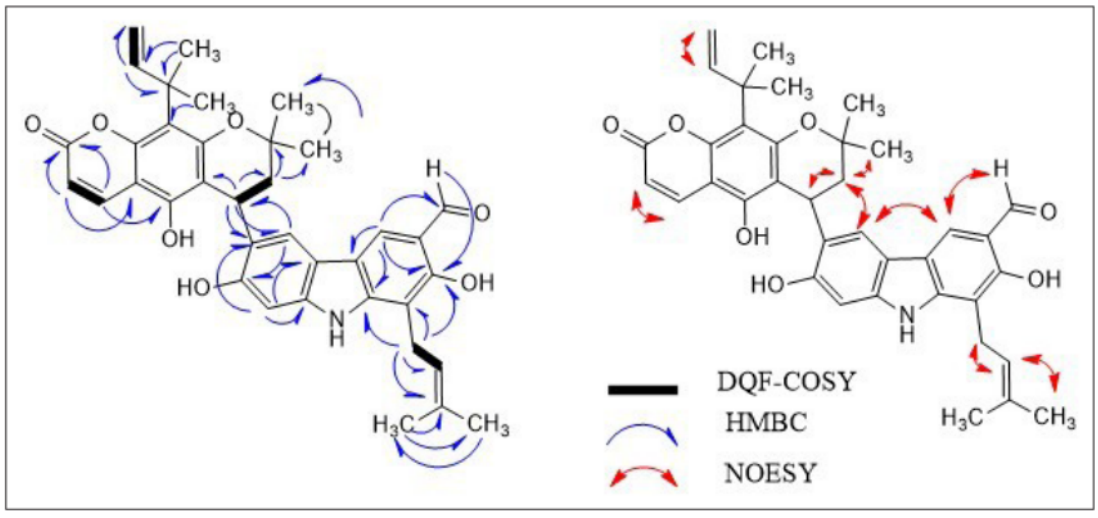

Figure 1. COSY (black bold), key HMBC (blue), and NOESY (red) correlation of **1**.

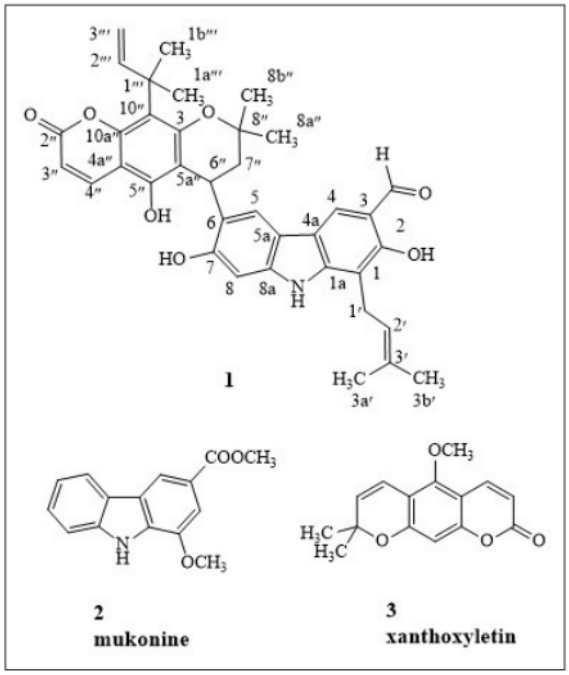

Figure 2. The structure of *Clausena excavata's* isolated compounds.

researches on maltase and sucrase α-glucosidase inhibition activity are strongly recommended.

Experimental

Plant Material

The collection of *C. excavata* was done in Myanmar, precisely on Pyin Ma Nar Township. The plants' substantial (voucher specimen MU-22032018) were collected on October 2016 under the authentication of Prof. Soe Myint Aye. Prof. Aye works as a botanist in the Botany Department of Mandalay University in Myanmar.[4]

Extraction and Isolation

The roots sample of *C. excavata* (3.6 kg) was air-dried before finally being extracted with 95% EtOH (12.0 L) for 14 days under ambient temperature. After removing the solvent, 156 g of extract was obtained. Afterward, as much as 100 g of the extract was partitioned by liquid-liquid extraction. It was successfully done 3 times by using the solvent of *n-hexane* and MeOH with the ratio of 1:1 in volumes. Furthermore, a vacuum liquid chromatography (VLC) was exposed to as much as 80.4 g of methanol portion over silica gel which was eluted with different mixtures of *n*-hexane and ethylacetate. It was done by slowly intensifying the gradient polarity until 7 combined fractions (MF-1 to -7) were acquired. Fraction MF-3.2 was exposed to silica gel column

Table 2. α-Glucosidase Inhibitory and the Isolated Compounds' (**1**-**3**) Radical Scavenging Activities.

Compound	α-Glucosidase, yeast IC_{50} (mM)	DPPH IC_{50} (mM)
1	0.22	NI
2	NI	NI
3	4.81	NI
Acarbose	4.89	-
Ascorbic acid	-	0.01

NI, no inhibition.

chromatography. There were 3 different solvent systems precisely the mixture between *n*-hexane, $CHCl_3$, and EtOAc with the ratio 100:5:20. As a result, there were 300 fractions gained and produced 6.8 mg of compound (**2**) and 15.3 mg of compound (**3**). A total of 25.6 g of fraction MF-6 was exposed to VLC with the mixtures *n-hexane*:EtOAc (EtOAc, 10%-100%) with gradient polarity. The outcome produced 23 subfractions, and after combining the same component fractions, it created MF-6.1 to -6.7. Silica gel column chromatography was exposed to Fraction MF-6.2.1.3 with various solvents ($CHCl_3$:MeOH/MeOH 5%) which produced a total of 99 fractions. After the recrystallization of fraction 82 to 88, a new compound (**1**, 10.6 mg) was obtained.

Carbazomarin-C, yellowish solid, mp. 251°C-252°C; UV (MeOH), λ_{max} (log ε) 335 (1.23), 279 (2.08), 228 (1.53). ^{1}H NMR (Methanol-d_4, 600 MHz), δ 9.75 (1H, s), 8.04 (1H, d, J = 9.6 Hz), 7.88 (1H, s), 7.41 (1H, s), 6.97 (1H, s), 6.35 (1H, dd, J = 17.4, 10.6 Hz), 6.00 (1H, d, J = 9.6 Hz), 5.41-5.24 (2H, m), 4.93 (2H, dd, J = 17.4, 1.2 Hz), 4.85 (2H, dd, J = 10.6, 1.2 Hz), 4.78-4.73 (3H, m), 3.54 (3H, d, J = 6.8 Hz), 3.35 (1H, s), 2.31 (2H, dd, J = 13.8, 7.8 Hz), 2.04 (3H, dd, J = 13.8, 10.1 Hz), 1.90 (0H, s), 1.83 (4H, s), 1.72 (6H, s), 1.68 (3H, s), 1.68 (3H, s), 1.41 (3H, s), 1.34 (3H, s) and ^{13}C NMR (151 MHz, Methanol-d_4) δ 195.78, 162.58, 159.54, 158.62, 156.75, 153.87, 150.76, 145.26, 144.77, 140.83, 132.02, 124.08, 123.68, 121.22, 117.73, 116.80, 114.78, 110.12, 109.76, 108.93, 107.65, 106.63, 103.99, 97.20, 76.47, 42.78, 41.66, 40.88, 40.52, 29.41, 28.91, 28.15, 24.49, 24.49, 23.75, 23.03, 22.33, 16.66.

Declaration of Conflicting Interests

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ORCID ID

Nanik S. Aminah https://orcid.org/0000-0002-2767-6006

Supplemental Material

Supplemental material for this article is available online.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5