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Propolis Components from Stingless Bees Collected on South Sulawesi, Indonesia, and Their Xanthine Oxidase Inhibitory Activity

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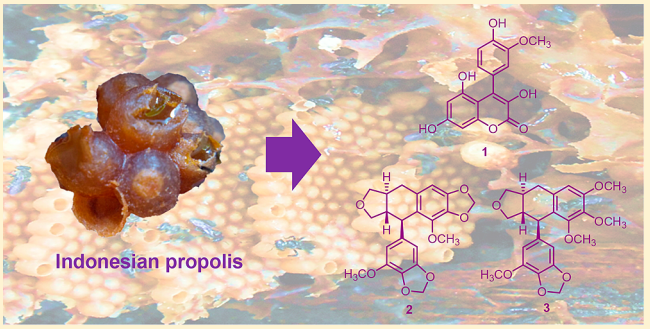
Supporting Information

ABSTRACT: Three new compounds, namely, 4-(4′-hydroxy-3′-methoxyphenyl)-3,5,7-trihydroxycoumarin (**1**) and sulawesins A (**2**) and B (**3**), were isolated from the propolis of stingless bees (*Tetragonula* aff. *biroi*) collected on South Sulawesi, Indonesia. In addition, five known compounds, glyasperin A, broussoflavonol F, (2*S*)-5,7-dihydroxy-4′-methoxy-8-prenylflavanone, (1′*S*)-2-*trans*,4-*trans*-abscisic acid, and (1′*S*)-2-*cis*,4-*trans*-abscisic acid, were identified. The structures of the new compounds were determined by a combination of methods that included mass spectrometry and NMR spectroscopy. The absolute configuration of sulawesin A (**2**), a new podophyllotoxin derivative, was determined by X-ray crystallography. The absolute configuration of sulawesin B (**3**) was also determined by the ECD calculation. 4-(4′-Hydroxy-3′-methoxyphenyl)-3,5,7-trihydroxycoumarin (**1**) and sulawesin A (**2**) were examined for xanthine oxidase (XO) inhibitory activity; **1** exhibited XO inhibitory activity, with an IC_{50} value of 3.9 μM.



Propolis is a natural resinous substance collected from the buds and exudates of certain trees and plants by honeybees, *Apis mellifera*. Propolis has been reported to display a variety of biological activities, including antibacterial, anti-inflammatory, antioxidant, and anticancer properties, and is used as a folk medicine in many regions of the world.[1−3] Generally, propolis is used in foods, beverages, and supplements to improve health and prevent conditions such as inflammation, heart disease, and cancer, as well as in cosmetics.[4−6]

The chemical components of propolis depend on the vegetation at the collection site, as honeybees collect resins from target plants grown near beehives as sources of propolis. For example, green propolis from Minas Gerais State, Brazil, contains many terpenoids and prenylated derivatives of *p*-coumaric acid, particularly artepillin C and (*E*)-3-prenyl-4-(dihydrocinnamoyloxy)cinnamic acid, as the young leaves of *Baccharis dracunculifolia* are the propolis source.[7] On the other hand, propolis from Europe and China contains many flavonoids and phenolic acid esters, such as pinocembrin, chrysin, and caffeic acid phenethyl ester, as the bud exudates of *Populus* species are the major propolis source.[8,9] Previously, we found that *Macaranga tanarius* is the source of propolis from Okinawa, which is the southernmost prefecture of Japan. Okinawan propolis contains many prenylflavonoids that exhibit strong antioxidant activities and are not present in the propolis from other regions.[10] Furthermore, differences in plant origins also affect propolis properties, such as biological activity, texture, flavor, and color.

In this study, we aimed to examine the propolis from stingless bees. Stingless bees belong to the Meliponini tribe.[11] There are more than 300 reported species in the Meliponini tribe, which are found in tropical regions of the world.[12] The nests of *Apis mellifera* honeybees are made from their beeswax, and their hives are coated with propolis as a sealant. On the other hand, the nests of stingless bees are constructed of propolis, because stingless bees do not produce hexagonal beeswax combs. The entire nests of stingless bees in tropical regions are referred to as "propolis" and used as the ingredients of soaps and mouthwashes. Despite this, the propolis from stingless bees has not been well studied.

With this in mind, the components of propolis from stingless bees (*Tetragonula* aff. *biroi*) collected on South Sulawesi, Indonesia, and their biological activities were studied to assess their potential utility. Three new compounds (**1**−**3**) and five known compounds were isolated, and their structures were determined by spectroscopic analysis. In addition, the absolute configuration of sulawesin A (**2**) was determined by X-ray structure analysis. Moreover, some of the isolated compounds were tested for xanthine oxidase (XO) inhibitory activity.

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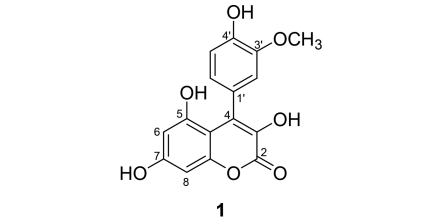
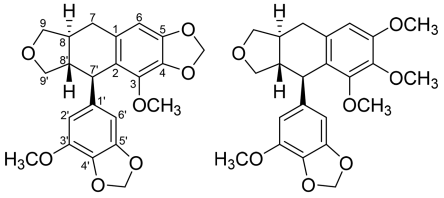
Herein, we report the determination of the structures of the isolated compounds, as well as XO inhibitory activity testing.

RESULTS AND DISCUSSION

Propolis from the stingless bee *Tetragonula* aff. *biroi* was extracted with 70% EtOH by stirring at room temperature. The extract was suspended in H_2O and successively partitioned with *n*-hexane and EtOAc to yield *n*-hexane-, EtOAc-, and H_2O-soluble fractions, respectively. Further separation and purification of the *n*-hexane and EtOAc fractions led to the identification of three new compounds (**1**–**3**). The five known compounds glyasperin A, broussoflavonol F, (2*S*)-5,7-dihydroxy-4′-methoxy-8-prenylflavanone, (1′*S*)-2-*trans*,4-*trans*-abscisic acid, and (1′*S*)-2-*cis*,4-*trans*-abscisic acid were also identified.



1



2 **3**

Compound **1** was isolated as a yellow powder. Its molecular formula was determined to be $C_{16}H_{12}O_7$ by high-resolution ESIMS (HRESIMS). The ^{1}H NMR spectrum of **1**, which is summarized in Table 1, revealed signals assignable to two *ortho*-coupled aromatic protons at δ_H 7.00 (d, J = 8.5, H-5′) and 7.75 (dd, J = 8.5, 2.0, H-6′) and two *meta*-coupled protons at δ_H 7.75 (H-6′) and 7.81 (d, J = 2.0, H-2′). These resonances suggest the presence of a 1,3,4-trisubstituted benzene ring. This interpretation was supported by the ^{13}C NMR spectrum of **1**, which revealed six signals assignable to aromatic carbons at δ_C 112.2 (C-2′), 116.0 (C-5′), 122.2 (C-6′), 122.4 (C-1′), 147.8 (C-3′), and 149.3 (C-4′) (Table 1). The ^{1}H NMR spectrum also exhibited signals assignable to two *meta*-coupled protons at δ_H 6.25 (d, J = 1.9, H-6) and 6.53 (d, J = 1.9, H-8). These resonances suggest the presence of another benzene ring. The ^{13}C NMR spectrum showed a typical deshielded resonance assignable to a conjugated ester group at δ_C 176.3 (C-2). The ^{1}H NMR spectrum also exhibited deshielded resonances assignable to four OH protons at δ_H 9.49 (3-OH), 9.80 (4′-OH), 10.84 (7-OH), and 12.53 (5-OH). These structural units and HMBC correlations from the 3-OH proton to carbons C-2, C-3, and C-4, from the 5-OH proton to carbons C-5, C-6, and C-10, from the 7-OH proton to carbons C-6, C-7, and C-8, from the H-6 proton to carbon C-10, and from the H-8 proton to carbon C-9 establish the presence of a 3,5,7-trihydroxycoumarin unit (Figure 1). A proton signal at δ_H 3.90 (s, 3′-OCH_3) and a carbon signal at δ_C 56.2 (3′-OCH_3) suggest the presence of a methoxy group. This structural unit and HMBC correlations from the 4′-OH proton to carbons C-3′, C-4′, and C-5′ and from the 3′-OCH_3 methyl protons to carbon C-3′ establish the presence of a 4′-hydroxy-3′-methoxyaryl moiety. HMBC correlations from H-2′ and H-5′ to carbon C-4 establish the attachment of a 4′-hydroxy-3′-methoxyaryl moiety at C-4. Based on these spectroscopic analyses, **1** was determined to be 4-(4′-hydroxy-3′-methoxyphenyl)-3,5,7-trihydroxycoumarin.

Compound **2** was obtained as a white powder. Its molecular formula was determined to be $C_{22}H_{22}O_7$ by HRESIMS. The nonequivalent methylene protons at δ_H 3.48 (dd, J = 10.1, 8.0, H-9) and 4.16 (t, J = 7.4, H-9) and δ_H 3.61 (dd, J = 10.2, 8.0, H-9′) and 3.94 (t, J = 7.4, H-9′) in the ^{1}H NMR spectrum of **2**, suggest that these methylene units are bonded to oxygen atoms (Table 1). These structural units and COSY correlations between protons H-7 (δ_H 2.69 and 2.89) and H-8 (δ_H 2.12), between protons H-8 and H-9 (δ_H 3.48 and 4.16), between protons H-8 and H-8′ (δ_H 2.00), between protons H-7′ (δ_H 3.83) and H-8′, and between protons H-8′ and H-9′ (δ_H 3.61 and 3.94) established the presence of an octahydroisobenzofuran skeleton (Figure 1). The ^{1}H NMR spectrum of **2** revealed signals assignable to three aromatic protons at δ_H 6.42 (s, H-6), 6.24 (s, H-2′), and 6.27 (s, H-6′). The signals at δ_H 5.89 (d, J = 1.0), 5.92 (d, J = 1.0), and 5.94 (s) were assigned to the methylene protons of the 4′,5′-OCH_2O and 4,5-OCH_2O units, respectively. The presence of two methoxy groups was indicated by the two proton signals at δ_H 3.89 (s, 3-OCH_3) and 3.35 (s, 3′-OCH_3), and the two carbon signals at δ_C 56.8 (3-OCH_3) and 58.5 (3′-OCH_3). These spectroscopic data and the HMBC correlations from the 4,5-OCH_2O methylene protons to carbons C-4 (δ_C 136.2) and C-5 (δ_C 148.1), from the 4′,5′-OCH_2O methylene protons to carbons C-4′ (δ_C 133.0) and C-5′ (δ_C 142.1), from the 3-OCH_3 protons to the C-3 carbon (δ_C 143.3), from the 3′-OCH_3 methyl protons to the C-3′ carbon (δ_C 148.7), from the H-6 proton (δ_H 6.42) to the C-5 carbon, and from the H-6′ proton (δ_H 6.27) to the C-5′ carbon suggested the presence of 3-methoxy-4,5-methylenedioxybenzene and 3′-methoxy-4′,5′-methylenedioxybenzene units (Figure 1). HMBC correlations from the H-6 proton to the C-1 carbon (δ_C 131.6), from the H-7 proton to carbons C-1 and C-6 (δ_C 103.5), and from the H-7′ proton to the C-2 carbon (δ_C 125.3) established the attachment of an octahydroisobenzofuran skeleton to the 3-methoxy-4,5-methylenedioxybenzene unit at C-1 and C-2. Moreover, HMBC correlations from the H-7′ proton to carbons C-1′ (δ_C 143.3), C-2′ (δ_C 100.7), and C-6′ (δ_C 106.1) and from the H-8′ proton to carbon C-1′ established the attachment of an octahydroisobenzofuran skeleton to the 3′-methoxy-4′,5′-methylenedioxybenzene at C-1′. Single crystals of **2** were obtained from 2-propanol and were subjected to single-crystal X-ray diffraction with Cu Kα radiation. The absolute configurations of the stereocenters in **2** were finally established as 8*S*, 7′*R*, 8′*S* with a Flack parameter of −0.09(5). The ORTEP drawing of **2** is shown in Figure 2. In the crystal, a C−H···π interaction between the 3′-methoxy-4′,5′-methylenedioxybenzene units was observed. On the other hand, a π–π interaction was not seen. Based on these spectroscopic and crystallographic analyses, **2** was determined to be a new podophyllotoxin derivative and assigned the name "sulawesin A".

Compound **3** was obtained as a colorless oil. Its molecular formula was determined to be $C_{23}H_{26}O_7$ by HRESIMS. The 1D NMR spectrum of **3** resembled that of **2** (Table 1). The differences of compounds **2** and **3** were substitutions on one aromatic ring (methylene dioxy and methoxy versus trimethoxy). The HMBC spectrum showed correlations from

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Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data for New Compounds 1−3 (δ in ppm, J in Hz)

	1[a]		2[b]		3[b]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1			131.6, C		132.4, C	
2	176.3, C		125.3, C		125.4, C	
3	136.3, C		143.3, C		152.5, C	
4	147.1, C		136.2, C		140.9, C	
5	161.1, C		148.1, C		152.4, C	
6	98.7, CH	6.25, d (1.9)	103.5, CH	6.42, s	107.6, CH	6.40, s
7	164.4, C		33.5, CH_2	2.69, dd (13.6, 6.8)	33.2, CH_2	2.65, dd (12.8, 5.2)
				2.89, dd (15.4, 3.8)		2.83, dd (15.4, 4.0)
8	94.1, CH	6.53, d (1.9)	41.7, CH	2.12, m	41.3, CH	2.05, m
9	156.6, C		72.7, CH_2	3.48, dd (10.1, 8.0)	72.7, CH_2	3.46, dd (10.0, 8.0)
				4.16, t (7.4)		4.14, t (7.4)
10	103.5, C					
1'	122.4, C		143.3, C		142.8, C	
2'	112.2, CH	7.81, d (2.0)	100.7, CH	6.24, s	106.1, CH	6.18, s
3'	147.8, C		148.7, C		143.5, C	
4'	149.3, C		133.0, C		133.2, C	
5'	116.0, CH	7.00, d (8.5)	142.1, C		148.9, C	
6'	122.2, CH	7.75, dd (8.5, 2.0)	106.1, CH	6.27, s	100.8, CH	6.16, s
7'			46.6, CH	3.83, d (10.3)	46.3, CH	3.74, obscured
8'			53.1, CH	2.00, m	52.7, CH	1.96, m
9'			72.5, CH_2	3.61, dd (10.2, 8.0)	72.6, CH_2	3.55, dd (10.0, 8.0)
				3.94, t (7.4)		3.90, t (7.4)
4,5-OCH_2O			101.0, CH_2	5.94, s		
4',5'-OCH_2O			101.2, CH_2	5.89, d (1.0)	101.3, CH_2	5.84, s
				5.92, d (1.0)		
3-OCH_3			56.8, CH_3	3.89, s	59.6, CH_3	3.16, s
4-OCH_3					60.5, CH_3	3.68, s
5-OCH_3					56.8, CH_3	3.78, s
3'-OCH_3	56.2, CH_3	3.90, s	58.5, CH_3	3.35, s	55.9, CH_3	3.79, s
3-OH		9.49, s				
5-OH		12.53, s				
7-OH		10.84, s				
4'-OH		9.80, s				

[a]Measured in DMSO-d_6. [b]Measured in $CDCl_3$.

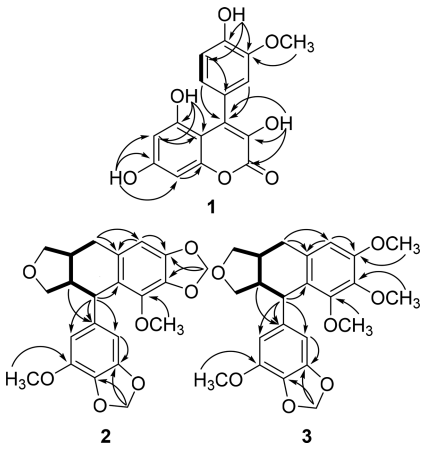

Figure 1. Connectivities (bold line) determined by COSY spectra and significant HMBC correlations (solid arrows) observed for new compounds 1−3.

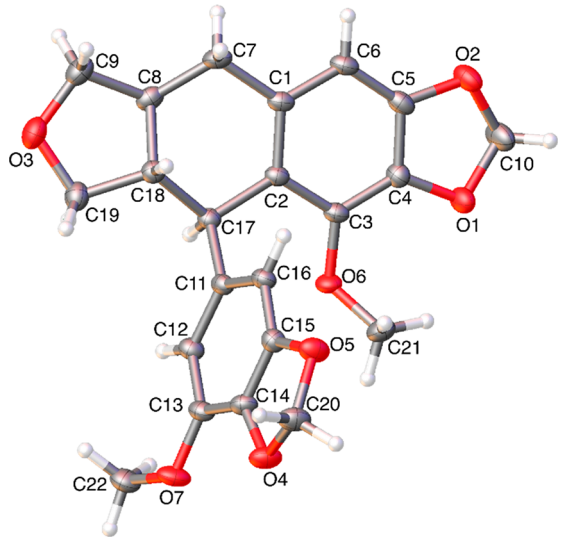

Figure 2. Molecular structure of 2, with displacement ellipsoids drawn at the 50% probability level. H atoms are shown as small spheres of arbitrary radius.

the 3-OCH_3 methyl protons (δ_H 3.16) to the C-3 carbon (δ_C 152.5), from the 4-OCH_3 methyl protons (δ_H 3.68) to the C-4 carbon (δ_C 140.9), and from the 5-OCH_3 methyl protons (δ_H 3.78) to the C-5 carbon (δ_C 152.4) (Figure 1). The HMBC

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and COSY spectrum of **3** showed similar correlations to **2**. Based on these spectroscopic analyses, **3** was determined to be a new podophyllotoxin derivative that was assigned the name "sulawesin B". The experimental electronic circular dichroism (ECD) spectrum of **3** showed one large positive cotton effect at 220 nm, and its λ_{max} and band shape were almost identical with those of **2** (Figure 3). Furthermore, the calculated ECD

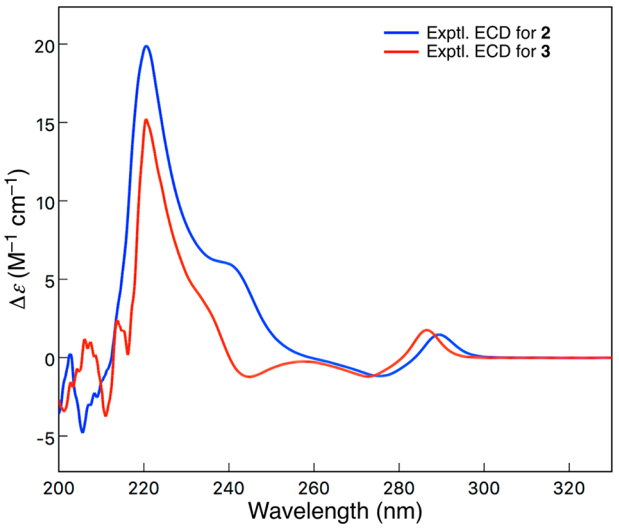

Figure 3. Experimental ECD spectra of **2** and **3** (MeOH).

spectrum of the (8*S*,7′*R*,8′*S*) diastereoisomer of **3** was in good accordance with the experimental one (Figure S25, Supporting Information). Hence, the absolute configuration of **3** was established as (8*S*,7′*R*,8′*S*).

From the 1D and 2D NMR, MS, ECD, and specific rotation data and comparisons with literature data, the known compounds were identified to be glyasperin A,[13] broussoflavonol F,[14] (2*S*)-5,7-dihydroxy-4′-methoxy-8-prenylflavanone,[15] (1′*S*)-2-*trans*,4-*trans*-abscisic acid,[16,17] and (1′*S*)-2-*cis*,4-*trans*-abscisic acid.[16,17]

Because 4-(4′-hydroxy-3′-methoxyphenyl)-3,5,7-trihydroxycoumarin (**1**) and sulawesin A (**2**) were isolated as new compounds in high yields, they were evaluated for XO inhibitory activity. As a result, sulawesin A (**2**) exhibited little XO inhibitory activity, whereas 4-(4′-hydroxy-3′-methoxyphenyl)-3,5,7-trihydroxycoumarin (**1**) showed potent XO inhibitory activity, with an IC_{50} value of 3.9 μM. This IC_{50} value is close to that of allopurinol, which is used as an antigout drug (IC_{50}: 1.0 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a DIP-1000 digital polarimeter (Jasco). UV−vis spectra were acquired using a V-560 UV/vis spectrophotometer (Jasco). ECD spectra were obtained using a J-600 spectrometer (Jasco) and a J-820 spectrometer (Jasco). IR spectra were recorded by an FT/IR-550 spectrometer (Jasco). 1D and 2D NMR spectra were acquired on a Bruker Biospin AVANCE-III (400 MHz) spectrometer, with chemical shifts expressed in ppm. The NMR spectra were referenced to residual solvent peaks (DMSO-d_6: ^{1}H NMR 2.49 ppm, ^{13}C NMR 39.7 ppm; $CDCl_3$: ^{1}H NMR 7.26 ppm, ^{13}C NMR 77.0 ppm; CD_3OD: ^{1}H NMR 3.30 ppm, ^{13}C NMR 49.0 ppm). HRESIMS spectra were acquired on a Thermo Fisher Scientific Q-Exactive HR-ESI-Orbitrap-MS. Silica gel column chromatography was carried out using silica gel 60N (230−400 mesh, Kanto Chemical). For RP-HPLC separations with a recycling system, a PU-1586 Intelligent prep pump (Jasco), UV-8010 detector (Tosoh), CAPCELL PAK UG 120 C18 column (5 μm, 20 × 250 mm, Shiseido), Shiseido CAPCELL PAK UG120 C18 column (5 μm, 10 × 250 mm), Shiseido CAPCELL PAK ACR C18 column (5 μm, 20 × 250 mm), and HPLC-grade solvents were used. For analytical HPLC, a PU-2089 Plus quaternary gradient pump (Jasco), an MD-4017 photodiode array detector (Jasco), and an AS-4050 HPLC autosampler (Jasco) were used. Data were analyzed using ChromNAV software (v.2, Jasco).

Biological Material. Stingless bee propolis (*Tetragonula* aff. *biroi*) was collected in December 2015 in North Luwu, South Sulawesi Province, Indonesia. The stingless bee species was identified by Dr. Sih Kahono from the Laboratorium Entomologi, Museum Zoologicum Bogorience, Pusat Penelitian Biologi LIPI (Entomology Laboratory, Museum Zoologicum Bogorience, Biology Research Center, The Indonesia Science Institute). A voucher sample of the propolis (1512RPPD01) studied in this paper has been deposited at PT RIN Biotek Indonesia, South Tangerang, Banten, Indonesia.

Extraction and Isolation. Stingless bee propolis (100 g) was extracted with 70% EtOH (1.3 L) with stirring at room temperature for 24 h, after which the solids were removed by filtration. The filtrates were concentrated at reduced pressure to give an EtOH extract (25 g). This extract was suspended in H_2O (300 mL) and successively partitioned with *n*-hexane (2 × 300 mL) and EtOAc (2 × 300 mL) to give *n*-hexane- (6.1 g), EtOAc- (4.1 g), and H_2O-soluble extracts, respectively. The *n*-hexane-soluble fraction (6.1 g) was subjected to silica gel column chromatography (20 × 400 mm), with *n*-hexane/EtOAc−MeOH gradient mixtures (4:1, 850 mL; 3:1, 1250 mL; 2:1, 200 mL; 1:1, 400 mL; 0:1, 200 mL; MeOH 400 mL) as eluents with 200 mL for each fraction, to yield 17 fractions (fr. 1, 799 mg; fr. 2, 2.44 g; fr. 3, 289 mg; fr. 4, 790 mg; fr. 5, 130 mg; fr. 6, 314 mg; fr. 7, 14 mg; fr. 8, 211 mg; fr. 9, 16 mg; fr. 10, 75 mg; fr. 11, 30 mg; fr. 12, 214 mg; fr. 13, 54 mg; fr. 14, 148 mg; fr. 15, 12 mg; fr. 16, 392 mg; fr. 17, 241 mg). The EtOAc-soluble fraction (4.1 g) was subjected to silica gel column chromatography (30 × 500 mm), with *n*-hexane/EtOAc−MeOH gradient mixtures (4:1, 400 mL; 3:1, 500 mL; 2:1, 300 mL; 1:1, 300 mL; 0:1, 300 mL; MeOH 300 mL) as eluents with 200 mL for each fraction, to yield nine fractions (fr. 18, 226 mg; fr. 19, 1.03 g; fr. 20, 404 mg; fr. 21, 237 mg; fr. 22, 453 mg; fr. 23, 222 mg; fr. 24, 564 mg; fr. 25, 268 mg; fr. 26, 853 mg). Fraction 6 was subjected to preparative RP-HPLC with H_2O−MeCN (40:60, 0.1% trifluoroacetic acid (TFA)) as the eluent to give (2*S*)-5,7-dihydroxy-4′-methoxy-8-prenylflavanone (1.3 mg, t_R: 15 min) and **2** (31.9 mg, t_R: 10 min). Fraction 8 was subjected to preparative RP-HPLC with H_2O−MeCN (40:60, 0.1% TFA) as the eluent to give glyasperin A (6.8 mg, t_R: 50 min), broussoflavonol F (6.8 mg, t_R: 30 min), and **3** (3.1 mg, t_R: 15 min). Fraction 22 was subjected to preparative RP-HPLC with H_2O−MeCN (75:25, 0.1% TFA) as the eluent to give **3** (17.0 mg, t_R: 15 min). Fraction 24 was subjected to preparative RP-HPLC with H_2O−MeCN (75:25, 0.1% TFA) as the eluent to give (1′*S*)-2-*trans*,4-*trans*-abscisic acid (3.6 mg, t_R: 20 min) and (1′*S*)-2-*cis*,4-*trans*-abscisic acid (3.9 mg, t_R: 25 min) followed by **1** (7.7 mg, t_R: 15 min) with H_2O−MeCN (50:50, 0.5% TFA) as the eluent. All preparative RP-HPLC separations were performed at a flow rate of 10 mL/min, and compounds were detected at a wavelength of 270 nm. The purity by HPLC of all isolated compounds at a wavelength of 270 nm is >98%.

*4-(4′-Hydroxy-3′-methoxyphenyl)-3,5,7-trihydroxycoumarin (**1**):* yellow powder; UV (MeOH) λ_{max} (log ε) 254 (4.17), 372 (4.19); IR ν_{max} (KBr) 3258, 2361, 1656, 1615, 1509 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6, 400 MHz), Table 1; HRESIMS *m*/*z* 315.0506 $[M - H]^-$ (calcd for $C_{16}H_{11}O_7$, 315.0505).

*Sulawesin A (**2**):* white powder; $[\alpha]_D^{30}$ +62.3 (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 282 (3.38); ECD (*c* 1.50 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 289 (1.47), 275 (−1.17), 240 (6.00), 220 (19.89), 205 (−4.80); IR ν_{max} (KBr) 2929, 2857, 1632, 1477 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$, 400 MHz), Table 1; HRESIMS *m*/*z* 399.1431 $[M + H]^+$ (calcd for $C_{22}H_{23}O_7$, 399.1444).

*Sulawesin B (**3**):* colorless oil; $[\alpha]_D^{29}$ +48.9 (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 278 (3.29); ECD (*c* 1.65 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 286 (1.76), 273 (−1.20), 245 (−1.22), 233 (3.81), 220

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(15.22), 213 (2.39), 211 (−3.76); IR ν_{max} (KBr) 2935, 1635, 1489 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 400 MHz), Table 1; HRESIMS m/z 415.1732 $[M + H]^+$ (calcd for $C_{23}H_{27}O_7$, 415.1757).

X-ray Crystallographic Data for 2. Crystals of compound **2** were obtained from 2-propanol, and the absolute configuration of **2** was determined from data collected on a Rigaku XtalLAB Synergy-S diffractometer with Cu Kα radiation (λ = 1.541 87) at T = 100.2(5) K. The structure was solved by the SHELXT method and refined based on full-matrix least-squares on F^2 using SHELXL.[18] Crystallographic data for sulawesin A (**2**): plates, colorless, crystal size 0.343 × 0.077 × 0.040 mm, $C_{22}H_{22}O_7$, M = 398.39, monoclinic space group, $P2_1$, a = 11.01570(10) Å, b = 6.75950(10) Å, c = 13.03660(10) Å, β = 106.3730(10)°, V = 931.348(18) Å^3, Z = 2, D_{calcd} = 1.421 g/m^3, 37 659 collected reflections (7.068° ≤ 2θ ≤ 153.24°), μ(Cu Kα) = 0.885 mm^{-1}, R_1 = 0.0322 for $I \geq 2\sigma(I)$, and wR_2 = 0. 0857 (all data), S = 1.081, Flack parameter = −0.09(5), Hooft parameter = −0.09(9). Crystallographic data for **2** have been deposited at the Cambridge Crystallographic Data Centre (CCDC number 1850729).

ECD Calculations for 3. Three-dimensional (3D) structure generation and conformational analysis of stereoisomers for **3** were carried out by use of a shell script previously reported.[19] Briefly, 300 energy-minimized 3D structures of the stereoisomers were generated from the 2D chemical structures by Open Babel and Balloon.[20,21] The single-point energy of each conformer was calculated with the PM7 Hamiltonian by MOPAC2016.[22] The several low-energy conformers were geometrically optimized with the B3LYP/6-31G(d,p) level of density functional theory (DFT) in the gas phase by Gaussian 09.[23] The theoretical calculation of ECD was conducted with the B3LYP/6-31G(d,p) level of time-dependent density functional theory (TDDFT) in MeOH using the conductor-like polarizable continuum model (CPCM) by Gaussian 09. The calculated ECD data were processed and visualized by GaussView 5.

XO Inhibitory Assays. Allopurinol, xanthine, and xanthine oxidase were purchased from Sigma-Aldrich (St. Louis, MO, USA). The XO inhibitory assays were carried out following a slightly modified, previously reported method.[24] The reaction medium, composed of 10 μL of 1 mM xanthine in DMSO and 160 μL of 12.5 mM phosphate buffer (pH 7.4), was preincubated at 37 °C for 5 min. XO buffer solution (0.020 units/mL, 20 μL) was added to the solution. After incubation at 37 °C for 10 min, 3% aqueous $HClO_4$ (25 μL) was added to terminate the reaction. To quantify the amount of uric acid produced, an aliquot (20 μL) of the solution was injected onto an HPLC column under the following conditions: column, 5 μm, 4.6 × 250 mm, Shiseido CAPCELL PAK UG120 C18; flow rate, 1.0 mL/min; eluent, 0.1% phosphoric acid in H_2O−MeOH (96:4, v/v); detection, 290 nm. Percent inhibition was calculated according to the following equation: inhibition (%) = [(peak area of uric acid in the control experiment) − (peak area of uric acid in the sample experiment)] × 100/(peak area of uric acid in the control experiment).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00541.

Additional figures (PDF)
X-ray crystal data (CIF)

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Notes

The authors declare no competing financial interest.

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