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Isolation of Sesquiterpenoids and Trypanocidal Constituents from *Artemisia adamsii*

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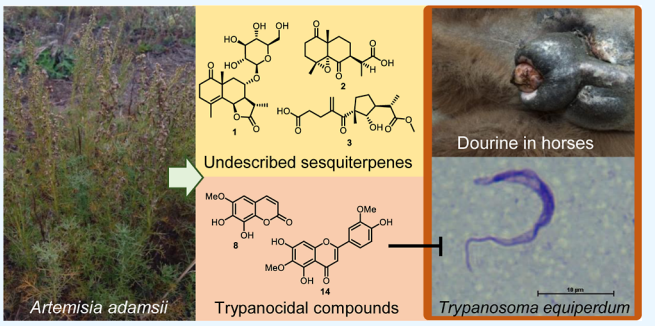
ABSTRACT: The Mongolian *Artemisia adamsii* (*A. adamsii*) plant is of particular interest from both medicinal and ecological perspectives. In this study, three previously undescribed sesquiterpenoids (**1**−**3**) were isolated, along with 25 known compounds, from naturally dried and browned aerial parts of *A. adamsii*, which were collected in the autumn from the pasturelands of Bayan Soum, Tuv Province, Mongolia. The chemical structures of the isolated undescribed compounds, including their relative and absolute configurations, were elucidated by high-resolution mass spectrometry, nuclear magnetic resonance spectroscopy, and single crystal X-ray crystallography. The trypanocidal activities of the isolated compounds against six species, namely *Trypanosoma brucei brucei*, *T. b. gambiense*, *T. b. rhodesiense*, *T. equiperdum*, *Trypanosoma congolense*, and *Trypanosoma evansi* were evaluated. Consequently, a coumarin (fraxetin) and a flavonoid (jaceosidin) were identified as exhibiting relatively potent activities. Quantitative analysis by high-performance liquid chromatography confirmed relatively high contents of these compounds in *A. adamsii*, indicating that they represent the main functional components of this plant. These results further demonstrate the potential medicinal and ecological significance of *A. adamsii*.



1. INTRODUCTION

In Mongolia, *Artemisia adamsii* Besseris (Asteraceae) is commonly found in degraded land and dry steppes based on soft soils.[1,2] In these areas, predominantly Artemisia plants, including *A. adamsii* (*A. adamsii*), thrive and are expected to facilitate the growth of other plant species.[3] Indeed, nomads recognized that these plants were initially distributed over degraded lands by overgrazing livestock and rodents such as voles. After a few years, these plants disappeared, allowing other plants to grow. Due to the arrival of grasses from other areas and subsequent entanglement with *A. adamsii*, this plant remains in place between the fall and winter months, providing valuable food to animals. Although pastoral livestock animals consume only small amounts of this plant in its green form, during the dry seasons (i.e., fall and winter), camels, horses, and cows consume it moderately.[4,5] Since 80% of Mongolia's agricultural sector is centered around animal husbandry, pastoral livestock and forage plants play a role in supporting the Mongolian economy and the nomadic inhabitants.[6] The characteristics of *A. adamsii* are therefore beneficial due to its ability to restore suitable pastures for agriculture. In addition to its environmental importance, *A. adamsii* is a key component of various traditional medicines. For instance, in Mongolian traditional medicine, the leaves and inflorescences of the plant are used to prepare a liquid for the treatment of sore throats and toothaches, while a jam has been used to relieve fever. In Tibetan medicine, the aerial parts of the plant have been used to prepare antipyretic prescriptions.[7] Although guaianolide sesquiterpenoids and some coumarins have been reported as key constituents of *A. adamsii*,[8] there is generally a lack of comprehensive scientific information regarding its other phytochemical components. Consequently, its ecological interactions and traditional uses have not been adequately supported by scientific evidence.

Trypanosomes are pathogenic protozoan parasites that can cause deadly human and animal diseases,[9] such as dourine, a sexually transmitted disease of horses that is caused by *Trypanosoma equiperdum*infection. Indeed, a nationwide surveillance of horse trypanosomes in Mongolia found that the overall seroprevalence of the disease in Mongolia was 4.8%.[10] In another study, *T. equiperdum* was isolated from the

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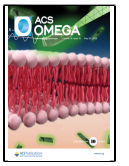

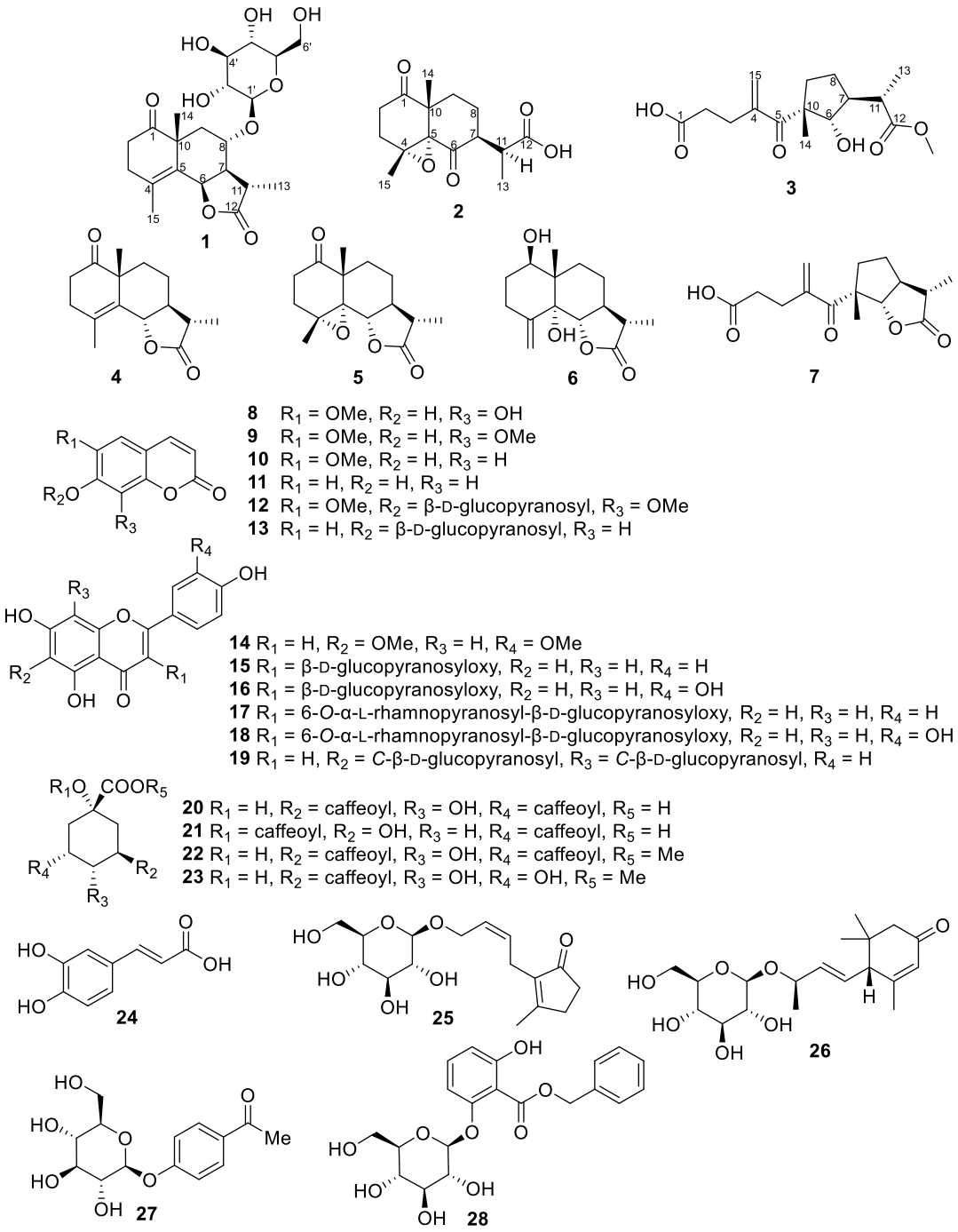

Figure 1. Chemical structures of isolated compounds **1**−**28**.

urethral tract of a Mongolian horse and was identified as a new strain, named *T. equiperdum* IVM-t1. This strain was adapted in vitro using soft agarose media.[11] Furthermore, a method was established for evaluating trypanocidal activity in horses.[12] Treatment with existing drugs has been tried with some success,[13] but various issues remain, and there is a requirement for more economic treatments that exhibit fewer side effects. As part of our research to discover trypanocidal substances, compounds obtained from *Artemisia sieversiana* were evaluated, and a number of previously undescribed sesquiterpenoids, trypanocidal flavonoids, and lignans were identified.[14] It is therefore desirable to identify and isolate active molecules from local plants for the treatment of trypanosomoses in Mongolia. Indeed, this would be of particular importance from the perspective of effectively utilizing local resources to resolve local problems.

In the present study, terpenoids, flavonoids, coumarins, and other compounds are isolated from *A. adamsii*, and their inhibitory activities against Mongolian *T. equiperdum* (in addition to other pathogenic species, such as *Trypanosoma brucei brucei*, *T. b. gambiense*, *T. b. rhodesiense*, *T. congolense*, and *T. evansi*) are evaluated. Overall, this work aims to search for potential pharmaceutical leads for curing protozoan diseases. In addition, this study is aimed at inspiring research into the ecological interactions of *A. adamsii* with animals and other

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Table 1. NMR Spectroscopic Data for Compounds 1−3

	1[a]				2[b]				3[b]			
position	δ_C	δ_H (J in Hz)	HMBC	NOESY	δ_C	δ_H (J in Hz)	HMBC	NOESY	δ_C	δ_H (J in Hz)	HMBC	NOESY
1	216.7				210.4				177.0			
2	36.3	2.62, m			34.2	2.65[c]	1		32.7	2.58[c]		
		2.35, m				2.30[c]				2.48[c]		
3	30.9	2.62, m			27.9	2.30[c]		15	28.3	2.58[c]	1,2,4,5,15	
		2.45, m				2.16[c]	4,5			2.48[c]		
4	140.1				63.9				145.2			
5	128.1				71.5				208.7			
6	78.9	5.66, d (6.0)	4,10	7,13	204.4				85.5	3.61, d (7.0)	5,8,9,11	11,14
7	49.8	2.22, dd (7.0, 6.0)		6,13	53.2	2.65, m	6	13	50.9	2.16, m	6,8,11,13	
8	77.8	3.80, m		11,14,1′	23.1	2.16[c]			25.2	1.82, m		8
						1.72, m		11,13,14		2.32[c]		8,11
9	38.9	2.09, m			32.7	2.16[c]		14	34.5	2.36[c]	10	9
		1.83, m				1.92, m				1.61, m		9
10	47.5				49.2				56.8			
11	42.3	2.85, q (7.5)	13	13	38.0	2.84, m	13	8,13	43.5	2.42, m	12	6,8,13
12	182.4				180.7				177.1			
13	15.6	1.38, d (7.5)	7,11,12	6,7,11	14.2	1.23 (d, 7.0)	7,11,12	7,8,11	15.2	1.15, d (7.0)	6,7,12	7,11
14	25.5	1.25, s	1,5,9,10	8	18.2	1.14, s	1,5,9,10	8	24.5	1.35, s	5,6,9,10	6,15
15	19.0	1.95, s	3,4,5		17.9	1.29, s	3,4,5	3	123.5	5.81, br s	3,4,5	14
										5.75, br s	3,4,5	
OMe									51.8	3.69, s	12	
1′	105.0	4.35, d (8.0)	8	8								
2′	75.3	3.09, dd (9.0, 8.0)										
3′	78.2	3.34, dd (9.0, 8.0)										
4′	71.4	3.25[c]										
5′	77.8	3.25[c]										
6′	62.7	3.83, dd (12.0, 2.0)										
		3.65, dd (12.0, 5.0)										

[a]In CD_3OD solution. [b]In $CDCl_3$ solution. [c]Unclear signal pattern due to overlapping.

plants, along with explorations of its potential therapeutic applications.

2. RESULTS AND DISCUSSION

2.1. Isolation and Structural Determination of the Undescribed and Known Compounds from *A. adamsii*.

Three new sesquiterpenoids (**1**−**3**) and 25 known compounds (**4**−**28**; Figure 1) were isolated from the extracts of dried *A. adamsii* areolas through HP-20 column chromatography and high-performance liquid chromatography (HPLC). The chemical structures of compounds **1**−**3** were investigated using a range of spectroscopic techniques, and their relative configurations were determined based on NOESY correlations. Compounds **4**−**6** were obtained as small rectangular prism crystals, and their structures and absolute/relative configurations were determined using X-ray crystallography. The absolute configurations of **1**−**3** were subsequently obtained based on a comparison with the data and conceivable biosynthetic pathways of the crystallized compounds. For example, all three crystals grown in this study had the same 7*S*,10*R*-configuration, and so it was expected that the series of obtained compounds would have the same configuration according to the general biosynthetic pathway of terpenoids. The known compounds were identified by comparison with reported spectroscopic data and with compounds that had been previously isolated in our laboratory. More specifically, these compounds were identified as gracilin (1-keto-6β,7α,11β-H-selin-4(5)-en-6,12-olide) (**4**),[15] martimin (**5**),[15] artemin (**6**),[16] santolinifolide A (**7**),[17] fraxetin (**8**),[18] isofraxidin (**9**),[19] isoscopoletin (**10**),[20] umbelliferone (**11**),[21] eleutheroside B1 (**12**),[22] cichoriin (**13**),[23] jaceosidin (**14**),[24] quercetin-3-*O*-β-D-glucopyranoside (**15**),[25] kaempferol 3-*O*-β-D-glucopyranoside (**16**),[25] kaempferol 3-*O*-rutinoside (**17**),[25] rutin (**18**),[25] vicenin-2 (**19**),[26] 3,5-di-*O*-caffeyol quinic acid (**20**),[27] 1,5-di-*O*-caffeyol quinic acid (**21**),[14] methyl 3,5-di-*O*-caffeoyl quinate (**22**),[28] chlorogenic acid methyl ester (**23**),[29] caffeic acid (**24**),[14] (*Z*)-5′-hydroxyjasmone-5′-*O*-β-D-glucopyranoside (**25**),[30] (6*R*,9*R*)-3-oxo-α-ionol-9-*O*-β-D-glucopyronoside (**26**),[31] picein (**27**),[32] and benzyl 2-*O*-β-D-glucopyranosyl-2,6-dihydroxybenzoate (**28**).[33] Notably, the chemical shifts obtained by nuclear magnetic resonance (NMR) spectroscopy were similar for the different structural isomers. Thus, the positions of the methoxy and hydroxy groups of trypanocidal compounds **8** and **14** were confirmed using two-dimensional (2D) NMR techniques based on HMBC and NOESY correlations (Figures S24−S35).

Compound **1** was acquired in the form of a white amorphous powder, and its optical rotation value was determined to be $[\alpha]_D^{20}$ −48.6 (*c* 1.4, MeOH). The observation of a sodium adduct $[M + Na]^+$ at *m*/*z* 449.1777 (calcd for $C_{21}H_{30}O_9Na^+$: 449.1787) by high-resolution (HR) fast atom bombardment (FAB) mass spectrometry (MS) indicated a molecular formula of $C_{21}H_{30}O_9$. In the ^{1}H NMR spectrum, resonances corresponding to three methyl groups

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were observed at δ_H 1.38 (3H, d, J = 7.5 Hz, H-13), 1.95 (3H, s, H-15), and 1.25 (3H, s, H-14), while signals corresponding to aliphatic protons (δ_H 5.66 (1H, d, J = 6.0 Hz, H-6), 3.80 (1H, m, H-8), 2.85 (1H, q, J = 7.5 Hz, H-11), 2.62 (overlapping, H-2 and H-3), 2.45 (1H, m, H-3), 2.35 (1H, m, H-2), 2.22 (1H, dd, J = 7.0 and 6.0 Hz, H-7), 2.09 (1H, m, H-9), and 1.83 (1H, m, H-9)) were also observed. Based on these proton resonances, their corresponding 10 carbon resonances defined from the HMQC spectrum, and the presence of two carbonyl signals (δ_C 216.7 (C-1) and 182.4 (C-12)), two olefinic carbon signals (δ_C 140.1 (C-4) and 128.1 (C-5)), and a quaternary carbon signal (δ_C 47.5 (C-10)), a sesquiterpenoid skeleton with a *cis*-lactone fusion junction was identified.[34] In the ^{1}H−^{1}H COSY spectrum of **1**, two coupled systems were clearly observed, i.e., (H-6, H-7, H-8 and H-9) and (H-11 and H-13). Furthermore, HMBC long-range coupling correlations from H-13 to C-7, C-11, and C-12; from H-14 to C-1, C-5, C-9, and C-10; and from H-15 to C-3, C-4, and C-5 were observed (Table 1 and Figure 2).

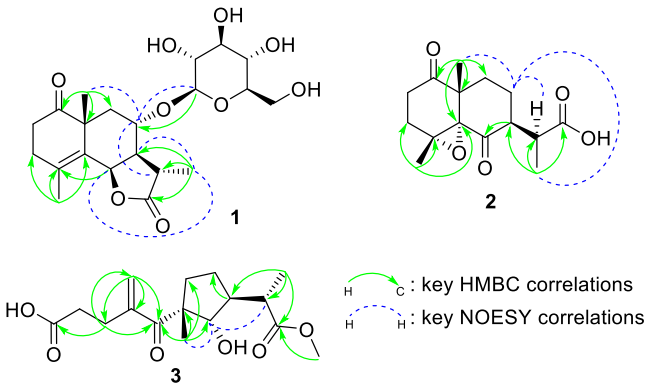

Figure 2. Key HMBC and NOESY correlations for compounds **1**−**3**.

These 2D correlations therefore indicated that the molecular structure of the sesquiterpenoid moiety was 4-hydroxy-3,5a,9-trimethyl-3a,5,5a,7,8,9b-hexahydronaphtho[1,2-*b*]furan-2,6-(3*H*,4*H*)-dione, as shown in Figure 1. X-ray crystallography results for **4**−**6** are shown in Figure 3.

Based on the general biosynthesis pathway concerning the carbon chain skeleton of terpenoids, the absolute configurations of C-7, C-10, and C-11 of **1** were hypothesized to be identical to those of **4**−**6**. Although the isolated compound **4** was identified as gracillin with an absolute configuration of 6*S*,7*S*,10*R*,11*S* (as determined by X-ray crystallography, see Figure 3), the NMR data suggested that compound **1** possesses the 6*R* configuration instead of 6*S*. More specifically, the observed coupling constant of 6.0 Hz reflects an (6*R*,7*R*)-configuration in **1**.[35] Furthermore, in the NOESY spectrum of **1**, the correlation between H-6 and H-13 implied a (6*R*,7*R*,11*S*)-configuration, as supported by the correlation between H-6 and H-7. In addition, NOE correlations between H-8 and H-11, and between H-8 and H-14 indicated that these protons existed in a β-orientation, thereby giving the 8*S* configuration (Figure 2). Consequently, the absolute configuration of **1** was determined to be (3*S*,3a*R*,4*S*,5a*R*,9b*R*)-4-hydroxy-3,5a,9-trimethyl-3a,5,5a,7,8,9b-hexahydronaphtho-[1,2-*b*]furan-2,6(3*H*,4*H*)-dione. Additionally, in the ^{1}H NMR spectrum of compound **1**, oxygenated proton resonances (δ_H 4.35, H, d, J = 8.0 Hz, H-1′; 3.09, H, dd, J = 9.0, 8.0 Hz, H-2′; 3.34, H, dd, J = 9.0, 8.0 Hz, H-3′; 3.25, 2H, overlapping, H-4′ and H-5′; 3.65, H, dd, J = 12.0, 5.0 Hz, H-6′; 3.83, H, dd, J = 12.0, 2.0 Hz H-6′) were observed. In the ^{13}C NMR spectrum, the six oxygenated carbons of the sugar moiety (δ_C 105.0, 75.3, 78.2, 71.4, 77.8 and 62.7, C-1′−6′) were assigned based on their chemical shifts and the corresponding HMQC spectra. Acid hydrolysis and modification prior to HPLC analysis indicated that compound **1** possesses a D-glucose moiety,[36] and the coupling constant of the β-anomeric proton (i.e., J = 8.0 Hz) confirmed that **1** contains a β-D-glucosyl moiety. The characteristic NOE cross-peak between the anomeric sugar proton (H-1′) and the H-8 atom of the aglycone confirmed attachment of the glucopyranosyl moiety to the C-8 position. Therefore, the chemical structure of **1** was determined as shown in Figure 1. Based on the Mongolian word, referring to Artemisia, this compound was named shariljin A.

Compound **2** was obtained as a colorless fine crystal and its molecular formula was determined to be $C_{15}H_{20}O_5$ based on the HRFABMS observation of a deprotonated ion $[M-H]^-$ at m/z 281.1383 (calcd for $C_{15}H_{21}O_5$ 281.1389). A total of 15 resonances were observed in the ^{13}C NMR spectrum, including three carbonyl signals (δ_C 210.4, 204.4, and 180.7) and three methyl signals (δ_C 18.2, 17.9, and 14.2). In addition, the presence of an epoxy moiety was confirmed by signals corresponding to two oxygenated carbon atoms, C-4 (δ_C 63.9) and C-5 (δ_C 71.5), as elucidated by the HMBC correlations (i.e., H_3-15/C-3, C-4, and C-5; H_3-14/C-5; and H_2-3/C-4 and C-5) (Table 1 and Figure 2). In the ^{1}H NMR spectrum, three methyl signals were observed at δ_H 1.23 (3H, d, J = 7.0 Hz, H-13), 1.14 (3H, s, H-14), and 1.29 (3H, s, H-15), and the HMBC correlations between H-13 and the C-7 (δ_C 53.2), C-11 (δ_C 38.0), and C-12 atoms indicates the presence of a isobutyric acid moiety attached to the C-7 position (Figure 1). These spectroscopic features of **2** suggest that this compound is an eudesmane-type sesquiterpenoid. Furthermore, long-range HMBC correlations from H-14 to C-1, C-5, C-9 (δ_C 32.7), and C-10 (δ_C 49.2) indicated that the molecular structure of **2** was 1a,4a-dimethyl-4,8-dioxooctahydro-3*H*-naphtho[1,8a-*b*]oxiren-7-yl)propanoic acid, as shown in Figure 1. The 7*S*,10*R* relative configuration of **2** was

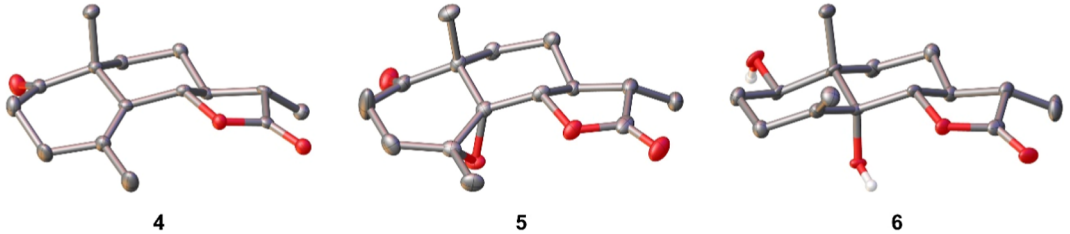

Figure 3. ORTEP diagrams for compounds **4**−**6**. The thermal ellipsoids are drawn at 50% probability. Carbon-bound H atoms and solvated MeOH are omitted for clarity.

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determined based on the NOESY correlations between H-8 and H-11, H-13, and H-14. Notably, compound **5** was also found to possess a similar epoxy group, while compound **6** contained a hydroxyl group at the C-5 position. The absolute configurations of these two compounds were also determined by X-ray crystallography (Figure 3). Based on the hypothesis that the biosynthesis pathway or the reaction mechanism of **2** is identical to those of **5** and **6**, an absolute configuration of 4*R*, 7*S*, 10*R*, 11*S* was proposed for **2**. Thus, **2** was determined to be (*S*)-2-((1a*R*,4a*R*,7*S*,8a*S*)-1a,4a-dimethyl-4,8-dioxooctahydro-3*H*-naphtho[1,8a-*b*]oxiren-7-yl)propanoic acid, and was named shariljin B.

Compound **3** was obtained as a pale brown amorphous solid with a $[\alpha]_D^{20}$ value of −2.9 (*c* 10.3, MeOH), and a molecular formula of $C_{16}H_{24}O_6$, as determined based on the observation of a sodium adduct $[M + Na]^+$ at *m/z* = 335.1471 (calcd for $C_{16}H_{24}O_6Na^+$ 335.1470). In the ^{13}C NMR spectrum of **3**, three carbonyl signals (δ_C 208.7, 177.1, and 177.0), two olefinic carbon signals (δ_C 145.2 and 123.5), and 11 aliphatic carbon signals (δ_C 85.5, 56.8, 51.8, 50.9, 43.5, 34.5, 32.7, 28.3, 25.2, 24.5, and 15.2) were observed. Based on an HMQC correlation with the methyl proton resonance at δ_H 3.69 (3H, s) and the HMBC long-range correlation between the methyl proton and the carbonyl carbon resonance at δ_C 177.1 (C-12), the resonance at δ_C 51.8 resonance was assigned to the methoxy carbon atom of the methyl ester. The features of the ^1H and ^{13}C NMR spectra of compound **3** were similar to those of seco-sesquiterpenoid lactones, including santolinifolide A (**7**)[17,35] and arvestonate C.[37] Furthermore, the HMBC correlations from H-13 (δ_H 1.15, 3H, d, *J* = 7.0 Hz) to C-7 (δ_C 50.9), C-11 (δ_C 43.5), and C-12 (δ_C 177.1); from H-14 (δ_H 1.35, 3H, s) to C-5 (δ_C 208.7), C-6 (δ_C 85.5), C-9 (δ_C 34.5), and C-10 (δ_C 56.8); and from H-15 (δ_H 5.81, 1H, br s; 5.75, 1H, br s) to C-3 (δ_C 28.3), C-4 (δ_C 145.2), and C-5 (δ_C 208.7) confirmed that the molecular structure of **3** was 4-(2-hydroxy-3-(1-methoxy-1-oxopropan-2-yl)-1-methylcyclopentane-1-carbonyl)pent-4-enoic acid (Table 1 and Figure 2). The NOESY correlations between H-6 and H-14, and between H-6 and H-11 indicated a *β*-orientation (Figure 2). Notably, a correlation between H-6 and H-7 was not observed, although the H-6/H-7 coupling constant was defined as 7.0 Hz, indicating that these two protons exist in an *E*-configuration. These spectral features of **3** are similar to those of arvestonate C,[37] suggesting that compound **3** has a similar molecular structure and identical relative configurations. However, it was considered that the described 6*R*,7*R*,10*S* absolute configuration of arvestonate C was not identical to that of **3**. More specifically, in this study, compound **7** was identified as the known compound santolinifolide A,[17,35] which was expected to possess the 6*S*,7*S*,10*R*-configuration, based on the general biosynthetic pathway in which C-7 and C-11 are both in the *S*-configuration. The experimental and calculated electronic circular dichroism (ECD) spectra of compound **3** are shown in Figure 4.

Considering these results, and upon comparison with the calculated ECD values, the 6*S*,7*S*,10*R* absolute configuration was determined for **3** based on the positive cotton effect at ∼222 nm and the negative cotton effect at ∼311 nm. Consequently, **3** was determined to be 4-((1*R*,2*S*,3*S*)-2-hydroxy-3-((*S*)-1-methoxy-1-oxopropan-2-yl)-1-methylcyclopentane-1-carbonyl)pent-4-enoic acid, and was named shariljin C.

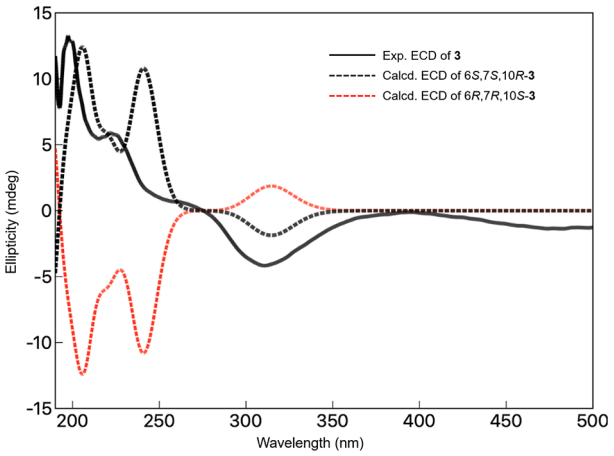

Figure 4. Experimental and calculated ECD spectra for compound **3**. Solid black line: experimental ECD spectrum; dashed black line: calculated ECD spectrum for the 6*S*,7*S*,10*R*-configuration of **3**; red dashed line: calculated ECD spectrum for the 6*R*,7*R*,10*S*-configuration of **3**.

2.2. Trypanocidal Activities of the Isolated Compounds. The trypanocidal activities of the isolated compounds (**1**−**27**) were evaluated against *T. brucei brucei*, *T. b. gambiense*, *T. b. rhodesiense*, *T. equiperdum*, *T. congolense*, and *T. evansi* (Table 2).

Although the crude extract and the frs.1A−1G showed no activities at 25 *μ*g/mL against any species, among the isolated compounds, terpenoids (**3** and **7**), a coumarin (**8**) and a flavonoid (**14**) showed relatively potent activities. Sesquiterpenoids with open-ring structures were also found to be active against *T. b. gambiense* and *T. congolense*. Notably, compounds **8** and **14** demonstrated moderate activities against all strains examined herein. This is interesting from a structure−activity relationship perspective because despite these compounds being active, the other coumarins (**9**−**13**) and flavonoids (**15**−**19**) investigated herein showed no activities (IC_{50} > 50 *μ*g/mL). It is also noteworthy that compounds **8** and **14** possess both a phenolic hydroxyl group and a methoxy group, similar to previously reported active compounds such as some flavonoids (e.g., chrysosplenetin and wogonin) and some oxazoles (e.g., 2-(2′-hydroxy-5′-*O*-methylphenyl)-5-(2″,5″-dihydroxyphenyl)-oxazole).[14,25,38,39] Compound **14** is a methoxylated flavonoid, similar to the previously reported active flavonoid chrysosplenetin, which is known to exhibit an inhibitory activity against *T. congolense*.[14] Indeed, the obtained results showed that **14** demonstrated trypanocidal activity against all species and strains evaluated herein (*T. b. brucei*: IC_{50} 4.47 *μ*g/mL; *T. b. gambiense*: IC_{50} 4.98 *μ*g/mL; *T. b. rhodesiense*: IC_{50} 3.25 *μ*g/mL; *T. equiperdum*: IC_{50} 12.8 *μ*g/mL; *T. congolense*: IC_{50} 3.43 *μ*g/mL; and *T. evansi*: IC_{50} 25.9 *μ*g/mL), thereby suggesting that the methoxyflavonoid compounds isolated from Artemisia plants may have potential for application in the treatment of these parasites. These two active compounds (i.e., **8** and **14**) produced relatively prominent HPLC peaks in the crude plant extract (Figure S43), indicating their abundance in this medium. In particular, quantitative HPLC analyses of **8** [0.033 mg/1.0 mg extract, 0.35% (w/w) of the dried aerial parts of *A. adamsii*] and **14** [0.042 mg/1.0 mg extract, 0.45% (w/w) of the dried aerial parts of *A. adamsii*] confirmed that these compounds are the key biologically active components that characterize *A. adamsii*.

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Table 2. IC_{50} ± SD (μg/mL) of Active Compounds Against Selected Trypanosomes[a]

sample[b]	Tbb GuTat3.1	Tbg IL1922	Tbr IL1501	Teq	Tc IL3000	Tev Tansui
3	N.D[c]	42.5 ± 12.0	N.D[c]	N.D[c]	26.5 ± 2.71	N.D[c]
7	N.D[c]	35.4 ± 11.7	N.D[c]	N.D[c]	17.1 ± 2.62	N.D[c]
8	4.86 ± 1.34	5.89 ± 1.81	5.48 ± 0.49	4.56 ± 1.36	5.57 ± 0.70	9.33 ± 4.04
14	4.47 ± 2.42	4.98 ± 1.80	3.25 ± 0.76	12.8 ± 3.79	3.43 ± 0.49	25.9 ± 7.48
suramine[9]	0.094 ± 0.0074	0.092 ± 0.0025	0.11 ± 0.015	0.094 ± 0.0010	10.25 ± 1.25	0.54 ± 0.083
pentamidine[9]	0.014 ± 0.00080	0.0049 ± 0.0011	0.0099 ± 0.0021	0.0017 ± 0.0042	0.11 ± 0.018	0.00033 ± 0.000064
niflutimox[9]	1.34 ± 0.57	1.32 ± 0.68	1.25 ± 0.46	0.42 ± 0.099	0.30 ± 0.063	0.75 ± 0.40
eflornithine[9]	7.03 ± 1.80	6.68 ± 2.34	8.38 ± 3.11	6.50 ± 1.85	2.94 ± 0.53	10.42 ± 3.20
diminazene[9]	0.020 ± 0.0061	0.0066 ± 0.011	0.013 ± 0.0026	0.081 ± 0.060	0.065 ± 0.0068	0.0033 ± 0.00064

[a]n = 3. [b]Compounds **1**, **2**, **4**−**6**, **9**−**13**, and **15**−**28** were inactive at 25 μg/mL against any species. [c]Not determined at 25 μg/mL.

Further evaluation of their biological activities is therefore desirable. Unfortunately, the activities of the compounds obtained in this study were lower than those of existing drugs. Consequently, there is still room for improvement, and this could potentially be achieved through the preparation of less-toxic analogues, such as in the case of previously described oxazole derivatives.[38] On the other hand, considering the current demand for economical treatments for Trypanosoma treatment, the results presented herein will be expected to contribute to the development of novel medications from plant resources.

3. CONCLUSIONS

In this study, aerial parts were isolated from the Mongolian *A. adamsii* plant in the autumn, and three undescribed sesquiterpenoids (**1**−**3**) were isolated along with 25 known compounds (**4**−**28**). The relative and absolute configurations of the obtained compounds were elucidated using nuclear magnetic resonance spectroscopy, electronic circular dichroism, and single crystal X-ray crystallography. Two seco-sesquiterpenoids, namely shariljin C (**3**) and santolinifolide A (**7**) exhibited moderate trypanocidal activities against *T. brucei gambiense* and *T. congolense*. In addition, one coumarin (fraxetin) and one flavonoid (jaceosidin), which were identified by quantitative high-performance liquid chromatography as the main constituents of *A. adamsii*, showed potent trypanocidal activities against *T. equiperdum* (the cause of Dourine), *T. b. brucei*, *T. b. gambiense*, *T. b. rhodesiense*, *T. congolense*, and *T. evansi*. It is expected that these results regarding the basic active constituents of *A. adamsii* that remain in the plant tissue as it withers in the autumn months will highlight its medicinal and ecological significance. In future work, the isolated flavonoids, lignans, and terpenoids from this species will be employed as lead compounds for the synthesis of more effective analogues, and their potential beneficial biological effects will also be evaluated.

4. METHODS

4.1. General Experimental Procedures. The optical rotations were recorded using a P-2300 polarimeter (Jasco Co., Tokyo, Japan). NMR spectroscopic data were recorded on JNM-AL400, JNM-ECZ400S/L1, and JNM-ECZ600R/S1 FT-NMR spectrometers (JEOL Ltd., Tokyo, Japan). The chemical shifts are expressed in ppm (δ) with respect to either the solvent peaks or tetramethylsilane (TMS) as an internal standard. The HRFABMS and HREIMS data were obtained on a JMS700 mass spectrometer (JEOL Ltd.). A porous polymer gel (Diaion HP-20; Mitsubishi Chemical Co., Tokyo, Japan) was used as the matrix to perform open column chromatography. Preparative HPLC was carried out on a Jasco 2089 instrument fitted with a UV detector (210 nm) (columns: TSKgel ODS-80Ts (Tosoh, Tokyo, Japan, 21.5 × 300 mm), Develosil C_{30}-UG-5 (Nomura Chemical, Aichi, Japan, 20 × 250 mm), Mightysil RP-18 GP (Kanto Chemical, Tokyo, Japan, 10 × 250 mm), and ODS-SM-50C-M (Yamazen Co., Osaka, Japan, 37 × 300 mm)). Acetone (99.5%), acetonitrile (99.5%), and methanol (MeOH, 99.5%) were obtained from Fujifilm Wako (Osaka, Japan), CD_3OD (98%, 99.8%D) and $CDCl_3$ (99.96%D, containing 0.03% (v/v) tetramethylsilane) were obtained from Merck (Darmstadt, Germany), while DMSO-d_6 (99.9%D) was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

4.2. Plant Materials. In October 2020, the aerial parts of *A. adamsii* Besser were gathered from Bayan Soum, Tuv province, Mongolia. The specimens were stored at the Laboratory of Bioorganic Chemistry and Pharmacognosy, National University of Mongolia (No. 103.33.46). Dr. Shagdar Dariimaa, who is affiliated with the Mongolian State University of Education, identified this plant species.

4.3. Extraction and Isolation. The dried aerial parts of *A. adamsii* (250 g) were extracted using a 4:1 mixture of acetone/H_2O (2 L, 3 × 72 h) at ∼20 °C. The concentrated extract (26.6 g) was then subjected to column chromatography using an HP-20 matrix with a mixture of H_2O and MeOH, MeOH, and acetone being used for elution. Seven fractions (Fr) were collected using different solvent compositions: Fr.1A (5.7 g) from H_2O; Fr.1B (0.56 g) from 4:1 H_2O/MeOH; Fr.1C (1.25 g) from 3:2 H_2O/MeOH, Fr.1D (4.2 g) from 2:3 H_2O/MeOH, Fr.1E (5.48 g) from 1:4 H_2O/MeOH, Fr.1F (5.65 g) from MeOH, and Fr.1G (3.78 g) from acetone. Fractions 1C−1F were subjected to column chromatography (ODS-SM-50C-M column) using a mixture of H_2O and MeOH as the mobile phase. Fractions 2A−2M from Fr.1F (3:2, 4:1, and 5:0, MeOH/H_2O), fractions 3A−3N from Fr.1E (2:3, 3:2, 4:1, and 5:0, MeOH/H_2O), fractions 4A−4I from Fr.1D (3:7, 1:1, 4:1, and 5:0, MeOH/H_2O), fractions 5A−5F from Fr.1C (3:7, 1:1, and 4:1, MeOH/H_2O and MeOH) were obtained accordingly. Subsequently, Fraction 2B (785.7 mg) was subjected to HPLC separation using an ODS-80Ts column (3:7, acetonitrile (MeCN)/H_2O containing 0.2% TFA as the mobile phase) and a C30-UG-5 column (9:11, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain **5** (2.0 mg, R_t = 25 min) and **7** (6.0 mg, R_t = 17 min). Fraction 2C (1498.4 mg) was obtained as a white powder, which was determined to be compound **4** (1498.4 mg). Fractions 2D and 2E (1431.2 mg) were mixed together, and the soluble part was subjected to HPLC separation using an ODS-80Ts column (2:3, MeCN/H_2O containing 0.2% TFA as the mobile phase) and a C_{30}-

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UG-5 column (11:14, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain **14** (10.9 mg, R_t = 30 min). Fraction 2I (66.4 mg) was subjected to HPLC separation using an ODS-80Ts column (3:7, MeCN/H_2O containing 0.2% TFA as the mobile phases and a C30-UG-5 column (9:11, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain **3** (11.3 mg, R_t = 19 min). Fraction 3D (453.6 mg) was subjected to HPLC separation using an ODS-50C column (2:3, MeOH/H_2O as the mobile phase), a ODS-80Ts column (1:4, MeCN/H_2O containing 0.2% TFA as the mobile phase), and a C30-UG-5 column (3:7, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain compounds **11** (7.7 mg, R_t = 27 min), **20** (25.4 mg, R_t = 33 min), **21** (6.9 mg, R_t = 20 min), and **28** (10.4 mg, R_t = 28 min). Fraction 3E (600.4 mg) was subjected to HPLC separation using an ODS-50C column (2:3, MeOH/H_2O as the mobile phase), a ODS-80Ts column (1:4, MeCN/H_2O containing 0.2% TFA as the mobile phase), and a C30-UG-5 column (3:7, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain **6** (6.0 mg, R_t = 36 min). Similarly, using a C30-UG-5 column and a 7:13 mixture of MeCN/H_2O containing 0.2% TFA as the mobile phase, compound **2** (6.4 mg, R_t = 20 min) was also obtained. Fraction 3F (501.4 mg) was subjected to HPLC separation using an ODS-80Ts column (1:4, MeCN/H_2O containing 0.2% TFA as the mobile phase) and a C30-UG-5 column (7:13, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain compounds **12** (17.0 mg, R_t = 22 min) and **22** (17.0 mg, R_t = 21 min). Fraction 4D (600.3 mg) was subjected to HPLC separation using an ODS-80Ts column (3:17, MeCN/H_2O containing 0.2% TFA as the mobile phase) and a C_{30}-UG-5 column (1:4, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain compounds **1** (8.5 mg, R_t = 39 min), **8** (72.9 mg, R_t = 41 min), **9** (4.7 mg, R_t = 29 min), **10** (2.0 mg, R_t = 32 min), **19** (5.7 mg, R_t = 34 min), **24** (98.9 mg, R_t = 28 min), and **25** (8.5 mg, R_t = 15 min). Fraction 4E (326.8 mg) was subjected to HPLC separation using an ODS-80Ts column (3:7 MeCN/H_2O containing 0.2% TFA as the mobile phase) and a C30-UG-5 column (1:3, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain compounds **15** (5.3 mg, R_t = 23 min), **18** (5.4 mg, R_t = 19 min), and **26** (10.2 mg, R_t = 24 min). Fraction 4F was subjected to HPLC separation using an ODS-80Ts column (2:3, MeCN/H_2O containing 0.2% TFA as the mobile phase) and a C_{30}-UG-5 column (1:3, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain **16** (16.6 mg, R_t = 29 min) and **17** (19.6 mg, R_t = 26 min). Fraction 5B (240.7 mg) was subjected to HPLC separation using an ODS-50C column (3:7, MeOH/H_2O as the mobile phase), a ODS-80Ts column (3:7, MeCN/H_2O containing 0.2% TFA as the mobile phase), and a C_{30}-UG-5 column (1:9, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain compounds **13** (5.2 mg, R_t = 80 min) and **27** (5.6 mg, R_t = 51 min). Fraction 5C (302.4 mg) was subjected to HPLC separation using an ODS-50C column (3:7, MeOH/H_2O as the mobile phase), a ODS-80Ts column (3:7, MeCN/H_2O containing 0.2% TFA as the mobile phase), and a C_{30}-UG-5 column (1:9, MeCN/H_2O containing 0.2% TFA as the mobile phase) to obtain **23** (6.4 mg, R_t = 63 min).

*4.3.1. Shariljin A (**1**).* Colorless amorphous powder, $[\alpha]_D^{20}$ −48.6 (*c* 1.4, MeOH), HRFABMS (positive) $[M + Na]^+$ at *m*/*z* = 449.1777 (calcd for $C_{21}H_{30}O_9Na^+$: 449.1787), ^{1}H NMR: (CD_3OD, 400 MHz) Table 1, ^{13}C NMR: (CD_3OD, 100 MHz) Table 1; IR ν_{max} (KBr) cm^{-1}: 3422, 2931, 1765, 1707, 1200, 1076, 1030.

*4.3.2. Shariljin B (**2**).* Colorless needle, mp 189 °C, $[\alpha]_D^{20}$ 7.1 (*c* 6.4, MeOH), HRFABMS (negative) $[M - H]^-$ at *m*/*z* = 281.1383 (calcd for $C_{15}H_{21}O_5^-$: 281.1389), ^{1}H NMR: ($CDCl_3$, 400 MHz) Table 1, ^{13}C NMR: ($CDCl_3$, 100 MHz) Table 1; IR ν_{max} (KBr) cm^{-1}: 3422, 3197, 2975, 2959, 2939, 1710, 1458, 1417, 1217, 1197, 1184, 1167.

*4.3.3. Shariljin C (**3**).* Pale yellow amorphous liquid, $[\alpha]_D^{20}$ −2.9 (*c* 10.3, MeOH), HRFABMS (positive) $[M + Na]^+$ at *m*/*z* 335.1471 (calcd for $C_{16}H_{24}O_6Na^+$: 335.1470), UV (MeOH) λ_{max} (log ε) 207 (2.92) nm, ECD (c 0.00080, MeOH) ([θ]) 215 (+1800), 222 (+2300), 311 (−1600) nm, ^{1}H NMR: ($CDCl_3$, 400 MHz) Table 1, ^{13}C NMR: ($CDCl_3$, 100 MHz) Table 1; IR ν_{max} (KBr) cm^{-1}: 3448, 2955, 1729, 1459, 1378, 1200, 1172, 1053.

*4.3.4. Gracilin (**4**).* Colorless needle, mp 114 °C.

*4.3.5. Maritimin (**5**).* Colorless needle, mp 197 °C.

*4.3.6. Artemin (**6**).* Colorless needle, mp 248 °C.

*4.3.7. Santolinifolide A (**7**).* Pale yellow amorphous liquid, $[\alpha]_D^{21}$ −0.5 (*c* 24.2, MeOH), HREIMS $[M]^+$ at *m*/*z* 280.1305 (calcd for $C_{15}H_{20}O_5^+$: 280.1311), UV (MeOH) λ_{max} (log ε) 223 (2.98) nm, ECD (c 0.00040, MeOH) ([θ]) 212 (+1000), 229 (+1100), 317 (−700) nm. ^{1}H NMR: ($CDCl_3$, 400 MHz) Figure S19, ^{13}C NMR: ($CDCl_3$, 100 MHz) Figure S20.

*4.3.8. Fraxetin (**8**).* Colorless powder, HREIMS $[M]^+$ at *m*/*z* 208.0368 (calcd for $C_{10}H_8O_5^+$: 208.0371), UV (MeOH) λ_{max} (log ε) 211 (4.60), 340 (4.09) nm. ^{1}H NMR: (CD_3OD, 400 MHz) Figure S24, ^{13}C NMR: (CD_3OD, 100 MHz) Figure S25.

*4.3.9. Jaceosidin (**14**).* Pale yellow powder, HREIMS $[M]^+$ at *m*/*z* 330.0743 (calcd for $C_{17}H_{14}O_7^+$: 330.0739), UV (MeOH) λ_{max} (log ε) 214 (4.58), 274 (4.22), 356 (4.41) nm. ^{1}H NMR: (DMSO-d_6, 400 MHz) Figure S30, ^{13}C NMR: (DMSO-d_6, 100 MHz) Figure S31.

4.4. Sugar Identification. The glycosidic moiety of compound **1** was identified using a previously described method.[36] More specifically, compound **1** (1.0 mg) was hydrolyzed using 3.5 N diluted HCl (Fujifilm Wako Pure Chemical Industries, Ltd. Osaka, Japan) at 80 °C for 1 h. The resulting solution was passed through a Diaion HP-20 column (5 mm × 50 mm), and the obtained eluate was concentrated. This concentrate was subsequently dissolved in pyridine (0.5 mL), and L-cysteine methyl ester (5 mg) was added. After heating for 1 h at 60 °C, *o*-tolyl isothiocyanate (20 μL) was added to the solution, and heating was continued at the same temperature for a further 1 h. The reaction mixture was then analyzed by HPLC (Thermo Acclaim C_{18}, 4.6 × 250 mm; CH_3CN/H_2O (1:3, v/v) containing 0.1% TFA, 1.0 mL/min; detection at 256 nm). The peak obtained for the derivative of **1** (R_t = 22.6 min) was compared with those of authentic L-glucose (R_t = 20.9 min) and D-glucose (R_t = 22.6 min) derivatives (Figure S42).

4.5. X-Ray Crystallography of Compounds 4−6. Compounds **4**−**6** were crystallized as colorless crystals from aqueous MeOH, and these crystals were subjected to single crystal X-ray diffraction measurements using an XtaLAB Synergy-*i* diffractometer (Rigaku Corporation, Tokyo, Japan) equipped with a Cu Kα radiation source (λ = 1.54184 Å) at 100 K. Data collection, cell refinement, and data reduction were performed using the CrysAlis^{Pro} program. The Olex2 and ShelXT structure-solution programs with intrinsic phasing were used to determine the structures of compounds **4**−**6**.[40,41] In addition, their structures were refined using the ShelXL

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refinement package with least-squares minimization.[42] The data can be obtained free of charge at http://www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Center (CCDC, 12 Union Road, Cambridge CB21EZ, UK).

4.5.1. Crystal Data for Compound 4. $C_{15}H_{20}O_3$, formula weight (FW) = 248.31, 0.624 × 0.586 × 0.263 mm^3 colorless block, monoclinic, Space group: $P2_1$, a = 5.6290(2) Å, b = 8.1496(2) Å, c = 14.4747(4) Å, β = 92.635(3)°, V = 663.31(3) $Å^3$, Z = 2, D_{calcd} = 1.243 g/cm^3, No. of reflections collected = 4847, No. of independent reflections = 2389, Restraints = 1, Parameters = 166, 2θ range for data collection: 6.112−136.374°, R indices ($I > 2\sigma(I)$): R_1 = 0.0497, wR_2 = 0.1334, R indices (all data): R_1 = 0.0507, wR_2 = 0.1348, Goodness-of-fit on F^2 = 1.063. Additional crystallographic data for this compound can be obtained from the CCDC (CCDC 2416892).

4.5.2. Crystal Data for Compound 5. $C_{15}H_{20}O_4$, FW = 264.31, 0.726 × 0.486 × 0.386 mm^3 colorless block, orthorhombic, Space group: $P2_12_12_1$, a = 5.79960(10) Å, b = 10.3030(2) Å, c = 22.1379(4) Å, V = 1322.81(4) $Å^3$, Z = 4, D_{calcd} = 1.327 g/cm^3, No. of reflections collected = 10,997, No. of independent reflections = 2400, Restraints = 0, Parameters = 175, 2θ range for data collection: 7.988−136.238°, R indices ($I > 2\sigma(I)$): R_1 = 0.0487, wR_2 = 0.1291, R indices (all data): R_1 = 0.0497, wR_2 = 0.1304, Goodness-of-fit on F^2 = 1.061. Additional crystallographic data for this compound can be obtained from the CCDC (CCDC 2416891).

4.5.3. Crystal Data for the MeOH Solvate of Compound 6. $C_{15}H_{23}O_4{\cdot}CH_3OH$, FW = 299.37, 0.92 × 0.531 × 0.369 mm^3 colorless block, monoclinic, Space group: $P2_1$, a = 9.0462(2) Å, b = 6.2570(2) Å, c = 14.2840(3) Å, β = 91.959(2)°, V = 808.03(4) $Å^3$, Z = 2, D_{calcd} = 1.230 g/cm^3, No. of reflections collected = 7352, No. of independent reflections = 2688, Restraints = 1, Parameters = 197, 2θ range for data collection: 6.192−136.464°, R indices ($I > 2\sigma(I)$): R_1 = 0.0798, wR_2 = 0.2283, R indices (all data): R_1 = 0.0810, wR_2 = 0.2312, Goodness-of-fit on F^2 = 1.056. Additional crystallographic data for this compound can be obtained from the CCDC (CCDC 2416893).

4.6. ECD Calculations. Conformational analysis was performed using a previously reported shell script.[43] More specifically, 500 energy-minimized three-dimensional structures of the stereoisomers of **3** were generated from the 2D chemical structures using Open Babel and Balloon.[44,45] Every 50th conformers were geometrically optimized in the gas phase using the B3LYP/TZVP level of theory in Gaussian 16.[46] The ECD calculations for **3** were conducted at the B3LYP/TZVP level of time-dependent density functional theory (TDDFT) in MeOH using the conductor-like polarizable continuum model (CPCM) of Gaussian 16. The ECD spectra were obtained from 45 calculated excitation energies and rotational strengths as the sum of the Gaussian functions centered at the wavelength of each transition with a parameter s, which represents the width of the band at a half height of 0.22 eV.

4.7. Quantitative HPLC Analysis of Compounds 8 and 14. HPLC analysis was performed using a reversed-phase HPLC system (Pump: PU4180, JASCO Co., Tokyo, Japan; column: Acclaim 120 C18, 5 μm, 120 Å, 4.6 mm × 250 mm, Thermo Scientific, USA; flow rate: 1.0 mL/min; detector: MD-4010 Photodiode array detector, PDA, JASCO Co.). For this purpose, the following solvent system was employed: Solvent A: CH_3CN containing 0.1% TFA; solvent B: H_2O containing 0.1% TFA; initial A/B ratio = 1:9; 0−40 min, A/B ratio increased to 4:6; 45 min, A/B ratio = 4:6; 45−53 min, A/B = 1:0. Solutions of compounds **8** and **14** (1.0, 0.5, 0.1, 0.05, and 0.01 mg/mL) were prepared, and the mean values obtained following analyses performed in triplicate were presented. The calibration curves for **8** (R_t = 17 min, PDA at 340 nm; y = 5857801x, R^2 = 0.997) and **14** (R_t = 41 min, PDA at 346 nm; y = 8783705x, R^2 = 0.999) were obtained. The injection volumes for the standard solutions and the *A. adamsii* extract (1.0 mg/mL) were 2 μL. The mean values obtained from the measurement of three extractions were determined to be 0.033 mg/1.0 mg extracts for compound **8** and 0.042 mg/1.0 mg extract for compound **14**.

4.8. Trypanocidal Activities of the Isolated Compounds. The trypanocidal activity assays were conducted in accordance with a previous report.[9] More specifically, the trypanocidal activities of the isolated compounds were primally evaluated against the *T. b. brucei* GUTat3.1 (isolated in Uganda), *T. b. gambiense* IL1922 (isolated from the Ivory Coast), *T. b. rhodesiense* IL1501 (isolated in Kenya), *T. b. rhodesiense* Chirundu (isolated in Zambia), *T. congolense* IL3000 (isolated in the Kenyan/Tanzanian border area), *T. evansi* Tansui (isolated in Taiwan), and *T. equiperdum* IVM-t1 (isolated in Mongolia) strains at active compound concentrations of 50 μg/mL. The IC_{50} (half-maximal inhibitory concentration) values of the active compounds were determined based on three independent experiments, using seven different concentrations (i.e., 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 μg/mL).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.5c00591.

^1H, ^{13}C, $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra of **1**−**3**, **7**, **8**, and **14**; HRFABMS data of **1**−**3** and HREIMS data of **7**, **8**, and **14**; and HPLC profiles of **1**, **8**, and **14** (PDF)

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Notes

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■ REFERENCES

(1) Yunatov, A. A. *Forage plants in the hayfields and pastures of the Mongolian People's Republic. Ulaanbaatar*; D. Sukhbaatar Printing House, 1968; pp 88−89.
(2) Shatar, S.; Altantsetseg, Sh. Chemical compounds, technological characteristics and importance of the essential oils of wormwood from Mongolian flora. *Ulaanbaatar, Eknmto printing* **2011**, 66−67.
(3) Kinugasa, T.; Ishibashi, K.; Miyawaki, M.; Gantsetseg, B. Germination characteristics and phytotoxic inhibition of germination in *Artemisia adamsii*, a low-palatability weed in the Mongolian steppe. *Seed Sci. Res.* **2019**, *29*, 197−203.
(4) Ulziikhutag, N.; Sanchir, Ch. *Identification Book for Forage Plants in Pastures and Hayfields of Mongolian People's Republic*; State publishing house: Ulaanbaatar, Mongolia, 1985; pp 409−4410.
(5) Samjaa, R.; Zöphel, U.; Peterson, J. J. The impact of the vole *Microtus brandti* on Mongolia steppe ecosystems. *Marbg. Geogr. Schriften* **2000**, *135*, 346−360.
(6) Dagys, K.; Agipar, B.; Tsolmon, S.; Ringler, C.; Bellisario, K.; Fanzo, J. Maximizing nutrition in key food value chains of Mongolia under climate change. *Food Policy* **2023**, *117*, 102468.
(7) Ligaa, U. *Medicinal plants of Mongolia used in Western and Eastern medicine*; KCA Press: Korea, 2005; pp 198−199.
(8) Bohlmann, F.; Hartono, L.; Jakupovic, J.; Huneck, S. Guaianolides related to arborescin from *Artemisia adamsii*. *Phytochemistry* **1985**, *24*, 1003−1007.
(9) Munsimbwe, L.; Suganuma, K.; Ishikawa, Y.; Choongo, K.; Kikuchi, T.; Shirakura, I.; Murata, T. Benzophenone glucosides and B-Type proanthocyanidin dimers from Zambian *Cassia abbreviata* and their trypanocidal activities. *J. Nat. Prod.* **2022**, *85*, 91−104.
(10) Mizushima, D.; Amgalanbaatar, T.; Davaasuren, B.; Kayano, M.; Naransatsral, S.; Myagmarsuren, P.; Otgonsuren, D.; Enkhtaivan, B.; Davkharbayar, B.; Mungun-Ochir, B.; Baatarjargal, P.; Nyamdolgor, U.; Soyolmaa, G.; Altanchimeg, A.; Zoljargal, M.; Nguyen, T.-T.; Battsetseg, B.; Battur, B.; Inoue, N.; Yokoyama, N.; Suganuma, K. Nationwide serological surveillance of non-tsetse-transmitted horse trypanosomoses in Mongolia. *Parasite Epidemiol. Control* **2020**, *10*, No. e00158.
(11) Suganuma, K.; Narantsatsral, S.; Battur, B.; Yamasaki, S.; Otgonsuren, D.; Musinguzi, S. P.; Davaasuren, B.; Battsetseg, B.; Inoue, N. Isolation, cultivation and molecular characterization of a new *Trypanosoma equiperdum* strain in Mongolia. *Parasite Vectors* **2016**, *9* (1), 481.
(12) Suganuma, K.; Yamasaki, S.; Molefe, N. I.; Musinguzi, P. S.; Davaasuren, B.; Mossaad, E.; Narantsatsral, S.; Battur, B.; Battsetseg, B.; Inoue, N. The establishment of *in vitro* culture and drug screening systems for a newly isolated strain of *Trypanosoma equiperdum*. *Int. J. Parasitol Drug* **2017**, *7*, 200−205.
(13) Davkharbayar, B.; Davaasuren, B.; Narantsatsral, S.; Battur, B.; Punsantsogvoo, M.; Battsetseg, B.; Mizushima, D.; Inoue, N.; Suganuma, K. Treatment Efficiency of Combination Therapy With Diminazene Aceturate and Quinapyramine Sulfate in a Horse With Dourine. *J. Equine Vet. Sci.* **2020**, *87*, 102905.
(14) Nurbek, S.; Murata, T.; Suganuma, K.; Ishikawa, Y.; Buyankhishig, B.; Kikuchi, T.; Byambajav, T.; Davaapurev, B.-O.; Sasaki, K.; Batkhuu, J. Isolation and evaluation of trypanocidal activity of sesquiterpenoids, flavonoids, and lignans in *Artemisia sieversiana* collected in Mongolia. *J. Nat. Med.* **2020**, *74*, 750−757.
(15) G González, A.; Gallindo, A.; Mansilla, H.; Gutiérrez, A. Structure of maritimin, a sesquiterpene lactone from *Artemisia maritima gallica*. *Phytochemistry* **1981**, *20*, 2367−2369.
(16) Trendafilova, A. B.; Todorova, M. N.; Gussev, C. V. Eudesmanolides from *Artemisia pontica*. *Phytochemistry* **1996**, *42*, 469−471.
(17) Jakupovic, J.; Tan, R. X.; Bohlmann, F.; Jia, Z. J.; Huneck, S. Seco- and Nor-sesquiterpene lactones with a new carbon skeleton from *Artemisia santolinifolia*. *Phytochemistry* **1991**, *30*, 1941−1946.
(18) Yasuda, T.; Fukui, M.; Nakazawa, T.; Hoshikawa, A.; Ohsawa, K. Metabolic fate of fraxin administered orally to rats. *J. Nat. Prod.* **2006**, *69*, 755−757. https://pubs.acs.org/doi/10.1021/np0580412

(19) Kanlayavattanakul, M.; Ruangrungsi, N.; Watanabe, T.; Ishikawa, T. Chemical constituents of *Pterocaulon redolens*. *Heterocycles* **2003**, *61*, 183−187.
(20) Jerezano, A.; Jiménez, F.; Cruz, M. del C.; Montiel, L. E.; Delgado, F.; Tamariz, J. New approach for the construction of the coumarin frame and application in the total synthesis of natural products. *Helv. Chim. Acta* **2011**, *94*, 185−198.
(21) Li, H.; Bos, A.; Jean, S.; Webster, D.; Robichaud, G. A.; Johnson, J. A.; Gray, C. A. Antimycobacterial 1,4-napthoquinone natural products from *Moneses uniflora*. *Phytochem. Lett.* **2018**, *27*, 229−233.
(22) Kozawa, M.; Matsuyama, Y.; Fukumoto, M.; Baba, K. Chemical studies of *Coelopleurum gmelinii* (D.C.) LEDEB. I. constituents of the root. *Chem. Pharm. Bull.* **1983**, *31*, 64−69.
(23) Kisiel, W.; Michalska, K. A new coumarin glucoside ester from *Cichorium intybus*. *Fitoterapia* **2002**, *73*, 544−546.
(24) Martínez, V.; Barberá, O.; Sánchez-Parareda, J.; Alberto Marco, J. Phenolic and acetylenic metabolites from *Artemisia assoana*. *Phytochemistry* **1987**, *26*, 2619−2624.
(25) Buyankhishig, B.; Murata, T.; Suganuma, K.; Batkhuu, J.; Sasaki, K. Hyaluronidase inhibitory saponins and a trypanocidal isoflavonoid from the aerial parts of *Oxytropis lanata*. *Fitoterapia* **2020**, *145*, 104608.
(26) Xie, C.; Veitch, N. C.; Houghton, P. J.; Simmonds, M. S. J. Flavone *C*-glycosides from *Viola yedoensis* MAKINO. *Chem. Pharm. Bull.* **2003**, *51*, 1204−1207.
(27) Basnet, P.; Matsushige, K.; Hase, K.; Kadota, S.; Namba, T. Four Di-*O*-caffeoyl quinic acid derivatives from propolis. Potent hepatoprotective activity in experimental liver injury models. *Biol. Pharm. Bull.* **1996**, *19*, 1479−1484.
(28) Choi, S. Z.; Choi, S. U.; Lee, K. R. Phytochemical constituents of the aerial parts from *Solidago virga-aurea* var. *gigantea*. *Arch. Pharm. Res.* **2004**, *27*, 164−168.
(29) Zhu, X.; Dong, X.; Wang, Y.; Ju, P.; Luo, S. Phenolic compounds from *Viburnum cylindricum*. *Helv. Chim. Acta* **2005**, *88*, 339−342.
(30) Kitajima, J.; Ishikawa, T.; Urabe, A. A new hydroxyjasmone glucoside and its related compounds from the leaf of thyme. *Chem. Pharm. Bull.* **2004**, *52*, 1013−1014.
(31) Kuang, H.-x.; Yang, B.-y.; Xia, Y.-g.; Feng, W.-s. Chemical constituents from the flower of *Datura metel* L. *Arch. Pharm. Res.* **2008**, *31*, 1094−1097.
(32) Jeon, S. H.; Chun, W.; Choi, Y. J.; Kwon, Y. S. Cytotoxic constituents from the bark of *Salix hulteni*. *Arch. Pharm. Res.* **2008**, *31*, 978−982.
(33) D'Abrosca, B.; DellaGreca, M.; Fiorentino, A.; Monaco, P.; Previtera, L.; Simonet, A. M.; Zarrelli, A. Potential allelochemicals from *Sambucus nigra*. *Phytochemistry* **2001**, *58*, 1073−1081.
(34) Pinhey, J. T.; Sternhell, S. Structure of α-hydroxysantonin and some aspects of the stereochemistry of related eudesmanolides and guaianolides. *Aust. J. Chem.* **1965**, *18*, 543−557.
(35) Marco, J. A.; Sanz-Cervera, J. F.; Pareja, J. M.; Sancenon, F.; Valles-Xirau, J. Sesquiterpene lactones from North African *Artemisia* species. *Phytochemistry* **1994**, *37*, 477−485.
(36) Tanaka, T.; Nakashima, T.; Ueda, T.; Tomii, K.; Kouno, I. Facile discrimination of aldose enantiomers by reversed-phase HPLC. *Chem. Pharm. Bull.* **2007**, *55*, 899−901.
(37) Tian, S.-H.; Zhang, C.; Zeng, K.-W.; Zhao, M.-B.; Jiang, Y.; Tu, P.-F. Sesquiterpenoids from *Artemisia vestita*. *Phytochemistry* **2018**, *147*, 194−202.
(38) Narita, K.; Suganuma, K.; Murata, T.; Kondo, R.; Satoh, H.; Watanabe, K.; Sasaki, K.; Inoue, N.; Yoshimura, Y. Synthesis and evaluation of trypanocidal activity of derivatives of naturally occurring 2,5-diphenyloxazoles. *Bioorg. Med. Chem.* **2021**, *42*, 116253.
(39) Nurbyek, S.; Buyankhishig, B.; Suganuma, K.; Ishikawa, Y.; Kutsuma, M.; Abe, M.; Sasaki, K.; Davaapurev, B.-O.; Batkhuu, J.; Murata, T. Phytochemical investigation of *Scutellaria scordiifolia* and its trypanocidal activity. *Phytochemistry* **2023**, *209*, 113615.
(40) Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *OLEX2*: A complete structure solution, refinement and analysis program. *J. Appl. Crystallogr.* **2009**, *42*, 339−341.
(41) Sheldrick, G. M. *SHELXT* - Integrated space-group and crystal-structure determination. *Acta Crystallogr., Sect. A:Found. Adv.* **2015**, *71*, 3−8.
(42) Sheldrick, G. M. Crystal structure refinement with SHELXL. *Acta Crystallogr., Sect. C:Struct. Chem.* **2015**, *71*, 3−8.
(43) Ishikawa, Y. A Script for automated 3-dimensional structure generation and conformer search from 2-dimensional chemical drawing. *Bioinformation* **2013**, *9*, 988−992.
(44) O'Boyle, N. M.; Banck, M.; James, C. A.; Morley, C.; Vandermeersch, T.; Hutchison, G. R. Open babel: an open chemical toolbox. *J. Cheminf.* **2011**, *3* (1), 33.
(45) Vainio, M. J.; Johnson, M. S. Generating conformer ensembles using a multiobjective genetic algorithm. *J. Chem. Inf. Model.* **2007**, *47*, 2462−2474. https://pubs.acs.org/doi/10.1021/ci6005646
(46) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. *Gaussian, 16*.. Revision, C.01; Gaussian, Inc.: Wallingford CT, 2016.

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