Note

Limonoids from the Stem Bark of *Khaya senegalensis*

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Six new limonoids with modified furan ring, khaysenelide A–F (1–6), together with six known limonoids (7–12) were isolated from the stem bark of *Khaya senegalensis*. The basic skeletons of these new limonoids belong to mexicanolide (1, 2) and rearrangement phragmalin (3–6), which were elucidated on the basis of spectroscopic methods including high resolution-electrospray ionization (HR-ESI)-MS, one and two dimensional (1D and 2D)-NMR and confirmed by single-crystal X-ray crystallography using CuK α radiation of 1 and 3. Their absolute configurations were determined by the X-ray crystallography data and comparison of their electronic circular dichroism spectra. The inhibitory effect on nitric oxide (NO) production in lipopolysaccharide-activated RAW264.7 macrophages of new compounds was also tested.

Key words *Khaya senegalensis*; Meliaceae; rearranged limonoid; X-ray

Meliaceae plants are attracting considerable interest because of the abundance and structural diversity of the limonoids in its plant members.^{1,2)} *Khaya senegalensis* (DES.) A. JUSS. (Meliaceae), belonging to the genus *Khaya*, was used traditionally in Africa for treatment of malaria and fever,³⁾ the crude extracts of whose bark showed the antifungal, anti-inflammatory and hypertensive effects.^{4–7)} Previous chemical investigations on it had afforded a series of rings B, D-*seco* limonoids, such as mexicanolide, phragmalin, and rearrangement phragmalin.^{8–12)} In the course of a search for limonoids with novel structure and significant biological activities, six new limonoids, khaysenelide A–F (1–6) (Fig. 1), along with six known limonoids were isolated from the stem bark of *K. senegalensis*. The basic skeleton of these limonoids were belong to mexicanolide (1, 2) and rearranged phragmalin (3–12), which were elucidated on the basis of spectroscopic methods including high resolution-electrospray ionization (HR-ESI)-MS, one and two dimensional (1D and 2D)-NMR and confirmed by the single-crystal X-ray crystallography using CuK α radiation of 1 and 3, which also was used to determine their absolute

configuration. The mechanism of modified furan rings in 1–6 was proposed, which also explained why the NMR signal of modified furan ring of compounds 1, 3, 4, 6 shrunk severely. The inhibitory effect on nitric oxide (NO) production in lipopolysaccharide-activated RAW264.7 macrophages of new compounds was also tested. Herein, the isolation and structural elucidation of these compounds are presented.

Results and Discussion

Khaysenelide A (1) was obtained as a colorless crystal methanol–water (MeOH–H₂O), and had the molecular formula of C₂₇H₃₄O₁₁ as determined by HR-ESI-MS at *m/z* 557.1989 [M+Na]⁺ (Calcd for C₂₇H₃₄O₁₁Na, 557.1993). The ¹H-NMR (Table 1) showed the presence of four tertiary methyls (δ_{H} 1.24, 1.16, 1.07, 0.90, each 3H, s), a methoxyl (δ_{H} 3.69, 3H, s). In heteronuclear multiple bond connectivity (HMBC) spectra, two interrelated methyl signals showed same correlations to C-3 and C-5, which indicated that these two methyls connected to a same carbon (Fig. 2). Company with other correlations, such as H-2 to C-4, C-8, and C-10, Me-19 to C-1, C-5,

Fig. 1. The Structures of Compounds 1–6

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Table 1. The ^1H -NMR (500MHz) Spectroscopic Data of Compounds **1–6** in $\text{DMSO}-d_6$

No.	KS-1 δ_{H} , mult, (J in Hz)	KS-2 δ_{H} , mult, (J in Hz)	KS-3 δ_{H} , mult, (J in Hz)	KS-4 δ_{H} , mult, (J in Hz)	KS-5 δ_{H} , mult, (J in Hz)	KS-6 δ_{H} , mult, (J in Hz)
2	3.02, d (9.5)	3.02, d (10.0)	4.29, dd (9.5, 6.5)	4.38, d (10.5)	4.36, dd (9.5, 7.0)	4.35, dd (9.5, 6.5)
3			3.23, dd (8.5, 6.5)		3.31 ^{a)}	3.30 ^{a)}
5	2.73, brs	2.73, brs	2.97, d (6.5)	3.05, d (8.5)	2.94, d (6.5)	2.96, d (6.5)
6	4.40, d (5.5)	4.40, d (5.5)	4.04, dd (7.5, 4.5)	4.13, dd (9.0, 5.0)	4.10, t (5.0)	4.09, t (5.5)
9	1.75, dd (14.0, 5.0)	1.75, m	2.05, m	2.30, d (8.5)	2.15, d (9.0)	2.14, d (9.0)
11 α	1.67, m	1.67, m	1.69, m	1.78, m	1.53, m	1.55, m
11 β	1.32, d (12.5)	1.33, m	1.85, m	1.99, m	1.67, m	1.65, m
12 α	1.20, m	1.20, m	1.12, m	1.21, m	0.71, m	0.67, m
12 β	1.57, d (14.5)	1.53, m	1.85, m	1.72, m	1.91, m	1.88, m
14	1.69, dd (6.5, 2.5)	1.69, dd (6.5, 2.5)				
15a	2.72, m	2.72, m	2.87, d (18.5)	2.93, d (19.0)	2.84, d (18.5)	2.83, d (18.5)
15b	2.70, m	2.70, m	2.67, d (18.5)	2.73, d (19.0)	2.68, d (18.5)	2.64, d (18.5)
17	5.22, s	5.22, s	5.31, brs	5.09, s	5.42, s	5.43, s
18	0.90, s	0.88, s	1.04, s	1.05, s	1.01, s	1.00, s
19	1.24, s	1.25, s	1.11, s	1.33, s	1.21, s	1.21, s
21			6.20, s ^{b)}	6.22, s ^{b)}		
22	7.51, s ^{b)}	7.51, s	6.22, s ^{b)}	6.25, s ^{b)}	7.51, s	7.51, s ^{b)}
23	6.12, s ^{b)}	6.12, s			6.12, s	6.12, s ^{b)}
28	1.16, s	1.16, s	0.94, s	0.91, s	1.01, s	1.01, s
29a	1.07, s	1.07, s	1.19, d (12.0)	2.06, d (12.5)	1.68, d (12.0)	1.68, d (11.5)
29b			1.71, d (12.0)	2.53, d (12.5)	2.10, d (12.0)	2.11, d (11.5)
30 α	2.20, dd (12.5, 10)	2.20, m	2.52, d ^{a)}	3.28, d (10.5)	3.04, d (9.5)	3.03, d (9.5)
30 β	2.45, d (14.5)	2.46, d (14.5)				
OMe-7	3.69, s	3.69, s	3.62, s	3.59, s	3.60, s	3.62, s
23-OMe		3.47, s			3.48, s	
OH-6	5.40, d (5.5)	5.41, d (5.5)			5.48, d (4.5)	5.51, brs
OH-8	4.79, s	4.81, s	4.93, s		5.25, s	5.20, s
OH-1			4.43			
OAc-1				2.01, s	1.96, s	1.96, s
OH-3			3.54, d (8.5)			

a) The signal of overlapped, b) The partial signal observed from HSQC.

Fig. 2. The Key HMBC and ROESY of the Compound **1**

and C-9, the characteristic [3,3,1] A/B ring system could be deduced. Meanwhile, in the HMBC, the strong correlations from H-17 to C-18, C-12, from Me-18 to C-17, C-14, and from H-14 to C-12, C-16 constructed the rings C and D in **1**. The aforementioned information and typical structural moieties indicated that **1** was a mexicanolide-type limonoid, except for a series of weak NMR signals for 23-hydroxybutenolide ring moiety instead of the typical signals for β -substituted furan

ring.^{13,14)} Fortunately, the good high quality single-crystal of **1** was obtained successfully, and the X-ray crystallography data (Fig. 3) confirmed that **1** was mexicanolide-type limonoid with a 23-hydroxybutenolide moiety at C-17 as our deduction, and the absolute configuration of **1** was also finally determined as 5*S*, 6*S*, 8*S*, 10*R*, 13*R*, 14*R*, 17*R*.

Khaysenelide B (**2**), a white amorphous powder, gave a molecular formula of $\text{C}_{28}\text{H}_{36}\text{O}_{11}$ (Calcd for $\text{C}_{28}\text{H}_{40}\text{NO}_{11}$

Fig. 3. Single-Crystal X-Ray Structure of Compound **1**

[M+NH₄]⁺) from the HR-ESI-MS, 14 mass units more than **1**, in accordance with the presence of an additional methylene group. The 1D-NMR data of **2** were similar to those of **1**, except for the presence of 23-methoxybutenolide moiety at C-17, which were supported by the HMBC correlations from H-17 to C-22, C-21, H-22 to C-21, 23, and from OMe-23 to C-23. The relative configuration of **2** was assigned from the rotating frame Overhauser enhancement spectroscopy (ROESY) spectrum. The correlations of H-5/H-11 β , H-11 β /H-17 indicated that such groups were cofacial, and these were assigned arbitrarily as β -oriented. The ROESY cross-peaks of Me-19/H-9, OH-8/H-9 and H-2/Me-29 showed the α -orientation of those groups. However, the available evidences were insufficient to determine the configuration at C-23. Therefore, the relative stereochemistry of compound **2** was established as shown.

Khaysenelide C (**3**), a colorless crystals (in MeOH–H₂O), possessed the molecular formula C₂₇H₃₄O₁₂ by HR-ESI-MS. The ¹H-NMR displayed the existence of three tertiary methyls (δ_{H} 1.11, 1.04, 0.94, each 3H, s), two geminal coupling methylenes [δ_{H} 2.87 (1H, d, J =18.5 Hz), 2.67 (1H, d, J =18.5 Hz)] and [δ_{H} 1.71 (1H, d, J =12.0 Hz), 1.19 (1H, d, J =12.0 Hz)], which implied that **3** was a rearranged phragmalin-type limonoid as khayanolide B.¹⁵⁾ Extensive analysis of 1D- and 2D-NMR data of **3** suggested a close similarity between **3** and khayanolide B,¹⁵⁾ sharing the same A₁, A₂, B, C, and D rings. The main differences were the signal absence of furan ring (E), however, degradation of ring E was unreasonable according to the HR-ESI-MS. Weak carbon and proton signals observed from 1D- and 2D-NMR, δ_{C} 169.4 (C-23), 120.3 (C-22), 98.7 (C-21) and δ_{H} 6.20 (H-22), 6.22 (H-21), indicated that the furan ring was modified to be 21-hydroxybutenolide moiety as trichanolide D.¹⁵⁾ With the X-ray crystallography data of **3** (Fig. 4), above deduce was confirmed and the structure of **3** was determined as depicted in Fig. 1, and the absolute configuration of it could be established as 1S, 2R, 3S, 4S, 5S, 6S, 8S, 10R, 13R, 14R, 17R.

Khaysenelide D (**4**), a white powder, its molecular formula was determined as C₂₉H₃₄O₁₃ on the basis of HR-ESI-MS at m/z 613.1889 [M+Na]⁺ (Calcd for C₂₉H₃₄O₁₃Na). The whole feature of the ¹H- and ¹³C-NMR spectral data (Tables 1, 2) indicated that **2** also possessed a rearranged phragmalin-type limonoid as khayanolide E.¹⁴⁾ The weak but similar carbon and proton signals about ring E indicated that **4** also possess 21-hydroxybutenolide moiety at C-17, which was in accordance with the molecular formula.¹⁴⁾ Thus, the structure of **4**

Fig. 4. Single-Crystal X-Ray Structure of Compound **3**Table 2. The ¹³C-NMR (125 MHz) Spectroscopic Data of Compounds **1**–**6** in DMSO-*d*₆

No.	KS-1	KS-2	KS-3	KS-4	KS-5	KS-6
1	210.0	210.0	83.2	89.7	90.7	90.7
2	53.4	53.4	72.6	74.0	71.9	71.9
3	214.4	214.5	77.9	204.9	77.4	77.5
4	49.5	49.6	42.2	50.2	43.7	43.6
5	45.2	45.2	40.3	39.6	38.9	39.1
6	69.5	69.5	70.9	69.3	71.0	70.9
7	175.8	175.8	174.7	172.9	174.6	174.6
8	71.8	71.8	86.4	86.2	85.8	85.8
9	60.0	59.9	54.4	53.7	54.7	54.6
10	48.8	48.8	58.9	60.6	60.5	60.5
11	22.7	22.6	16.2	15.9	15.9	15.9
12	34.6	34.7	25.2	25.2	25.4	25.1
13	34.4	34.5	37.3	37.1	37.3	37.4
14	50.6	50.5	80.5	83.0	80.4	80.6
15	26.1	26.1	31.9	32.6	31.6	31.6
16	169.2	169.1	169.2	168.5	169.4	169.4
17	75.2	75.0	80.5	80.0	78.3	78.3
18	23.4	23.6	14.6	14.3	13.8	13.8
19	23.9	23.9	18.0	17.6	18.4	18.4
20	130.5 ^{a)}	133.8	— ^{c)}	— ^{c)}	133.0	130.3 ^{a)}
21	169.2 ^{a)}	169.0	98.7 ^{b)}	— ^{c)}	169.2	169.1 ^{a)}
22	149.5 ^{b)}	149.5	120.8 ^{b)}	121.0 ^{b)}	149.7	149.4 ^{b)}
23	102.4 ^{b)}	102.5	169.4 ^{a)}	169.3 ^{a)}	102.6	102.1 ^{b)}
28	23.4	23.5	19.3	14.7	19.2	19.2
29	23.2	23.2	45.2	40.2	41.2	41.2
30	38.2	38.2	62.9	57.9	58.1	58.2
OMe-7	52.1	52.1	51.2	51.5	51.1	51.1
OMe-23		56.1			56.2	
OCOCH ₃ -1				169.8	169.8	169.8
OCOCH ₃ -1				21.7	21.8	21.8

a) The partial signal based on ¹³C-NMR and reference. b) The partial signal observed from HSQC. c) Signal can not be observed clearly from 1D- and 2D-NMR.

was determined as depicted in Fig. 1, and the absolute configuration of carbon skeleton was also as same as **3** based on the similar circular dichroism (CD) spectra between compounds **3** and **4** (Fig. 6).

Khaysenelide E (**5**), a white powder, was shown to have the molecular formula C₃₀H₃₈O₁₃ from its HR-ESI-MS. Based on the ¹H-NMR spectroscopy data, three tertiary methyls [(δ_{H} 1.21, CH₃, s), (δ_{H} 1.01, CH₃×2, s)], a C-29-methylene [δ_{H} 2.11 (1H, d, J =12.0 Hz), 1.68 (1H, d, J =12.0 Hz)], a C-15-methylene

Fig. 5. The ECD Spectra of Compounds 1 and 2

Fig. 6. The ECD Spectra of Compounds 3 and 4

$[\delta_{\text{H}}$ 2.84 (1H, d, $J=18.5$ Hz), 2.68 (1H, d, $J=18.5$ Hz)], compound **5** was also a rearranged phragmalin-type limonoid as **3**. Extensive analyses of 1D-NMR data (Tables 1, 2) revealed that **5** shared the common A_1 , A_2 , A_3 , C, and D rings with 1-*O*-acetylkhayanolide B.¹⁵⁾ However, the characteristic signals for a furan moiety were absent in the NMR spectra of **5**, instead of two singlets at δ_{H} 7.51 and 6.12 and four carbon signals at δ_{C} 102.6, 133.0, 149.7, and 169.2, which were in agreement with the presence of a 23-hydroxybutenolide moiety as compound **2**. Further HMBC correlations, from H-17 to C-20 and C-21, H-22 to C-17, C-20, C-21 and C-23, OMe-23 to C-23, indicated that 23-methoxybutenolide moiety connected at C-17 in **5**. The relative configuration of **5** was established by ROESY spectrum, in which correlations of H-5/H-12 β , H-17/12 β indicated that these groups were on the same side of the molecule, and they were arbitrarily assigned as β -oriented. The ROESY correlations of Me-19/H-9, OH-8/H-9 implied that they were α -oriented. Thus, the structure of **5** was determined as depicted in Fig. 1.

Khaysenelide F (**6**), a white powder, was given the molecular formula $C_{29}H_{36}O_3$ from HR-ESI-MS, 14 mass units less than **5**. The whole feature of the ^1H - and ^{13}C -NMR spectral data indicated that **6** possessed the same rearranged phragmalin-type limonoid skeleton as **5**. The weak but similar carbon and proton signals (Tables 1, 2) about ring E indicated that **6** also possessed 23-hydroxybutenolide moiety at C-17 as **5**, except for the absence of OMe-23. Thus, the structure of **6** was determined to be 23-*O*-demethoxy derivative of **5**, and named khaysenelide F.

The structures of known compounds were identified as khayanolide B (**7**),¹⁴⁾ 1-*O*-deacetyl-2 α -hydroxykhayanolide E (**8**),¹⁶⁾ 1-*O*-deacetylkhayanolide E (**9**),¹⁴⁾ senegalensins A (**10**),¹¹⁾ khaysenagain E (**11**),¹²⁾ khaysenagain I (**12**)¹⁷⁾ by comparison of their physical and spectroscopic data with those of literatures.

Compounds **1**–**12** was evaluated for inhibitory effect on NO production in lipopolysaccharide-activated RAW264.7 macrophages. Unfortunately, they did not show potent activity (IC_{50} value more than $50\ \mu\text{M}$).

Experimental

General Experimental Procedures Optical rotations were measured with a JASCO P-1020 polarimeter (JASCO, Tokyo, Japan). CD spectra were obtained on a JASCO J 810 spectropolarimeter (JASCO, Tokyo, Japan). UV spectra were recorded on a UV-2450 UV/Vis spectrophotometer (Shimadzu, Tokyo, Japan). X-Ray were measured on a Bruker Smart CCD with a graphite monochromator with $\text{CuK}\alpha$ radiation ($\lambda=1.54184\ \text{\AA}$) at 291(2)K (Bruker, Karlsruhe, Germany). NMR spectra were recorded on a Bruker AV III-500 NMR instrument (^1H : 500 MHz, ^{13}C : 125 MHz) with tetramethylsilane (TMS) as internal standard (Bruker, Karlsruhe, Germany). HR-ESI-MS was obtained on an Agilent 6520B Q-TOF spectrometer (Agilent Technologies, Santa Clara, CA, U.S.A.). Column chromatography (CC) was carried out on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), MCI gel (Mitsubishi Chemical Corp., Tokyo, Japan), Sephadex LH-20 (Pharmacia, Uppsala, Sweden) and MPLC (Beijing H&E Co., Ltd., Beijing, China). Preparative HPLC was carried out using a Shimadzu LC-6A instrument with a SPD-10A detector (Shimadzu, Tokyo, Japan) using a shim-pack RP- C_{18} column (20 $\times$ 200 mm).

Plant Material The stem bark of the *Khaya senegalensis* were collected from Xishuangbanna, Yunnan Province, China in May 2009, and were authenticated by Professor Mian Zhang, Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. 2009-KS-LQP) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation The air-dried stem bark of the *K. senegalensis* (3.5 kg) were exhaustively extracted with 95% ethanol (EtOH) (3 h $\times$ 3) in room temperature. The EtOH extract was concentrated under reduced pressure to obtain a residue (314 g), which was suspended in H_2O and successively partitioned with petroleum ether (PE), acetyl acetate (EtOAc), respectively. The EtOAc extract (53.5 g) was subjected to column chromatography (CC) on silica gel eluted with a gradient of CH_2Cl_2 –MeOH (100:1, 50:1, 25:1, 10:1, 5:1) to give eight fractions (Frs. A–H). Fraction E (8.0 g) was separated by silica gel (PE–EtOAc, 5:1, 2:1, 1:1, 1:2) to obtain five subfractions E1–E5. E3 was chromatographed over silica gel (CH_2Cl_2 –MeOH, 25:1) to give five subfractions, E31–E35. E32 was subjected to Sephadex LH-20 (MeOH), MPLC with a continuous gradient of MeOH– H_2O (30:70, 50:50, v/v, 10 mL/min) and *prep*-HPLC (MeOH– H_2O , 40:60, v/v, 10 mL/min) to afford compound **4** (3 mg). E4 was separated on silica gel using a gradient of CH_2Cl_2 –MeOH (40:1, 20:1) to yield four fractions E41–E44. E41 was separated by Sephadex LH-20 (MeOH), and further chromatographed over *prep*-HPLC (MeOH– H_2O , 40:60, v/v, 10 mL/min) to obtain compounds **5**

(4mg) and **6** (4mg). E5 was isolated by CC on ODS (MeOH–H₂O, 30:70–90:10, v/v) to obtain four subfractions E5a–E5d. E5a was dissolved with methanol to crystallize. The crystal was subsequently subjected to *prep*-HPLC (acetonitrile–H₂O, 25:75, v/v, 10mL/min) to give compounds **1** (5mg) and **3** (4mg).

Fraction F (8.0g) was chromatographed over silica gel eluting with a gradient of CH₂Cl₂–MeOH (100:1, 40:1, 20:1, 10:1) to yield four subfractions Fa–Fd. Fb was separated by silica gel (PE–EtOAc, 4:1, 2:1, 1:2) to obtain five subfractions FbA–FbE. FbE was separated by ODS (MeOH–H₂O, 30:70–100:0) to give six subfractions FbEa–FbEf. FbEa was subjected to silica gel (CH₂Cl₂–MeOH, 40:1) to obtain seven subfractions FbEa1–FbEa7. FbEa4 was chromatographed on *prep*-HPLC (MeOH–H₂O, 40:60, v/v, 10mL/min) to obtain compound **11** (4mg). FbEa7 was subjected to *prep*-HPLC (MeOH–H₂O, 50:50, v/v, 10mL/min) to obtain compounds **10** (4mg) and **12** (5mg). Fraction G (4.0g) was separated by silica gel eluting with a gradient of PE–acetone (6:1, 3:1, 1:1, 1:2) to give six subfractions G1–G6. G4 was subjected to Sephadex LH-20 (MeOH) and further performed on *prep*-HPLC (acetonitrile–H₂O, 30:70, v/v, 10mL/min) to give compounds **7** (10mg), **8** (6mg), and **9** (9mg). G5 was chromatographed over Sephadex LH-20 (MeOH), silica gel (CH₂Cl₂–MeOH, 20:1) and further subjected to *prep*-HPLC (acetonitrile–H₂O, 15:85, v/v, 10mL/min) to yield compound **2** (5mg).

Khaysenelide A (**1**): Colorless needles, mp 275–277; $[\alpha]_D^{25} +25.5$ ($c=0.15$ MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 206 (3.29) nm; IR (KBr) $\nu_{\max}$: 3450, 2923, 1735, 1700, 1640, 1616, 1401, 1087; ¹H- and ¹³C-NMR data, see Tables 1 and 2; ESI-MS m/z 552 [M+NH₄]⁺, 533 [M–H][–]; HR-ESI-MS m/z 557.1989 [M+Na]⁺ (Calcd for C₂₇H₃₄O₁₁Na, 557.1993).

Khaysenelide B (**2**): White amorphous powder; $[\alpha]_D^{25} +28.4$ ($c=0.06$ MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 204 (3.68) nm; IR (KBr) $\nu_{\max}$: 3442, 2933, 1738, 1703, 1642, 1623, 1387, 1068; ¹H- and ¹³C-NMR data, see Tables 1 and 2; ESI-MS m/z 571 [M+Na]⁺; HR-ESI-MS m/z 566.2591 [M+NH₄]⁺ (Calcd for C₂₈H₄₀NO₁₁, 566.2596).

Khaysenelide C (**3**): Colorless needles, mp 238–240; $[\alpha]_D^{25} +1.51$ ($c=0.07$ MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 207 (3.79) nm; IR (KBr) $\nu_{\max}$: 3437, 2944, 1727, 1650, 1628, 1460, 1378, 1036; ¹H- and ¹³C-NMR data, see Tables 1 and 2; ESI-MS m/z 568 [M+NH₄]⁺; HR-ESI-MS m/z 568.2391 [M+NH₄]⁺ (Calcd for C₂₇H₃₈NO₁₂, 568.2389).

Khaysenelide D (**4**): White amorphous powder; $[\alpha]_D^{25} +41.2$ ($c=0.07$ MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 207 (3.82) nm; IR (KBr) $\nu_{\max}$: 3445, 2943, 1730, 1638, 1618, 1448, 1388, 1032; ¹H- and ¹³C-NMR data, see Tables 1 and 2; ESI-MS m/z 608 [M+NH₄]⁺; HR-ESI-MS m/z 613.1889 [M+Na]⁺ (Calcd for C₂₉H₃₄O₁₃Na, 613.1892).

Khaysenelide E (**5**): White amorphous powder; $[\alpha]_D^{25} -10.4$ ($c=0.10$ MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 205 (3.54) nm; IR (KBr) $\nu_{\max}$: 3455, 2937, 1736, 1638, 1621, 1436, 1383, 1028; ¹H- and ¹³C-NMR data, see Tables 1 and 2; ESI-MS m/z 641 [M+Cl][–]; HR-ESI-MS m/z 629.2206 [M+Na]⁺ (Calcd for C₃₀H₃₈O₁₃Na, 629.2205).

Khaysenelide F (**6**): White amorphous powder; $[\alpha]_D^{25} +15.2$ ($c=0.07$ MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 205 (3.84) nm; IR (KBr) $\nu_{\max}$: 3438, 2923, 1718, 1632, 1619, 1436, 1391, 1035; ¹H- and ¹³C-NMR data, see Tables 1 and 2; ESI-MS m/z 591 [M–H][–]; HR-ESI-MS m/z 615.2045 [M+Na]⁺ (Calcd for

C₂₉H₃₆O₁₃Na, 615.2048).

Single-Crystal X-Ray Crystallography of 1 The colorless crystals of **1** were obtained from MeOH–H₂O. Crystal data were obtained on a Bruker Smart CCD with a graphite monochromator with CuK α radiation ($\lambda=1.54184$ Å) at 291(2)K. The structure was solved by direct methods using the SHELXS-97 and expanded using difference Fourier techniques, refined with SHELXS-97. Crystal data of **1**: C₂₇H₃₄O₁₁ (M=534.54); monoclinic (0.32×0.24×0.22 mm³); space group *P*2₁; unit cell dimensions $a=7.5563(2)$ Å, $b=14.0622(3)$ Å, $c=12.2074(3)$ Å, $\beta=99.500(2)^\circ$, $V=1297.09(5)$ Å³; $Z=2$; $D_{\text{calcd}}=1.369$ mg/mm³; $\mu=0.893$ mm^{–1}; 9280 reflections measured ($9.60 \leq 2\theta \leq 139.94$); 4530 unique ($R_{\text{int}}=0.0162$) which were used in all calculations; the final refinement gave $R_1=0.0321$ ($I>2\sigma(I)$) and $wR_2=0.0863$ (all data); flack parameter=0.03(15).

Single-Crystal X-Ray Crystallography of 3 The X-ray data of **3** were collected at 291(2)K with CuK α radiation ($\lambda=1.54184$ Å) on a Bruker Smart CCD diffractometer. Crystal data: C₅₄H₆₈O₃₃ (1245.08); triclinic (0.32×0.21×0.20 mm³); space group *P*1; unit cell dimensions $a=8.2245(2)$ Å, $b=12.9811(3)$ Å, $c=13.6097(3)$ Å, $\beta=90.972(2)^\circ$, $V=1428.15(6)$ Å³; $Z=1$; $D_{\text{calcd}}=1.448$ mg/mm³; $\mu=1.045$ mm^{–1}; 27440 reflections measured ($6.536 \leq 2\theta \leq 143.948$); 10024 unique ($R_{\text{int}}=0.0246$) which were used in all calculations; the final refinement gave $R_1=0.0615$ ($I>2\sigma(I)$) and $wR_2=0.01748$ (all data); flack parameter=0.07(5).

NO Production Bioassay The protocol for NO production bioassays was provided in the previously published paper.¹⁸⁾ *N*-Monomethyl-L-arginine was used as the positive control. All the experiments were performed in three replicates.

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Conflict of Interest The authors declare no conflict of interest.

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