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Cycloartane Glycosides from *Sutherlandia frutescens*

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Abstract

Four new cycloartane glycosides, sutherlandiosides A–D (**1–4**), were isolated from the South African folk medicine *Sutherlandia frutescens* and their structures established by spectroscopic methods and X-ray crystallography as 1*S*,3*R*,24*S*,25-tetrahydroxy-7*S*,10*S*-epoxy-9,10-*seco*-9,19-cyclolanost-9(11)-ene 25-*O*-β-D-glucopyranoside (**1**), 3*R*,7*S*,24*S*,25-tetrahydroxycycloartan-1-one 25-*O*-β-D-glucopyranoside (**2**), 3*R*,24*S*,25-trihydroxycycloartane-1,11-dione 25-*O*-β-D-glucopyranoside (**3**), and 7*S*,24*S*,25-trihydroxycycloart-2-en-1-one 25-*O*-β-D-glucoyranoside (**4**). Compound **1** represents the first secocycloartane skeleton possessing a 7,10-oxygen bridge. Compounds **2–4** are also the first examples of naturally occurring cycloartanes with a C-1 ketone functionality. Biosynthetic considerations and chemical evidence suggest that the presence of the C-1 ketone in **2** may facilitate the ring opening of the strained cyclopropane system.

Sutherlandia frutescens (L.) R. Br (Fabaceae), native to South Africa with a folk name of Cancer Bush, is known as a multipurpose medicinal plant. Infusions and decoctions of the plant are widely used in South Africa to treat gastrointestinal problems such as stomachaches and intestinal ailments and as a tonic to relieve eye troubles and chicken pox.[1] It has also been used by traditional healers to treat cancers, inflammation, and viral diseases.[2] Recently, this plant has been utilized to treat people suffering from AIDS.[3] Some reports show that the crude extract of the plant possesses anticancer,[4–7] antistaphylococcal, and radical-scavenging activities as well as inhibitory activity against HIV target enzymes.[8,9] The plant extract also shows promise for the treatment of type 2 diabetes.[10] Various commercial products containing powdered plant material or the extract of the plant are on the market for sale as dietary supplements. To assess the safety of this medicinal plant, a clinical study has recently been conducted in South Africa, indicating that

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Supporting Information Available: X-ray ORTEP drawing, key HMBC and NOE correlations of compound **1**, key HMBC and ROESY correlations of compound **2**, and NMR spectra of compounds **1–4** and **8** are available free of charge via the Internet at http://pubs.acs.org.

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consumption of 800 mg of the leaf powder capsules daily for 3 months was tolerated by healthy adults without apparent adverse effects.[11] Despite extensive biological and pharmacological studies, little is known about the chemical constituents of this medicinally important herbal plant except for one report describing three compounds: L-canavanine, pinitol, and γ-aminobutyric acid.[5] The current study was attempted to investigate other chemical constituents of this plant, leading to the identification of four new cycloartane glycosides, namely, sutherlandiosides A–D (**1–4**).

Sutherlandiosides A–D (**1–4**) were obtained from the *n*-BuOH-soluble portion of the MeOH extract of the leaves of *S. frutescens* by column chromatography on silica gel and reversed-phase silica gel.

Results and Discussion

The molecular formula of sutherlandioside A (**1**) was determined as $C_{36}H_{60}O_{10}$ by HRESIMS, which provided a pseudomolecular ion peak at *m/z* 675.4042 $[M + Na]^+$, in conjunction with its ^{13}C NMR spectrum displaying 36 resonances. A DEPT NMR experiment permitted differentiation of the 36 resonances into seven methyl, nine methylene, 14 methine, and six quaternary carbons, of which 30 were attributed to a triterpene skeleton and six to a β-glucopyranosyl moiety (Table 1).[12,13] The β configuration of the glucopyranose was evident by its anomeric proton resonance at δ_H 5.24 (d, J = 7.6 Hz, H-1) in the ^{1}H NMR spectrum (Table 2). Regarding the aglycone moiety, typical resonances include six tertiary methyl groups at $\delta_{H/C}$ 0.71/15.5, 1.57/21.7, 1.54/24.6, 1.17/27.4, 1.03/24.0, and 0.75/20.0, one secondary methyl at $\delta_{H/C}$ 0.95/19.2, four oxygenated methine at $\delta_{H/C}$ 3.83 (br, s)/77.0, 3.86 (d, J = 9.7 Hz)/78.7, 4.28/72.0, and 4.30/75.8, two oxygenated quaternary carbons at δ_C 81.3 and 84.8, and one double bond at δ_C 135.9 (C) and 121.1 (CH), indicating a polyhydroxylated cycloartane-like triterpene skeleton.[12–14] Unlike most cycloartane compounds that show characteristic proton resonances for H-19 in the extremely upfield region up to δ_H 0.3,[14] the presence of a significantly downfield shifted H-19 at δ_H 2.69 and 2.89 with a large coupling constant of 16.1 Hz indicated that **1** might possess a 9,10-secocycloartane skeleton.[15] This was confirmed as follows. The chemical shifts of a set of carbon resonances, corresponding to the side chain at C-17 (from C-20 to C-27) and the β-glucopyranosyl moiety, are completely identical to those of known glycosides that possess a 24*S*,25-dihydroxy side chain in which the β-D-glucopyranosyl moiety is attached to the tertiary C-25 OH group.[13] The location of the OH groups at C-1 and C-3 on ring A was indicated by DQF-COSY, showing a coupling network from H-1 → H-2 → H-3. In the HMBC spectrum of **1**, the characteristic H-19 at δ_H 2.89 correlated with one oxygenated carbon at δ_C 84.8 (C), two olefinic carbons at δ_C 135.9 (C) and 121.1 (CH), and a methine at δ_C 53.1 that was assumed to be C-8. This indicated that C-10 (δ_C 84.8) might be oxygenated and a double bond located between C-9 and C-11. The remaining oxygenated tertiary carbon at δ_C 75.8 correlated with a proton at δ_H 4.30 in the HMQC spectrum. This proton showed cross-peaks with an olefinic carbon at δ_C 135.9 (C) and the quaternary carbon at δ_C 84.8 (C-10) in the HMBC spectrum, establishing the location of an oxygen atom at C-7 on ring B and the formation of an oxygen bridge between C-7 and C-10. The presence of the epoxy group, instead of free OH groups at C-7 and C-10, is consistent with the HRESIMS data. All other HMBC correlations that provide key C–C connectivity information support the planar structure of **1**. It is noted that the two geminal protons of H-2 in the ^{1}H NMR spectrum have very small couplings with H-1 and H-3 since both protons of H-2 showed a broad doublet at δ_H 2.20 (J = 14.0 Hz) and 2.40 (J = 14.0 Hz), respectively. This indicates that both H-1 and H-3 are equatorially oriented, positioned between the two geminal protons, and thus the OH groups at C-1 and C-3 should be α-oriented. Further evidence was derived from the NOE correlation between H-1 and H-19β and between H-3 and both Me-28 (weaker) and Me-29 (stronger) in the ROESY spectrum of **1**. The epoxy group between C-7 and C-10 was

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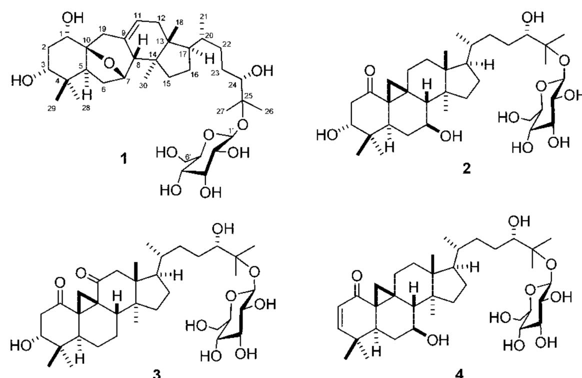
deduced to be β-oriented on the basis of the NOE correlations between H-7 and Me-30, between H-6α and Me-30, and between H-5 and Me-28 and H-19α. Other key NOE correlations included H-8β and Me-18 as well as anomeric H-1 and Me-26/Me-27. A comprehensive analysis of the 2D NMR data of **1** facilitated the assignments of the proton and carbon resonances, which are shown in Tables 1 and 2. The absolute configuration of **1** including C-24 and the sugar moiety was determined from the anomalous X-ray scattering from the oxygen atoms, with the refinement of 2658 Bijvoet pairs. The Flack absolute configuration parameter was 0.06(16),[16] where a value close or equal to zero represents the correct structure. Also, the deviation parameter *u*, being 0.016, satisfies the requirement that "$u < 0.04$ implies a strong inversion-distinguishing power".[17] Thus, the structure of **1** was characterized as 1*S*,3*R*,24*S*,25-tetrahydroxy-7*S*,10*S*-epoxy-9,10-*seco*-9,19-cyclolanost-9(11)-ene 25-*O*-β-D-glucopyranoside.



Sutherlandioside B (**2**) as the major glycoside of this plant was obtained as colorless needles from methanol in a yield of 1.95%. Its molecular formula was determined as $C_{36}H_{60}O_{10}$ by ^{13}C NMR data and HRESIMS. A DEPT NMR experiment permitted differentiation of the 36 resonances into seven methyl, 10 methylene, 12 methine, and seven quaternary carbons. The presence of a β-glucopyranosyl moiety in **2** was clear by comparison of its ^{1}H and ^{13}C NMR data with those of **1** (Tables 1 and 2). The characteristic resonances due to the aglycone moiety were observed for an isolated methylene of the cyclopropane system at $\delta_{H/C}$ 0.86, 1.60/24.4, six tertiary methyl groups at $\delta_{H/C}$ 0.99/16.0, 1.04/21.7, 1.09/18.9, 1.26/25.6, 1.54/24.6, and 1.57/21.6, and a secondary methyl at $\delta_{H/C}$ 0.95/19.3, indicating a cycloartane-type triterpene skeleton.[12–14,18] The side chain at C-17 including the β-glucopyranosyl moiety attached to the 25-hydroxy group in **2** is the same as that of compound **1** since their ^{1}H and ^{13}C NMR chemical shifts are identical (Tables 1 and 2). This suggested that the difference between **1** and **2** lies in their aglycone moieties. In comparison with **1**, which has a double bond between C-9 and C-11, the absence of such a double bond in **2** was clear simply based on the lack of olefinic proton and carbon resonances in its 1D ^{1}H and ^{13}C NMR spectra, and the presence of a carbonyl group in **2** was evident from the absorption at ν_{max} 1686 cm^{-1} in its IR spectrum and the carbon resonance at δ_C 210.7 in its ^{13}C NMR spectrum. The carbonyl is located at C-1 since it showed an HMBC correlation with H-19. H-19 protons also had correlations with C-11 (δ_C 29.2). The location of the OH groups at C-3 and C-7 was confirmed by relevant HMBC correlations such as H-2 and C-3 and H-8 and C-7. The orientation of the OH groups at C-3 and C-7 was determined as α and β, respectively, by NOE evidence, since H-3 had a weak correlation with α-orientated Me-28 and a strong correlation with β-orientated Me-29, while H-7 had a strong correlation with Me- 30. Other NOE correlations (refer to Figure S4 in the Supporting Information) supported the structure shown and facilitated the NMR signal assignments of **2**. It is noted that the two geminal protons of H-2 have similar coupling constants (~4 Hz) to H-3, which is equatorially oriented, indicating that the conformation of ring A is slightly different from

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that of compound **1**. Thus, the structure of **2** is defined as 3*R*,7*S*,24*S*,25-tetrahydroxycycloartan-1-one 25-*O*-β-D-glucopyranoside.

Sutherlandioside C (**3**) had a molecular formula of $C_{36}H_{58}O_{10}$ deduced from its ^{13}C NMR data and HRESIMS. The NMR data of **3** resembled those of **2**. Typical differences were the presence of an additional carbonyl group and the lack of an oxygenated carbon in **3**. The two carbonyls gave IR absorptions at 1708 and 1671 cm^{-1}. The HMBC spectrum indicated that the additional carbonyl was positioned at C-11 since both protons of H-19 (δ_H 1.32 and 2.26) showed correlations with both carbonyls (δ_C 205.6 and 209.3). In addition, the carbonyl at C-11 caused shifting of H-12 signals downfield to δ_H 2.72 and 2.88 (ABq, J = 16.4 Hz) when compared to H-12 at δ_H 1.54 and 1.84 in **2**. The absence of a C-7 OH group in **3** was confirmed by 2D NMR data, which supported its structure as 3*R*,24*S*,25-trihydroxycycloartane-1,11-dione 25-*O*-β-D-glucopyranoside.

Sutherlandioside D (**4**) had a molecular formula of $C_{36}H_{58}O_9$ on the basis of its ^{13}C NMR data and HRESIMS. The ^{1}H and ^{13}C NMR spectra of **4** were similar to those of **2**. However, the presence of an α, β-unsaturated ketone system was indicated by the NMR [δ_C 129.6, 159.1, 200.9; δ_H 6.11, 6.59 (1H each, d, J = 10 Hz)], IR (ν_{max} 1655 cm^{-1}), and UV (λ_{max} 222 nm). It appeared that this system was located in ring A since the NMR signals for the remaining protons and carbons were almost superimposable on those of **2** (Tables 1 and 2). The position of this double bond between C-2 and C-3 was confirmed by the HMBC spectrum, showing correlations of H-3 at δ_H 6.59 with the carbonyl carbon at δ_C 200.9, a quaternary carbon at δ_C 37.0 (C-4), and a tertiary carbon at δ_C 42.5 (C-5). This compound is simply a dehydration product of **2**. All the NMR data of **4**, assigned by DQF-COSY, HMQC, and HMBC, were satisfactorily consistent with the structure shown as 7*S*,24*S*,25-trihydroxycycloart-2-en-1-one 25-*O*-β-D-glucopyranoside.

Compound **1** is a novel natural product and, to our knowledge, represents the first secocycloartane skeleton possessing a 7,10-oxygen bridge. Compounds **2–4** are also the first examples of naturally occurring cycloartanes with a C-1 ketone functionality. The presence of the C-1 ketone functionality may facilitate the opening of the strained cyclopropane ring system. From the biosynthetic viewpoint, **1** may derive from **2**, which could undergo a ring opening between C-9 and C-10, generating two unsaturated C_9–C_{11} and C_1–C_{10} systems in the presumed intermediate **5**. Epoxidation of the C_9–C_{10} double bond in **5** may lead to **6**. An intramolecular nucleophilic attack of the C-7 OH group in **6** to the epoxide at C-10 could form the oxygen bridge in **7** (Scheme 1). Dehydration of **7**, followed by reduction could afford **1**. This could explain the retention of the C-7 configuration in **1**.

The opening of the cyclopropane ring in **2** was chemically achieved by treating the compound with 2 N HCl in dioxane, affording a new aglycone (**8**) possessing two double bonds, along with D-glucose. The structure of **8** was established as 7*S*,24*S*,25-trihydroxy-9,10*R*-*seco*-9,19-cyclolanost-2(3),9(11)-diene by the analysis of its IR, HRESIMS, and NMR data including COSY, HMQC, HMBC, and ROESY. The conformation of **8** obtained from Chem3D ultra 10.0 (Figure 1) nicely explains the similar coupling constants (3.2 and 4.8 Hz (Table 2)) between the two protons of H-19 and H-10β, which are positioned between them. The H-10 β configuration was supported by the key NOE correlation between H-10 and Me-29. Scheme 2 shows the possible mechanism for the formation of **8** from **2**. Thus, **2** was subjected to acid-catalyzed dehydration between C-2 and C-3 and the cleavage of the glucosyl unit at the OH group of C-25 to afford intermediate **9**, which underwent ring opening to generate two additional double-bond systems in the key intermediate **10**, which underwent an enol–ketone tautomerism in a selective manner to furnish **5**.

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Compounds **1–4** were tested for antimicrobial activity against *Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, Staphylococcus aureus,* methicillin-resistant *S. aureus, Pseudomonas aeruginosa,* and *Mycobacterium intracellulare* and antimalarial activity against *Plasmodium faciparum* (D6 clone and W2 clone) by the methods described in previous publications.[19,20] No activities were observed at the highest test concentrations, which were 20 μg/mL for antimicrobial activity or 40 μg/mL for antimalarial activity.

In conclusion, four new cycloartane glycosides have been isolated from the title plant, and a possible mechanism for the acid-catalyzed ring opening of the strained cyclopropane system in the C-1 ketone-containing cycloartanes is proposed. These compounds, in particular the major glycoside, sutherlandioside B (**2**), may serve as chemical markers for the commercial dietary supplements prepared from this herbal medicine.

Experimental Section

General Experimental Procedures

The 1D and 2D NMR (DQF-COSY, HMQC, HMBC, ROESY) spectra were recorded on a Varian Oxford AS400 spectrometer operating at 400 (^{1}H) and 100 (^{13}C) MHz or on a Bruker Avance DRX 500 FT spectrometer operating at 500 (^{1}H) and 125 (^{13}C) MHz. The chemical shift values are relative to the internal standard TMS. High-resolution HRESIMS were obtained on an Agilent Series 1100 SL mass spectrometer. IR spectra were recorded on a Bruker, Tensor 27 FT-IR spectrometer. Optical rotations were measured on a Rudolph Research Autopol IV polarimeter. Column chromatography was performed using normal-phase silica gel (J. T. Baker, 40 μm) and reversed-phase silica gel (RP-18, J. T. Baker, 40 μm). Semipreparative HPLC was conducted on an ODS (Prodigy) column (250 × 10 mm, 10 μm) using a UV detector at 215 nm. TLC was performed on silica gel sheets (Alugram Sil G/UV_{254}, Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F_{254S}, Merck, Germany). Visualization: 10% H_2SO_4 followed by heating.

Plant Material

The leaves of *Sutherlandia frutescens* were collected in South Africa. A voucher specimen is in the National Center for Natural Products Research at The University of Mississippi (voucher # 3222).

Extraction and Isolation

The air-dried leaves (2 kg) were extracted with MeOH at room temperature (3.0 L × 24 h × 4). The combined extracts were concentrated *in vacuo* (under 45 °C) to obtain 433.6 g of dry extract, a portion of which (102.0 g) was suspended in water and extracted with hexanes, $CHCl_3$, and *n*-BuOH (saturated with H_2O) sequentially. The *n*-BuOH phase was evaporated to dryness *in vacuo* to give a brown residue (29.75 g). The residue was subjected to silica gel chromatography using a stepwise gradient mixture of $CHCl_3$/MeOH/H_2O (9:1:0, 6:1:0, and 7:3:0.5) as eluent to give fractions A–T. Fraction H, in MeOH, yielded crystals of compound **2** (919 mg). Fraction E (1075 mg) was applied to an RP-18 column using 60%–70% MeOH to give subfractions E1–E18 and compound **1** (58.3 mg). Subfraction E7 was subjected to reversed-phase (ODS) semipreparative HPLC using 30% MeCN in H_2O (2 mL/min), yielding compounds **3** (12.0 mg) and **4** (5.8 mg).

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Sutherlandioside A (1)

colorless needles (from EtOH); mp 210–220 °C; $[\alpha]^{20}_{D}$ +17.8 (*c* 1.00, MeOH); IR (NaCl) ν_{max} 3344, 2915, 2358 cm^{-1}; NMR data, Tables 1 and 2; HRESIMS *m/z* 675.4042 (calcd for $[C_{36}H_{60}O_{10} + Na]^+$, 675.4079).

Sutherlandioside B (2)

colorless needles (from MeOH); mp 158 °C; $[\alpha]^{20}_{D}$ −26.8 (*c* 1.00, MeOH); IR (NaCl) ν_{max} 3279, 2950, 2362, 1686 cm^{-1}; NMR data, Tables 1 and 2; HRESIMS *m/z* 675.4112 (calcd for $[C_{36}H_{60}O_{10} + Na]^+$, 675.4079).

Sutherlandioside C (3)

white powder; $[\alpha]^{20}_{D}$ +15.0 (*c* 0.41, MeOH); IR (NaCl) ν_{max} 3389, 2933, 2361, 1708, 1671 cm^{-1}; NMR data, Tables 1 and 2; HRESIMS *m/z* 673.3961 (calcd for $[C_{36}H_{58}O_{10} + Na]^+$, 673.3922), 651.4143 (calcd for $[C_{36}H_{58}O_{10} + H]^+$, 651.4103).

Sutherlandioside D (4)

white powder; $[\alpha]^{20}_{D}$ +58.0 (*c* 0.79, MeOH); IR (NaCl) ν_{max} 3405, 2955, 2361, 1655 cm^{-1}; UV (MeOH) λ_{max} (log ε) 222 (4.04) nm; NMR data, Tables 1 and 2; HRESIMS *m/z* 657.4116 (calcd for $[C_{36}H_{58}O_9 + Na]^+$, 657.3973), 635.4194 (calcd for $[C_{36}H_{58}O_9 + H]^+$, 635.4154).

Acid Hydrolysis of 2

Compound **2** (31 mg) in 2 N HCl/dioxane was refluxed at 100 °C for 2 h. After cooling, the reaction mixture was diluted with H_2O and then extracted with $CHCl_3$ four times. The combined extracts were evaporated to dryness to yield a residue (23.2 mg). The residue was subjected to silica gel chromatography using the isocratic $CHCl_3$/MeOH (49:1) solvent system as eluent to give compound **8** (6.6 mg). The water layer was passed through an Amberlite MB-150 column eluting with H_2O, and the eluent was concentrated to dryness to give D-glucose (5.9 mg): $[\alpha]^{20}_{D}$ +43.2 (*c* 0.45, H_2O) (lit.[19] $[\alpha]^{20}_{D}$ +53.8).

Compound 8

white powder; $[\alpha]^{20}_{D}$ 83.6 (*c* 0.35, MeOH); IR (NaCl) ν_{max} 3337, 2960, 2360, 1663 cm^{-1}; UV (MeOH) λ_{max} 224 nm; NMR data, Tables 1 and 2; HRESIMS *m/z* 473.3679 (calcd for $[C_{30}H_{48}O_4 + H]^+$, 473.3636).

X-ray Crystallography of 1

Colorless needles of **1** were obtained from slow evaporation of a solution in EtOH. A single crystal, with approximate dimensions of 0.18 × 0.16 × 0.10 mm, was used for data collection on a Bruker Smart Apex II system,[22] using Cu Kα radiation with a graphite monochromator and fine-focus sealed tube. The crystal was kept at 100 K under a stream of cooled nitrogen gas from a KRYO-FLEX low-temperature device. Sutherlandioside A (**1**), $C_{36}H_{60}O_{10} \cdot H_2O$, MW = 670.87, crystallized along with one molecule of water in the asymmetric unit, monoclinic space group $P2_1$, a = 13.4595(2) Å, b = 6.78720(10) Å, c = 20.1461(3) Å, β = 104.7850(10)°, V = 1779.46 (5) Å^3, Z = 2. Goodness of fit (GooF) = 1.024, Flack parameter = 0.06(16), determined from the refinement of 2658 Friedel pairs. Data collection, indexing, and initial cell refinements were all carried out using APEX II software. Frame integration and final cell refinements were done using SAINT software.[23] The final cell parameters were determined from least-squares refinement on 8837 reflections, with R value = 0.0669, $wR(F^2)$ = 0.091. Structure solution, refinement, graphics, and generation of publication materials were performed by using SHELXTL, V6.12 software.[24] Hydrogen atoms were

placed at their expected chemical positions using the HFIX command and were included in the final cycles of least-squares with isotropic U_{ij}'s related to the atom's ridden upon. The supplementary crystallographic data can be obtained free of charge from The Cambridge Crystallographic Data Centre, reference number CCDC 673458, via www.ccdc.cam.ac.uk/data_request/cif.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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24. SHELXTL V6.12. Madison, WI: Bruker AXS, Inc. Analytical X-ray Systems; 2002.

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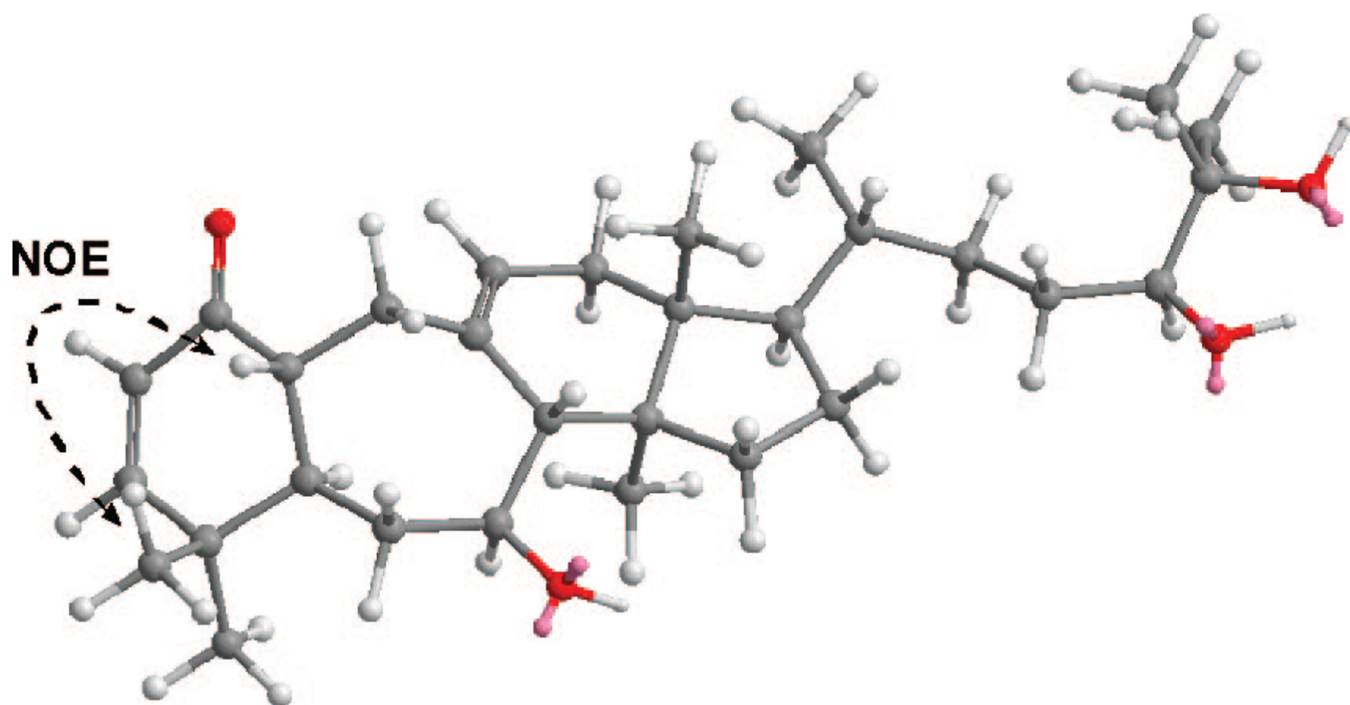

Figure 1.

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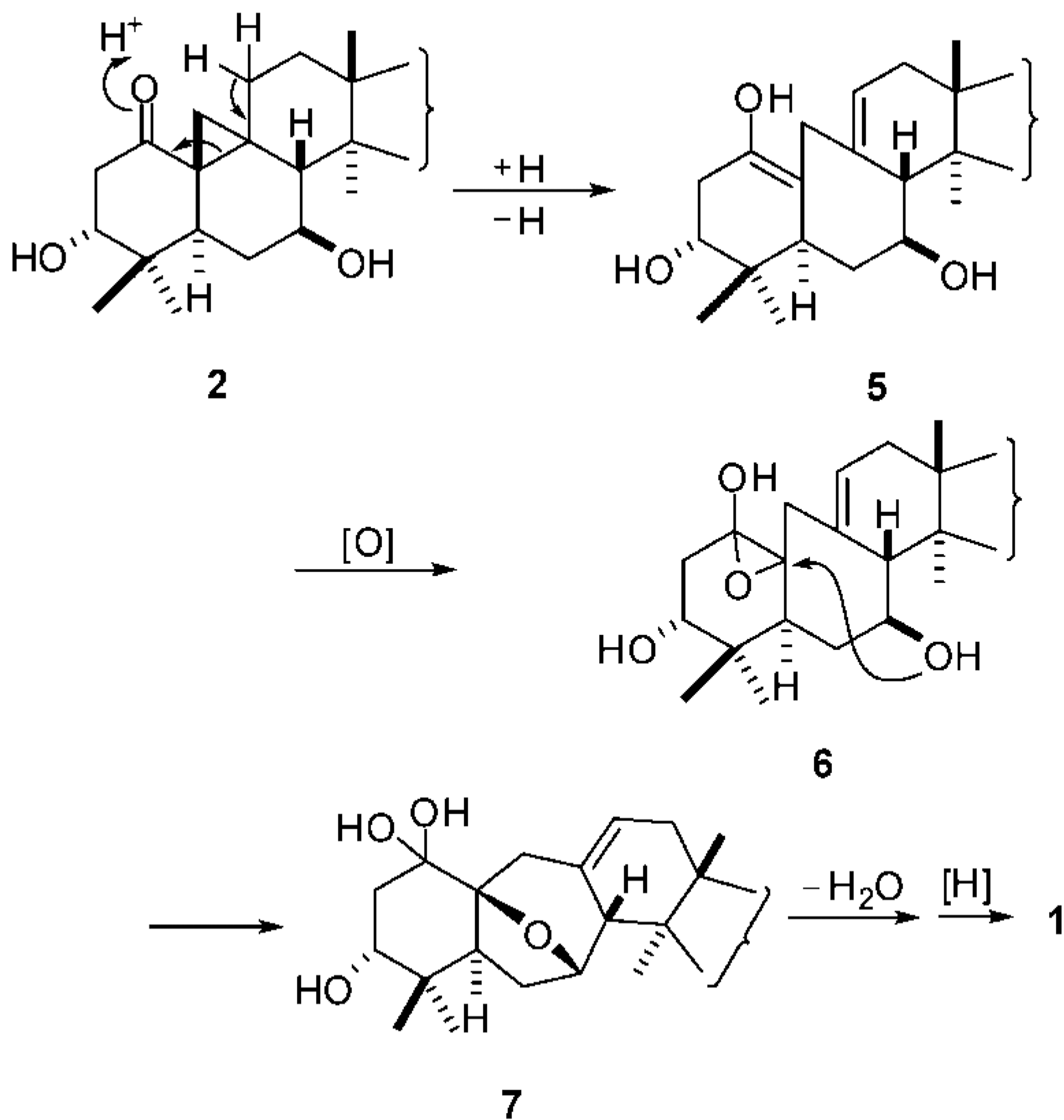

Scheme 1.

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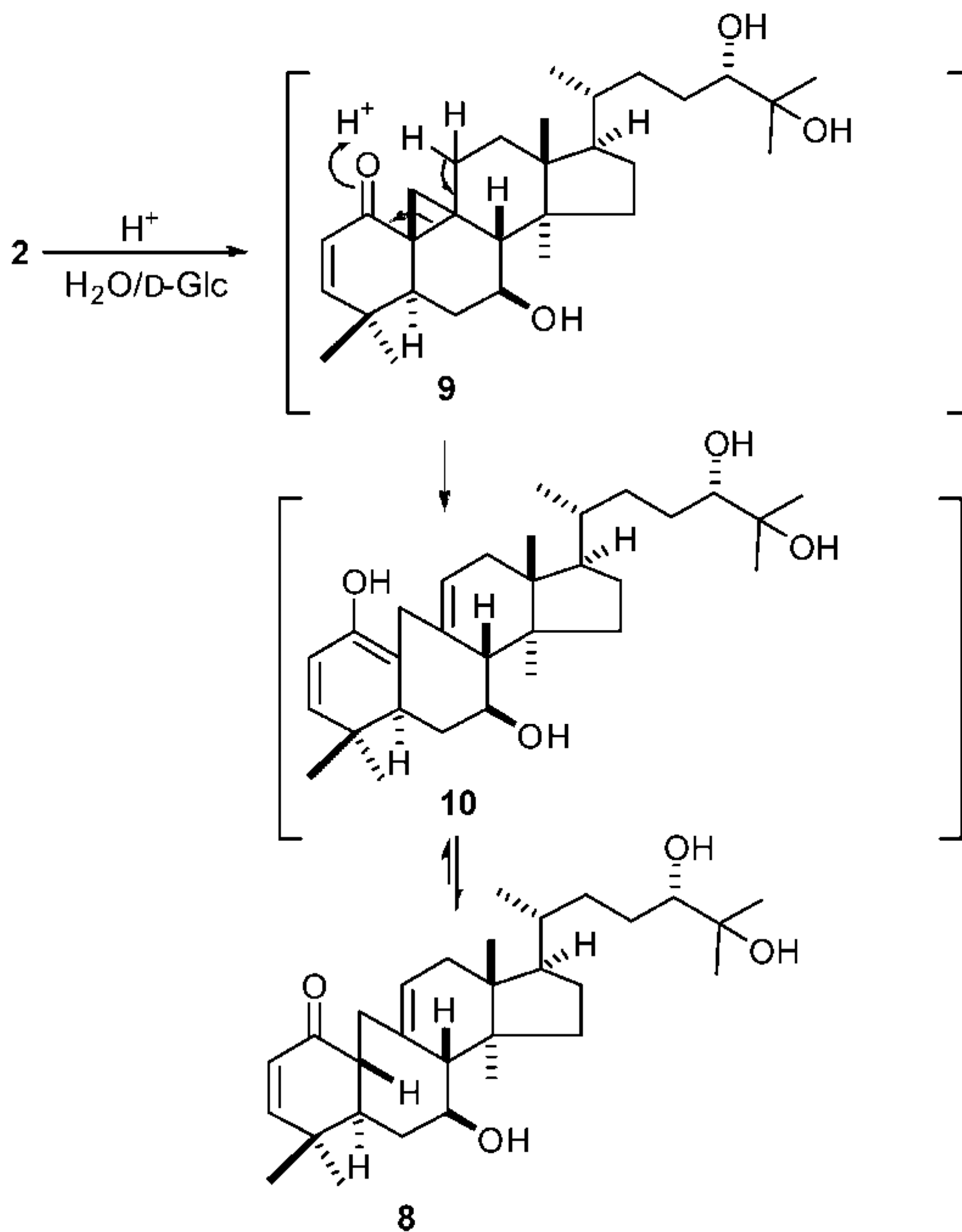

Scheme 2.

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Table 1

^{13}C NMR Data for Compounds **1–4** in Pyridine-d_5 and **8** in $CDCl_3$ (δ, ppm)[a]

position	1		2		3		4		8	
1	72.0	d	210.7	s	205.6	s	200.9	s	200.6	s
2	31.8	t	48.8	t	47.3	t	129.6	d	126.3	d
3	77.0	d	78.5	d	80.5	d	159.1	d	159.8	d
4	37.7	s	39.9	s	40.8	s	37.0	s	36.8	s
5	46.1	d	38.7	d	43.8	d	42.5	d	44.8	d
6	30.9	t	31.5	t	30.8	t	31.5	t	35.3	t
7	75.8	d	68.4	d	23.8	t	68.0	d	72.0	d
8	53.1	d	51.5	d	39.6	d	52.8	d	55.4	d
9	135.9	s	28.7	s	33.5	s	31.9	s	133.5	s
10	84.8	s	39.4	s	48.6	s	35.9	s	47.1	d
11	121.1	d	29.2	t	209.3	s	25.8	t	127.3	d
12	37.6	t	33.6	t	52.6	t	34.9	t	37.7	t
13	45.7	s	45.9	s	46.7	s	45.8	s	45.5	s
14	45.4	s	50.1	s	49.3	s	49.8	s	48.4	s
15	34.4	t	34.5	t	34.2	t	33.4	t	28.9	t
16	28.8	t	28.4	t	28.4	t	29.6	t	28.5	t
17	51.0	d	52.4	d	51.7	d	52.5	d	50.4	d
18	15.5	q	16.0	q	17.0	q	16.3	q	15.2	q
19	42.8	t	24.4	t	25.6	t	29.1	t	33.8	t
20	37.2	d	37.4	d	37.2	d	37.5	d	36.6	d
21	19.2	q	19.3	q	19.3	q	19.3	q	18.7	q
22	35.0	t	34.9	t	34.7	t	34.9	t	39.4	t
23	29.6	t	29.5	t	29.6	t	28.5	t	33.7	t
24	78.7	d	78.7	d	78.6	d	78.7	d	79.7	d
25	81.3	s	81.3	s	81.3	s	81.3	s	73.4	s
26	21.7	q	21.6	q	21.7	q	21.6	q	23.4	q
27	24.6	q	24.6	q	24.6	q	24.6	q	26.7	q
28	27.4	q	25.6	q	26.0	q	28.0	q	27.9	q

position	1		2		3		4		8	
29	24.0	q	21.7	q	21.5	q	21.4	q	20.0	q
30	20.0	q	18.9	q	19.1	q	19.1	q	18.4	q
1′	99.0	d	99.0	d	99.1	d	99.0	d		
2′	75.7	d	75.7	d	75.7	d	75.7	d		
3′	79.0	d	79.0	d	79.1	d	79.0	d		
4′	72.1	d	72.0	d	72.1	d	72.0	d		
5′	78.6	d	78.4	d	78.7	d	78.6	d		
6′	63.1	t	63.0	t	63.1	t	63.0	t		

[a]Spectra were acquired at 150 MHz for 1 and 100 MHz for **2–4** and **8**. Multiplicity was obtained from DEPT spectra.

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Table 2

^{1}H NMR Data for Compounds **1–4** in Pyridine-d_5 and **8** in $CDCl_3$ (δ, ppm; *J*, Hz)[a]

position	1	2	3	4	8
1	4.28				
2a	2.20 α	2.81 dd (13.6, 4.4) α	2.63	6.11 d (9.6)	5.81 d (10.1)
2b	2.40 br d (14.1) β	3.23 dd (13.8, 4.2) β	3.22 dd (12.0, 2.8)		
3	3.83 br s	4.03	4.06	6.59 d (9.6)	6.54 d (9.7)
5	2.34 br d (8.5)	2.90 dd (13.4, 3.4)	2.64	2.41 dd (11.6, 3.8)	1.56
6a	1.95 β	1.66 β	2.01	1.48	1.51
6b	2.20 α	2.17 α	2.01	2.10	1.66
7	4.30	3.97	1.22, 1.43	4.00	3.90 ddd (5.5, 3.5, 2.0)
8	2.64 br s	2.43 d (4.0)	2.06	2.42 d (4.4)	2.12
10					2.23 dt, (12.8, 4.8)
11a	5.41 d (2.3)	1.99 #x003B2;		2.25 dd (16.4, 7.6)	5.53 br t (2.4)
11b		2.37 dd (12.8, 2.8) α		2.96 dt (15.2, 4.0)	
12a	1.94 d (13.5) β	1.54	2.72 d (16.4)	1.60	1.94 d (4.8)
12b	2.12 d (17.0) α	1.84	2.88 d (16.4)	1.83	2.06
15	1.30, 1.36	1.55, 1.86	1.24, 1.27	1.53, 1.55	1.50, 1.60
16	1.36, 1.94	1.36 t (14.0), 1.84	1.22, 1.34	1.06, 1.89	1.35, 1.89
17	1.60	1.60	1.73	1.58	1.53
18	0.71 s	0.99 s	0.80 s	0.98 s	0.68 s
19a	2.69 d (16.1) β	0.86 d (4.4) α	1.32	1.16 d (3.6)	2.58 dd (14.8, 4.8)
19b	2.89 d (16.1) α	1.60 d (4.0) β	2.26 d (3.6)	1.67 d (4.0)	2.67 dd (14.4, 3.2)
20	1.43	1.46	1.34	1.45	1.37
21	0.95 d (6.2)	0.95 d (6.0)	0.87 d (6.1)	0.94 d (6.0)	0.87 d (6.0)
22a	1.14 *pro-R*	1.12	1.14	1.11	1.72
22b	2.20 *pro-S*	2.17	2.13	2.16	2.02
23a	1.48	1.46	1.44	1.33	0.96
23b	1.87	1.83	1.84	1.94	1.78
24	3.86 br d (9.7)	3.84 br d (9.2)	3.84 br d (10.4)	3.84 br d (9.6)	3.24 dd (10.0, 1.6)
26	1.57 s	1.57 s	1.57 s	1.55 s	1.12 s

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position	**1**	**2**	**3**	**4**	**8**
27	1.54 s	1.54 s	1.54 s	1.53 s	1.17 s
28	1.17 s	1.26 s	1.23 s	1.03 s	1.11 s
29	1.03 s	1.04 s	1.11 s	0.91 s	1.00 s
30	0.75 s	1.09 s	1.12 s	1.01 s	0.83 s
1′	5.25 d (7.9)	5.24 d (7.6)	5.26 d (8.0)	5.24 d (8.0)	
2′	4.05 t (7.1)	4.04 t (7.4)	4.03	4.03	
3′	4.27	4.28	4.27	4.26	
4′	4.26	4.22	4.25	4.22	
5′	3.99	4.03	4.01	3.98	
6′ a	4.35 dd (11, 5.5)	4.34 dd (11.6, 5.2)	4.37 dd (11.5, 5.1)	4.33 dd (11.6, 5.4)	
6′ b	4.54 br d (11)	4.54 br d (10.8)	4.57 br d (11.3)	4.52 dd (12.0, 2.0)	

[a] Data recorded at 600 MHz for **1**, 400 MHz for **2–4** and **8**. Assignments were based on 2D NMR including DQF-COSY, HMQC, HMBC, and ROESY. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses. For overlapped signals, only chemical shift values are given.