Constituents of *Senecio chionophilus* with Potential Antitubercular Activity

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Two new sesquiterpenoids, (1*S*,4*S*,5*R*,10*R*)-1-hydroxy-6-isobutyryloxy-10*H*-9-oxofuranoeremophilane (**1**) and 1α-hydroxy-6*β*-(2ξ-methylbutyryloxy)-10α*H*-9-oxofuranoeremophilane (**2**), along with 21 known constituents, were isolated from the *n*-hexane and dichloromethane extracts of the above-ground biomass and roots of *Senecio chionophilus*. The structures of **1** and **2** were elucidated on the basis of spectroscopic evidence and chemical transformation methods. The absolute configuration of **1** was determined by Mosher ester methodology. All of the isolates were evaluated for their antitubercular potential against *Mycobacterium tuberculosis* in a microplate Alamar Blue assay. Compound **2**, 6*β*-angeloyloxy-1α-hydroxy-10α*H*-9-oxofuranoeremophilane (**3**), and 4′-hydroxyacetophenone (**6**) exhibited mild antitubercular activity at minimum inhibitory concentrations of 119, 114, and 121 *μ*g/mL, respectively.

Senecio chionophilus Phil. (Asteraceae) is a small woody shrub with erect stems, hairy leaves, and yellow flowers, indigenous to Argentina and Chile, where it grows in the Andes Mountains above 1500 m.[1] Infusions of its aerial parts and roots are traditionally used in Chile for treating heavy colds and runny noses (Montenegro, personal communication). Up to the present, no phytochemical or biological activity information has been reported on this plant. As part of a collaborative search for novel antitubercular principles of plant and microbial organisms from dryland biodiversity of Latin America,[2,3] *n*-hexane- and dichloromethane-soluble extracts of the above-ground biomass and roots of *S. chionophilus* exhibited inhibitory effects on the growth of *Mycobacterium tuberculosis* H37Rv in a microplate Alamar Blue assay (MABA) system with minimum inhibitory concentrations (MICs) of 50 and 100 *μ*g/mL, respectively. Fractionation of these two extracts led to the isolation of two new furanoeremophilanes, **1** and **2**, along with 21 known compounds. The structures of **1** and **2** were elucidated by spectroscopic evidence and chemical transformation methods. The absolute configuration of **1** was determined by Mosher ester methodology in a modified procedure in an NMR tube.[4,5] All of the isolates were evaluated for their antitubercular potential as pure compounds, and the results are described herein.

Results and Discussion

Compound **1** was obtained as a white amorphous powder and gave a protonated molecular ion ($[M + H]^+$) at *m/z* 335.1861 by high-resolution FABMS, consistent with an elemental formula of $C_{19}H_{26}O_5$. This compound exhibited UV maxima at 205 and 285 nm, suggestive of the presence of considerable conjugation in the molecule. In the IR spectrum, absorption bands at 3490 cm^{-1} (OH), 1739 cm^{-1} (an ester carbonyl), and 1658 cm^{-1} (a conjugated keto) were apparent.[6] Comparison of the ^1H NMR data (Table 1) of **1** with those of 1α-hydroxy-6*β*-isobutyryloxy-10α*H*-9-oxofuranoeremophilane (**5**),[7] which was also obtained in the present investigation, indicated that they are a pair of isomers, with the only difference being in the configuration at C-10. The furanoeremophilane skeleton of **1** was confirmed by the observed HMBC correlations (H-12/C-7, C-8, C-11; H_3-13/C-7, C-11, C-12; H_3-14/C-3, C-4, C-5; and H_3-15/C-4, C-5, C-6, C-10) (Table 3). The hydroxyl group in **1** was located at C-1 rather than C-6 as a result of the observed $^1H-^1H$ COSY cross-peaks in $CDCl_3$ between OH-1 ($\delta_H = 4.95$, brs) and H-1 ($\delta_H = 3.91$, m), which was further correlated with H-10 ($\delta_H = 3.16$, brs) and H_2-2 ($\delta_H = 1.59$ and 1.96). The relative configuration of **1** was determined by a ROESY experiment (Figure 1), which gave diagnostic correlations from H-1*β* to H-2*β*, H-10*β*, and CH_3-5*β*, as well as H-6α to CH_3-4*β* and CH_3-5*β*, leading to the assignment of H-1*β*, H-4α, H-6α, H-10*β*, and CH_3-5*β*, respectively. A *cis*-fused A/B ring junction in **1** was established by the observed ROESY correlations between H-10 and H_3-15 as well as the chemical shift of H_3-15 at $\delta_H = 1.07$ in $CDCl_3$, rather than at $\delta_H < 1.00$ in the *trans*-fused series.[8,9] The placement of the 6*β*-isobutyryloxy group in **1** was confirmed by the observation of a relatively high-field shift of H_3-14 at $\delta_H = 0.82$ due to its quasi 1,3-diaxial interaction with H-6α in the *cis*-fused A/B ring series.[8] In addition, the broad singlet splitting pattern for H-10 of **1** confirmed the assignment of a *cis*-disposition for H-1*β*/H-10*β* in the *cis*-fused ring series, instead of a doublet ($J = 10$ Hz) indicating an *anti*-configuration for H-1*β*/H-10α in the *trans*-fused series.[7] Furthermore, a steroidal-like conformation of **1** was established on the basis of the observed spatial ROESY correlations between H-1*β* and CH_3-5, along with H-6α and CH_3-4 (Figure 1).[8] The absolute configuration of the stereogenic center at C-1 in **1** was determined by a convenient Mosher ester procedure,[4,5] which was performed directly in NMR tubes using deuterated pyridine as a solvent. Compound **1** was treated with (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride to obtain its (*S*)- (**1s**) and (*R*)-esters (**1r**), respectively (see Experimental Section). Analysis of $\Delta\delta_H = \delta_H$ (**1s**) $- \delta_H$ (**1r**) data in C_5D_5N (Table 1) showed positive differences in chemical shifts for the protons at C-10 and C-15 and negative effects for the protons at C-2 and C-3. These effects indicated that the absolute configuration at C-1 of **1** is *S*. Accordingly, the absolute configurations at C-4, C-5, and C-10 were

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Table 1. ^{1}H NMR Data of Compounds **1**, **1s**, **1r**, **2**, and **2a**[a]

position	**1**[b]	**1**[c]	**1s**[c]	**1r**[c]	$\Delta\delta_{H(1s-1r)}$	**2**[b]	**2a**[b]
1	3.91 m	4.57 brs	5.75 br s	5.77 br s	S[d]	4.17 m (5.1)	5.29 m
2	1.59 o	1.80 m	1.99 m	1.99 m	0	1.39 o	1.39 o
	1.96 d-like (11)	1.80 m	1.80 m	1.98 m	−0.18	2.05 ddd (5.3, 11)	2.12 t-like (11)
3	1.39−1.50 o	1.31 m (4.3, 11)	1.24 o	1.38 d-like (14)	−0.14	1.46 o	1.44 o
	1.39−1.50 o	2.84 o	2.17 t-like (15)	2.50 t-like (14)	−0.33	1.46 o	1.44 o
4	1.54 o	1.90 o	1.86 o	1.86 o	0	1.84 o	1.86 o
6	6.00 brs	7.66 o	6.76 s	7.14 brs	−0.38	6.35 s	6.38 s
10	3.16 brs	2.83 brs	3.07 brs	3.01 brs	+0.06	2.39 d (9.6)	2.67 d (10)
12	7.43 d (1.0)	7.60 o	7.68 brs	7.28 brs	+0.40	7.38 q (1.1)	7.33 br s
13	2.04 d (1.0)	1.90 s	1.86 s	1.86 s	0	1.89 d (1.1)	1.87 br s
14	0.82 d (6.2)	0.96 d (7.0)	0.92 d (7.0)	0.95 d (7.0)	−0.03	0.89 (6.8)	0.90 (6.8)
15	1.07 s	1.13 s	1.07 s	1.05 s	+0.02	0.99 s	0.99 s
OH-1	4.95 brs	6.81 br s				4.50 br s	
2′	2.56 sep (7.0)	2.72 sep (7.0)	2.66 sep (7.0)	2.72 sep (7.0)	−0.06	2.43 m (7.0)	2.43 sep (7.0)
3′	1.17 d (7.0)	1.24 d (7.0)	1.19 d (7.0)	1.24 d (7.0)	−0.05	1.89 o, 1.55 o	1.87 o, 1.50 o
4′	1.13 d (7.0)	1.22 d (7.0)	1.19 d (7.0)	1.23 d (7.0)	−0.04	0.99 t (7.4)	0.99 o
5′						1.23 d (7.2)	1.21 d (7.2)
$COCH_3$-1							2.02 s

[a] Spectra were recorded at 300.1 MHz, and values are reported in parts per million relative to TMS. Coupling constants in parentheses are reported in hertz. Multiplicities are as follows: brs, broad singlet; d, doublet; ddd, doublet of double doublets; m, multiplet; o, unclear due to overlapping signals; q, quartet; s, singlet; sep, septet; t, triplet. Assignments are based on ^{1}H−^{1}H COSY, HMQC, HMBC, and ROESY experiments. [b] Data in $CDCl_3$. [c] Data in C_5D_5N. [d] Absolute configuration at C-1 of **1**.

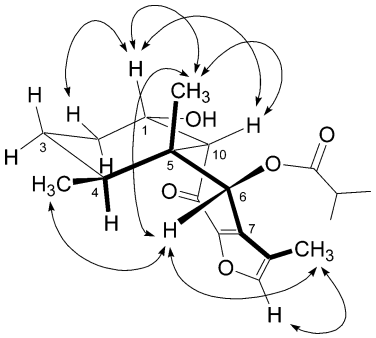


Figure 1. Selected ROESY correlations observed for **1**.

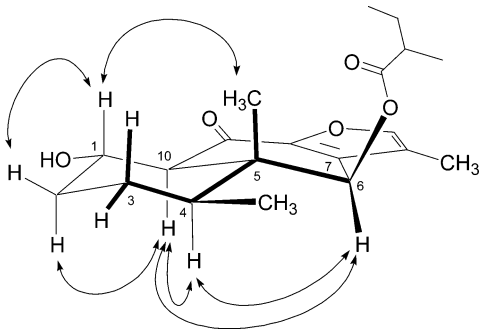


Figure 2. Selected ROESY correlations observed for **2**.

Table 2. ^{13}C NMR Data of Compounds **1**, **2**, **2a**, **4**, **4a**, and **5**[a]

position	**1**	**2**	**2a**	**4**	**4a**	**5**
1	69.3 d	66.4 d	67.3 d	66.4 d	67.3 d	66.3 d
2	31.8 t	32.8 t	31.2 t	32.8 t	31.2 t	32.8 t
3	29.7 t	30.1 t	29.7 t	30.2 t	29.7 t	30.1 t
4	33.0 d	42.0 d	41.8 d	41.6 d	41.4 d	41.9 d
5	47.1 s	50.9 s	51.1 s	51.0 s	51.3 s	50.9 s
6	67.9 d	74.6 d	74.6 d	74.6 d	74.7 d	74.7 d
7	135.2 s	135.9 s	133.5 s	136.2 s	133.8 s	136.0 s
8	147.5 s	146.6 s	147.2 s	146.4 s	147.1 s	146.5 s
9	190.3 s	189.2 s	184.7 s	189.2 s	184.7 s	189.2 s
10	53.6 d	60.8 d	57.9 d	60.7 d	57.9 d	60.7 d
11	121.5 s	121.1 s	120.7 s	121.1 s	120.6 s	121.1 s
12	145.9 d	145.9 d	144.9 d	146.0 d	144.9 d	146.0 d
13	7.8 q	8.9 q	9.0 q	8.9 q	8.8 q	8.9 q
14	15.4 q	17.9 q	17.7 q	17.7 q	17.5 q	17.8 q
15	15.8 q	8.9 q	8.8 q	8.7 q	8.7 q	8.8 q
1′	176.3 s	175.8 s	175.8 s	172.7 s	172.7 s	176.3 s
2′	34.2 d	41.1 d	41.1 d	43.4 t	43.4 t	34.5 d
3′	19.1 q	25.7 t	25.7 t	24.6 d	24.6 d	18.7 q
4′	18.9 q	11.7 q	11.7 q	22.7 q	22.7 q	18.6 q
5′		15.5 q	15.5 q	22.7 q	22.7 q	
$COCH_3$			170.5 s		170.5 s	
$COCH_3$			21.2 q		21.2 q	

[a] Spectra were recorded in $CDCl_3$ at 75.5 MHz, and values are reported in parts per million relative to TMS. Multiplicities were determined by ^{13}C NMR and DEPT experiments and are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet. Assignments are based on ^{1}H−^{1}H COSY, HMQC, HMBC, and ROESY experiments.

deduced as *S*, *R*, and *R*, respectively, on the basis of their relative configuration. All the ^{1}H and ^{13}C NMR signals were completely assigned by interpretation of the ^{1}H−^{1}H COSY, HSQC, and HMBC spectra. Thus, the structure of **1** was determined as (1*S*,4*S*,5*R*,10*R*)-1-hydroxy-6-isobutyryloxy-10*H*-9-oxofuranoeremophilane.

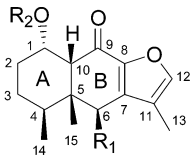
Compound **2** was obtained as a colorless amorphous solid, and its molecular formula was deduced as $C_{20}H_{28}O_5$ from the HRFABMS data at *m*/*z* 349.2010 $[M + H]^+$ ($C_{20}H_{29}O_5$, calcd for 349.2015). As documented in the Experimental Section, **2** also exhibited UV, IR, and ^{1}H NMR (Table 1) features similar to those of **5** and was therefore recognized as an analogue of the latter compound, with the only difference occurring in the ester group at C-6. A 2-methylbutyryloxy group of **2** was identified by observation of its characteristic ^{1}H NMR signals within one spin system for two methyls at $\delta_H = 0.99$ (t, $J = 7.4$ Hz, H_3-4′) and 1.23 (d, $J = 7.2$ Hz, H_3-5′), one methylene at $\delta_H = 1.55$ and 1.89 (overlapping, H_2-3′), and one methine at $\delta_H = 2.43$ (m, $J = 7.0$ Hz, H-2′). The presence of this ester side chain was confirmed by the ^{13}C and DEPT NMR data of **2** (Table 2) indicating an ester carbonyl at $\delta_C = 175.8$ (C-1′), two methyls at $\delta_C = 11.7$ (C-4′) and 15.5 (C-5′), a methylene at $\delta_C = 25.7$ (C-3′), and a methine at $\delta_C = 41.1$ (C-2′). The 2-methylbutyryloxyl group was located at C-6 as a result of observed HMBC correlations between H-6 and C-4, C-5, C-7, C-8, C-15, and C-1′ (Table 3). The configuration of the 2-methylbutyryloxy unit could not be determined by the present NMR experiments. The relative configuration of **2** including the *trans*-fused A/B ring system was determined by a ROESY experiment (Figure 2), which gave diagnostic spatial correlations from H-1β to H-2β and CH_3-5β, as well as H-10α to H-2α, H-4α, and H-6α, leading to the assignment of H-1β, CH_3-5β, H-4α, H-6α, and H-10α, respectively.

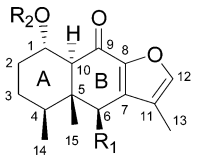
Table 3. Observed HMBC Correlations of **1**, **2**, and **2a** (300.1 MHz, $CDCl_3$)

H	C of **1**	C of **2**	C of **2a**
2	NO[a]	NO[a]	4, 10
6	NO[a]	4, 5, 7, 8, 15, 1′	4, 5, 7, 8, 15, 1′
10	NO[a]	1, 5, 6, 9, 15	1, 5, 6, 9, 15
12	7, 8, 11	7, 8, 11	7, 8, 11
13	7, 11, 12	7, 11, 12	7, 11, 12
14	3, 4, 5	3, 4, 5	3, 4, 5
15	4, 5, 6, 10	5, 6, 10	5, 6, 10
2′	1′, 3′, 4′	3′, 4′, 5′	NO[a]
3′	1′, 2′	1′, 2′, 4′, 5′	NO[a]
4′	1′, 2′	2′, 3′	2′, 3′
5′		1′, 2′, 3′	1′, 2′, 3′
acetate CH_3			acetate CO

[a] HMBC correlations were not observed in the spectra.

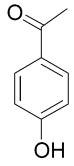
Furthermore, a coupling constant of 9.6 Hz between H-1 and H-10 in **2** indicated that these two neighboring protons existed in a diaxial orientation,[7] and this required a steroidal-like conformation, which was further confirmed by the above-mentioned ROESY correlations between H-1β and CH_3-5, as well as H-4α and H-10α (Figure 2).[8] Moreover, an acetate derivative (**2a**) was generated after compound **2** was treated with pyridine and acetic anhydride by a routine procedure to confirm the presence of a free hydroxy group in **2**. Therefore, **2** was identified as 1α-hydroxy-6β-(2ξ-methylbutyryloxy)-10α*H*-9-oxofuranoeremophilane, and all the NMR signals of **2** and **2a** were unambiguously assigned in Tables 1−3.





1 R_1 = a, R_2 = H
1s R_1 = a, R_2 = (*S*)-MTPA
1r R_1 = a, R_2 = (*R*)-MTPA

2 R_1 = b, R_2 = H
2a R_1 = b, R_2 = $COCH_3$
3 R_1 = c, R_2 = H
4 R_1 = d, R_2 = H
4a R_1 = d, R_2 = $COCH_3$
5 R_1 = a, R_2 = H



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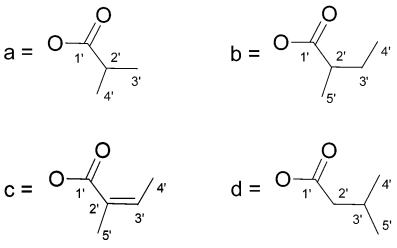

Twenty-one known compounds were isolated from the *n*-hexane- and dichloromethane-soluble extracts of the above-ground biomass and roots of *S. chionophilus*, as described in the Experimental Section, and were identified as 6β-angeloyloxy-1α-hydroxy-10α*H*-9-oxofuranoeremophilane (**3**),[10] 1α-hydroxy-6β-isovaleryloxy-10α*H*-9-oxofuranoeremophilane (**4**),[11] 1α-hydroxy-6β-isobutyryloxy-10α*H*-9-oxofuranoeremophilane (**5**),[7] 4′-hydroxyacetophenone (**6**),[12] 1α-acetoxy-6β-angeloyloxy-10α*H*-9-oxofuranoeremophilane,[6] 1α-acetoxy-6β-isobutyryloxy-10α*H*-9-oxofuranoeremophilane,[13] α-amyrenone,[14] β-amyrenone,[14] α-amyrin,[14] β-amyrin,[14] 1α-angeloyloxy-6β-isobutyryloxy-10α*H*-9-oxofuranoeremophilane,[13] 6β-angeloyloxy-10α*H*-9-oxofuranoeremophilane,[6] caryophyllene 4β,5α-epoxide,[15] daucosterol,[16] 1α-hydroxy-6β-propionyloxy-10α*H*-9-oxofuranoeremophilane,[17] 6β-isobutyryloxy-10α*H*-9-oxofuranoeremophilane,[6] lupenone,[14] lupeol,[14] β-sitosterol,[18] taraxasterol,[14] and taraxasterone,[14] by comparison of their physical and spectroscopic data (mp, $[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, DEPT, 2D NMR, and MS) with reported data. The ^{13}C NMR data of **4** and its acetate (**4a**) along with **5** have not been published; thus the complete assignments of their ^{13}C NMR data were performed and are reported in Table 2.

The occurrence of these isolates of *S. chionophilus* is in agreement with the fact that terpenoids, especially furanoeremophilanes, are widespread in the genus *Senecio*.[19] However, it is noteworthy that in the present investigation we did not obtain any pyrrolizidine alkaloids (PA), which are reported to be one of the most characteristic secondary metabolites in other species in the same genus.[20] The absence of PA in *S. chionophilus* was further confirmed by their negative detection in the methanolic extract when TLC plates were sprayed with Dragendorff's reagent. It is well known that pyrrolizidine alkaloids in *Senecio* species cause hepatotoxicity to livestock feeding on this plant.[21−23] The lack of PA in *S. chionophilus* may contribute to its ethnobotanical use to treat heavy colds by the local population in southern Chile.

All of the 23 compounds obtained from *S. chionophilus* along with two *O*-acetyl derivatives (**2a** and **4a**) were evaluated for their antitubercular potential against *M. tuberculosis*, according to established protocols.[3] The results showed that compounds **2**, **3**, and **6** exhibited mild inhibitory activity with MIC values of 119, 114, and 121 μg/mL, respectively, when compared with the MIC of 0.03 μg/mL for rifampin, a positive control used for the microplate Alamar Blue assay.[3] In contrast, the other 22 compounds were not regarded as active in this assay, since they did not meet the threshold MIC value of <128 μg/mL. This is the first evidence that sesquiterpenoids of the furanoeremophilane type may be considered as potential antitubercular leads. In addition, by comparison of the antitubercular activities of betulinic, oleanolic, and ursolic acids with MICs of 32, 64, and 32 μg/mL,[24] respectively, with those of individual inactive analogues found in the current investigation, the occurrence of a carboxylic group in a polycyclic triterpene skeleton appears to be necessary for the observed antimycobacterial activity.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Scientific melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco P-1010 polarimeter. UV spectra were measured on a Varian Cary 50 Conc UV−visible spectrophotometer. IR (as a film on a diamond cell) was obtained on a Thermo Nicolet Avatar 360 FT-IR spectrometer. HRFABMS data were recorded on a JEOL HX110A mass spectrometer using a mixed matrix of 50% glycerol, 25% thioglycerol, 24% 3-nitrobenzyl alcohol, and 1% trifluoroacetic acid. Low-resolution EIMS data were obtained with a Varian Saturn 2100T GC−MS workstation including data system software (Version 5.2) interfaced to a 3900-GC, a 2000-MS detector, and a 1079-injector. The gas chromatograph was fitted with a WCOT Fused Silica Chrompack capillary column packed with CP Sil 8 CB (30 m × 0.25 mm). A 2 μL aliquot of compound solution at 1 mg/mL

in CH_2Cl_2 was injected into the capillary column. Ultrapure helium at 1.2 mL/min was used as the carrier gas, and the injector, transfer line, and trap temperatures were 250, 250, and 200 °C, respectively. The column oven was temperature programmed at 80 °C for 5 min and then 280 °C at 10 °C/min, followed by 280 °C for 20 min. In turn, NMR spectra were recorded at room temperature on a Bruker Avance 300 NMR spectrometer in 5 mm NMR tubes with TMS as the internal standard. Standard pulse sequences were employed for the measurement of 2D NMR spectra ($^1H-^1H$ COSY, HSQC, HMBC, and ROESY). Column chromatography (CC) was conducted on silica gel (32−63 or 63−200 μm, Scientific Adsorbents Incorporated, Atlanta, GA) and Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden). Semipreparative revered-phase HPLC was performed on a Varian LC system equipped with a 9012 solvent delivery system and a 9065 Polychrom detector, using 10 × 250 mm, 10 μm, Econosphere C_{18} (Alltech, Deerfield, Illinois) or Lichrospher RP18 (Column Engineering, Ontario, Canada) columns. Semipreparative normal-phase HPLC was carried out on a Varian LC system equipped with a 9002 solvent delivery system and a Star 9040 RI detector, using a 10 × 250 mm, 10 μm Econosphere NH_2 column. A middle pressure LC (MPLC) system was set up with a Büchi 688 chromatography pump (Büchi Labortechnik AG, Flawil, Switzerland), a Büchi Borosilikat 3.3 glass column (2 × 45 cm) packed with C_{18} packing silica gel (15 g, 5 μm, Aldrich, Milwaukee, WI), and a Spectra/Chrom CF-1 fraction collector (Spectrum Chromatography Inc., Houston, TX). Analytical TLC was performed on Whatman Diamond K6F silica gel 60A (250 μm) and Merck RP-18 WF_{254S} (200 μm) plates. Compounds were visualized on TLC plates by dipping in phosphomolybdic acid (Aldrich) or vanillin-sulfuric acid reagents followed by charring at 110 °C for 5−10 min.

Plant Material. Aerial parts and roots of *Senecio chionophilus* Phil. were collected in October 2001 in Volcan Villarrica, IX Región, Chile (39°22′ S; 71°56′ W) and identified by Gloria Montenegro. A voucher specimen (No. 1096) has been deposited in the herbarium at the Pontificia Universidad Católica de Chile, Santiago, Chile. Intellectual Property Rights Agreements for plant collections and collaborative research have been fully executed between the University of Arizona and Pontificia Universidad Católica de Chile.

Extraction and Isolation. The milled plant material (480 g) was extracted by maceration with MeOH (1.7 L × 3). After filtration and evaporation of the solvent in vacuo, the resultant extract was diluted with H_2O to afford a 90% aqueous MeOH solution (0.5 L) and then partitioned with *n*-hexane (0.5 L × 3) and $CH_2Cl_2-H_2O$ (1:1, 1 L × 3), sequentially, to afford dried *n*-hexane-soluble (5.2 g) and CH_2Cl_2-soluble (5.8 g) residues. The *n*-hexane and CH_2Cl_2 extracts were found to inhibit the growth of *M. tuberculosis* with MIC values of 50 and 100 μg/mL, respectively. The *n*-hexane extract (5.2 g) was subjected to silica gel CC (208 g, 63−200 μm) by elution with a step gradient of *n*-hexane−acetone (150:1, 50:1, 30:1, 20:1, 10:1, 0:100, each 3 L) to give six pooled fractions (1−6), respectively. Fraction 1 (1.25 g) was applied to CC (SiO_2, 150 g, 63−200 μm) by elution of *n*-hexane−CH_2Cl_2 to afford four further fractions, 1-1 (720 mg, 2.5:1, 2 L), 1-2 (240 mg, 2:1, 1 L), 1-3 (130 mg, 1.5:1, 2 L), and 1-4 (30 mg, 1:2, 1 L). Purification of fraction 1-2 (60 mg) by HPLC (C_{18} column, CH_3CN-H_2O, 94:6, 3.5 mL/min, 200 nm) to afford lupenone (17 mg, t_R = 15.6 min), taraxasterone (12 mg, t_R = 18.2 min), β-amyrenone (8 mg, t_R = 19.8 min), and α-amyrenone (7 mg, t_R = 21.8 min). Caryophyllene 4β,5α-epoxide (20 mg) was separated from fraction 1-3 by CC on Sephadex LH-20−acetone and silica gel (*n*-hexane−EtOAc, 80:1). Fractionation of fraction 2 (380 mg) by CC (SiO_2, 60 g, 32−63 μm, *n*-hexane−CH_2Cl_2, 6:10) gave two further fractions, 2-1 and 2-2. Purification of fraction 2-1 (80 mg) (C_{18} column, CH_3CN-H_2O, 91:9, 4.0 mL/min, 200 nm) afforded lupeol (45 mg, t_R = 22.2 min), taraxasterol (18 mg, t_R = 28.4 min), β-amyrin (13 mg, t_R = 30.2 min), and α-amyrin (5 mg, t_R = 34.5 min). 6β-Angeloyloxy-10α*H*-9-oxofuranoeremophilane (24 mg, t_R = 12.6 min) was purified from fraction 2-2 by HPLC (NH_2 column, *n*-hexane−EtOAc, 85:15, 2.5 mL/min, RI detector). 6β-Isobutyryloxy-10α*H*-9-oxofuranoeremophilane (100 mg) was isolated from fraction 3 (150 mg) by CC on Sephadex LH-20−acetone. Fractionation of fraction 4 (560 mg) over silica gel (78 g, 32−63 μm, *n*-hexane−CH_2Cl_2−EtOAc, 40:40:1, 2 L) afforded β-sitosterol (150 mg) and a further fraction, 4-1 (79 mg), which gave 1α-angeloyloxy-6β-isobutyryloxy-10α*H*-9-oxofuranoeremophilane (4 mg, t_R = 5.7 min) followed by CC on Sephadex LH-20−acetone and HPLC (C_{18} column, CH_3CN-H_2O, 75:25, 4.0 mL/min, 200 nm). Fractionation of fraction 5 (700 mg) by CC on Sephadex LH-20−acetone gave three further fractions (5-1 to 5-3). 1α-Acetoxy-6β-isobutyryloxy-10α*H*-9-oxofuranoeremophilane (25 mg, t_R = 6.2 min) and 1α-acetoxy-6β-angeloyloxy-10α*H*-9-oxofuranoeremophilane (8 mg, t_R = 7.0 min) were obtained from fraction 5-2 by HPLC (C_{18} column, CH_3CN-H_2O, 65:35, 4.0 mL/min, 200 nm) after CC (SiO_2, *n*-hexane−CH_2Cl_2−EtOAc, 25:25:1). Fractionation of fraction 5-3 (300 mg) by MPLC (MeOH−H_2O, 50:50, 1.7 mL/min, 4 L) sequentially resulted in the purification of **1** (20 mg), 1α-hydroxy-6β-isobutyryloxy-10α*H*-9-oxofuranoeremophilane (**5**, 65 mg), 6β-angeloyloxy-1α-hydroxy-10α*H*-9-oxofuranoeremophilane (**3**, 30 mg), **2** (6 mg), and 1α-hydroxy-6β-isovaleryloxy-10α*H*-9-oxofuranoeremophilane (**4**, 10 mg). In turn, the CH_2Cl_2 extract (5.0 g) was subjected to Sephadex LH-20 CC by elution with MeOH to obtain four pooled fractions (7−10). Fraction 8 (1.7 g) was further subjected to silica gel CC (130 g, 63−200 μm) by elution with a step gradient of CH_2Cl_2−MeOH (400:1, 200:1, 5:1, each 2 L) to afford three fractions (8-1, 8-2, and 8-3), respectively. Fractionation of fraction 8-2 (700 mg) by silica gel CC (80 g, 63−200 μm) with CH_2Cl_2−EtOAc (60:1, 40:1, 40:1, 25:1, 1:1, each 1 L) as mobile phase afforded five further fractions (8-2-1 to 8-2-5), respectively. 1α-Acetoxy-6β-isobutyryloxy-10α*H*-9-oxofuranoeremophilane (120 mg) was purified from fraction 8-2-1 (200 mg) by MPLC (MeOH−H_2O, 60:40, 1.5 mL/min, 3 L). Compound **1** (40 mg) was purified from fraction 8-2-2 (45 mg) by Sephadex LH-20−actone. Fractionation of fraction 8-2-4 (190 mg) by MPLC (MeOH−H_2O, 50:50, 1.7 mL/min, 4 L) sequentially resulted in the isolation of 1α-hydroxy-6β-propionyloxy-10α*H*-9-oxofuranoeremophilane (3 mg), 1α-hydroxy-6β-isobutyryloxy-10α*H*-9-oxofuranoeremophilane (**5**, 90 mg), 6β-angeloyloxy-1α-hydroxy-10α*H*-9-oxofuranoeremophilane (**3**, 19 mg), **2** (3 mg), and 1α-hydroxy-6β-isovaleryloxy-10α*H*-9-oxofuranoeremophilane (**4**, 4 mg). Fractionation of fraction 8-3 (950 mg) by Sephadex LH-20−MeOH afforded a fraction (108 mg), which was further fractionated by silica gel CC with CH_2Cl_2−MeOH (30:1) as mobile phase to give daucosterol (12 mg). 4′-Hydroxyacetophenone (**6**, 20 mg) was purified from fraction 9 (120 mg) by silica gel CC (12 g, 63−200 μm, CH_2Cl_2−MeOH, 300:1, 0.5 L).

(1*S*,4*S*,5*R*,10*R*)-1-Hydroxy-6-isobutyryloxy-10*H*-9-oxofuranoeremophilane (1): white powder; mp 143−145 °C; $[\alpha]^{22}_D$ −140.0° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (4.36), 285 (4.19) nm; IR (film) ν_{max} 3490, 1739, 1658, 1640, 1458, 1414, 1387, 1258, 1185, 1149, 1090, 1056, 1021, 965, 925, 802 cm^{-1}; GC-EIMS *m*/*z* 334 $[M]^+$ (2), 306 (1), 264 (20), 246 (14), 229 (100), 189 (12), 179 (24), 161 (14), 133 (4), 109 (8), 85 (5); HRFABMS *m*/*z* 335.1861 $[M + 1]^+$ (calcd for $C_{19}H_{27}O_5$, 335.1858); ^1H NMR and ^{13}C NMR, see Tables 1 and 2, respectively.

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 1 by a Convenient Mosher Ester Procedure.[5] Compound **1** (1.7 mg) was transferred into a clean NMR tube and dried completely in vacuo. Deuterated pyridine (0.5 mL) and a catalytic amount of *N*,*N*-dimethyl-4-aminopyridine (DMAP, 0.2 mg) were then added to the NMR tube and mixed into one solution. After (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (12 μL) was added into the NMR tube immediately under a N_2 gas stream, the NMR tube was sealed and carefully shaken to mix the reactants evenly. The reaction to produce the (*R*)-MTPA ester derivative (**1r**) of **1** was found to be complete after 10 h at room temperature when the reaction in the NMR tube was monitored directly by ^1H NMR experiments. ^1H NMR data (pyridine-d_5, 300.1 MHz) of **1r** in Table 1 were assigned on the basis of observed δ and *J* values

compared with **1**, HMQC, and $^1H-^1H$ COSY correlations. In the same manner described for **1r**, the (*S*)-MTPA ester derivative (**1s**) of **1** was obtained in another NMR tube after the reactants inclusive of **1** (1.7 mg) and DMAP (0.2 mg) in pyridine-d_5 were treated with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (12 μL) for 10 h at room temperature. ^1H NMR data (pyridine-d_5, 300.1 MHz) of **1s** are shown in Table 1.

1α-Hydroxy-6*β*-(2*ξ*-methylbutyryloxy)-10α*H*-9-oxofuranoeremophilane (2): colorless amorphous solid; $[\alpha]^{22}_D$ −22.6° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (3.98), 220 (sh, 3.71), 280 (3.97) nm; IR (film) ν_{max} 3509, 1732, 1668, 1462, 1411, 1134, 1061, 962, 786 cm^{-1}; GC-EIMS *m*/*z* 349 $[M + 1]^+$ (86), 331 (21), 284 (2), 263 (86), 246 (100), 228 (95), 213 (76), 199 (47), 189 (62), 179 (81), 161 (45), 137 (34), 109 (29); HRFABMS *m*/*z* 349.2010 $[M + 1]^+$ (calcd for $C_{20}H_{29}O_5$, 349.2015); ^1H NMR and ^{13}C NMR, see Tables 1 and 2, respectively.

1α-Acetoxy-6*β*-(2*ξ*-methylbutyryloxy)-10α*H*-9-oxofuranoeremophilane (2a). Compound **2** (3.0 mg) was dissolved in 0.2 mL of anhydrous pyridine in a 4 mL vial, then 0.1 mL of acetic anhydride was added to the vial, which was sealed and kept at room temperature for 12 h. After evaporating the solvents in vacuo, the mixture was then purified by HPLC (RP-18 column, CH_3CN-H_2O, 58:42, 5.0 mL/min, 282 nm) to afford **2a** (3 mg, t_R = 10.6 min): white powder; mp 137−140 °C; $[\alpha]^{22}_D$ −88.4° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (4.09), 240 (sh, 3.58), 280 (4.06) nm; IR (film) ν_{max} 1733, 1687, 1462, 1414, 1375, 1359, 1247, 1130, 1026, 956, 895 cm^{-1}; GC-EIMS *m*/*z* 349 (100), 331 (13), 263 (43), 246 (45), 228 (41), 213 (27), 199 (23), 189 (32), 179 (22), 161 (20), 151 (14), 137 (15), 109 (17); HRFABMS *m*/*z* 391.2126 $[M + 1]^+$ (calcd for $C_{22}H_{31}O_6$, 391.2121); ^1H NMR and ^{13}C NMR, see Tables 1 and 2, respectively.

6*β*-Angeloyloxy-1α-hydroxy-10α*H*-9-oxofuranoeremophilane (3): colorless crystals (MeOH); mp 146−149 °C; $[\alpha]^{22}_D$ −40.2° (*c* 0.1, $CHCl_3$); HRFABMS *m*/*z* 347.1852 $[M + 1]^+$ (calcd for $C_{20}H_{27}O_5$, 347.1858); UV, IR, EIMS, and ^1H NMR data were in agreement with the reported literature values.[10]

4′-Hydroxyacetophenone (6): colorless rods ($CHCl_3$−MeOH); mp 112−114 °C (lit.[12] 104−109 °C); UV, IR, ^1H NMR, ^{13}C NMR, and EIMS data were consistent with literature values.[12]

Microplate Alamar Blue Assay (MABA). Antimycobacterial activity was determined against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in a microplate Alamar Blue assay system as described previously.[3] The minimum inhibitory concentration (MIC) value of <128 μg/mL was considered as active. The antitubercular drug rifampin was used as a positive control.

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