Antitubercular Activity of Triterpenoids from *Lippia turbinata*

Gerald A. Wächter,[†] Susanne Valcic,[†] Scott G. Franzblau,[‡] Enrique Suarez,[§] and Barbara N. Timmermann*[,†]

Department of Pharmacology and Toxicology, College of Pharmacy, The University of Arizona, 1703 E. Mabel Street, Tucson, Arizona 85721-0207, Institute for Tuberculosis Research, College of Pharmacy (MC 781), University of Illinois at Chicago, 833 S. Wood Street, Chicago, Illinois 60612-7231, and Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto de Recursos Biologicos, Las Cabanas y Los Reseros s/n, 1712 Villa Udaondo, Castelar, Provincia de Buenos Aires, Argentina

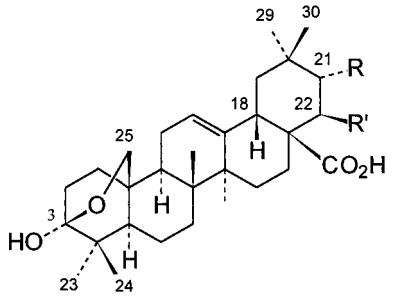
Received May 31, 2000

Assay-guided fractionation of the antitubercular MeOH−CH_2Cl_2 extract obtained from *Lippia turbinata* led to the isolation of four novel triterpenoids—3*β*,25-epoxy-3α,21α-dihydroxy-22*β*-(3-methylbut-2-en-1-oyloxy)olean-12-ene-28-oic acid (**1**); 3*β*,25-epoxy-3α,21α-dihydroxy-22*β*-angeloyloxyolean-12-ene-28-oic acid (**2**); 3*β*,25-epoxy-3α,21α-dihydroxy-22*β*-tigloyloxyolean-12-ene-28-oic acid (**3**); and 3*β*,25-epoxy-3α-hydroxy-22*β*-(2-methylbutan-1-oyloxy)olean-12-ene-28-oic acid (**4**)—together with the known triterpenoids lantanilic acid (**5**), camaric acid (**6**), lantanolic acid (**7**), and rehmannic acid (**8**). The MIC values of **1**−**8** for growth inhibition of *Mycobacterium tuberculosis* were determined in the radiorespirometric BACTEC system.

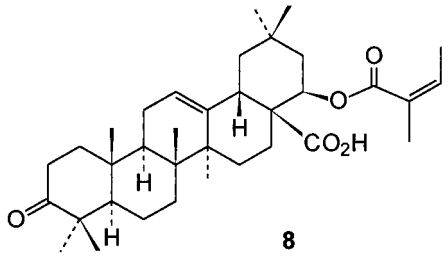
As part of our investigation for possible medicinal uses of plants from arid and semiarid regions of Latin America,[1] we routinely screen the obtained plant extracts for growth inhibition of the H_{37}Rv strain (ATCC 27294) of *Mycobacterium tuberculosis* using the radiorespirometric BACTEC 460 system, as previously described.[2] So far, this has led to the identification of a novel antitubercular diterpene alcohol from the Chilean species *Azorella madreporica*,[3] and recently we reported the antitubercular activities of a number of pentacyclic triterpenoids, isolated from a variety of plants.[4] Zeorin (6α,22-dihydroxyhopane), obtained from *Sarmienta scandens*, was the strongest inhibitor of mycobacterial growth in a series of triterpenoids whose MIC values ranged from 8 to more than 128 μM.[4] Zeorin is the first pentacyclic antitubercular triterpenoid whose activity is comparable to those of previously reported tetracyclic triterpenoids.[5,6] We now have observed a complete inhibition of the growth of *M. tuberculosis* by a MeOH−CH_2Cl_2 extract obtained from the aerial parts of *Lippia turbinata* (Verbenaceae) at 100 μg/mL. Antimycobacterial activity has, so far, not been reported for *Lippia* species, which are known to show insecticidal,[7] antiplasmodial,[8] antibacterial, and antifungal[9] activities as well as hypotensive[10] and muscle relaxant effects.[11] Chemical studies of *Lippia* species have often focused on monoterpenoid and sesquiterpenoid components of their essential oils due to the presence of the intensely sweet sesquiterpenoid hernandulcin in *L. dulcis*.[12] Other natural products isolated from *Lippia* include phenylpropanoids,[13] flavonoids,[14] and naphthoquinoids.[15] Triterpenoids from *L. rehmanni* have been connected to the toxicity of this plant to sheep.[16] Here we describe the assay-guided isolation of antitubercular triterpenoids from *L. turbinata*.

Results and Discussion

Fractionation of the CH_2Cl_2−MeOH extract obtained from aerial parts of *L. turbinata* was carried out using a Si gel column with Me_2CO−hexane mixtures of increasing polarity. Antitubercular activity was found to be concentrated in fractions eluting with 20−60% Me_2CO in hexane. In addition to the four novel triterpenoids—3*β*,25-epoxy-3α,21α-dihydroxy-22*β*-(3-methylbut-2-en-1-oyloxy)olean-12-ene-28-oic acid (**1**); 3*β*,25-epoxy-3α,21α-dihydroxy-22*β*-angeloyloxyolean-12-ene-28-oic acid (**2**); 3*β*,25-epoxy-3α,21α-dihydroxy-22*β*-tigloyloxyolean-12-ene-28-oic acid (**3**); and 3*β*,25-epoxy-3α-hydroxy-22*β*-(2-methylbutan-1-oyloxy)olean-12-ene-28-oic acid (**4**), the known triterpenoids lantanilic acid (**5**), camaric acid (**6**), lantanolic acid (**7**), and rehmannic acid (**8**) were isolated from these fractions. The MIC values of all eight compounds against the H_{37}Rv strain of *M. tuberculosis* and the toxicity of the most active compound against Vero cells[6] were determined.



1: R = OH, R' = 3-methylbut-2-en-1-oyloxy
2: R = OH, R' = angeloyloxy
3: R = OH, R' = tigloyloxy
4: R = H, R' = 2-methylbutan-1-oyloxy
5: R = H, R' = 3-methylbut-2-en-1-oyloxy
6: R = H, R' = angeloyloxy
7: R = H, R' = H



8

Compound **1** was obtained as an amorphous solid. Its molecular composition was determined as $C_{35}H_{52}O_7$ by

* To whom correspondence should be addressed. Tel.: (520) 626-2481. Fax: (520) 626-4063. E-mail: btimmer@pharmacy.arizona.edu.
[†] University of Arizona.
[‡] University of Illinois at Chicago.
[§] INTA.

10.1021/np000267b CCC: $20.00
Published on Web 12/07/2000

Table 1. ^{13}C NMR, DEPT, HSQC, and ^{1}H NMR Data of **1**−**4**[a]

	1		**2**		**3**		**4**	
position	^{13}C and DEPT	HSQC and ^{1}H	^{13}C and DEPT	HSQC and ^{1}H	^{13}C and DEPT	HSQC and ^{1}H	^{13}C and DEPT	HSQC and ^{1}H
1 α	35.8 t	1.23 m (ovl[b] 5)	35.8 t	1.21 m (ovl 5)	35.8 t	1.22 m (ovl 5)	35.7 t	1.20 m (ovl 15α, 30)
β		2.20 ddd 12.3/12.3/5.2		2.18 ddd 12.3/12.3/5.1		2.19 ddd 12.3/12.3/5.1		2.18 ddd 12.4/12.4/5.2
2 α	30.8 t	2.36 ddd 12.3/12.3/5.2	30.8 t	2.34 ddd 12.1/12.1/5.1 (ovl 16*β*)	30.8 t	2.35 ddd 12.3/12.3/5.1 (ovl 16*β*)	30.8 t	2.35 m (ovl 2′)
β		2.05 m (ovl 11α)		2.02 m (ovl 11α, 5′)		2.04 m (ovl 11α)		2.05 m (ovl 11α, 16*β*)
3	97.8 s		97.8 s		97.8 s		97.8 s	
4	40.7 s		40.6 s		40.7 s		40.6 s	
5	50.6 d	1.26 m (ovl 1α, 23)	50.6 d	1.23 m (ovl 1α, 23)	50.6 d	1.24 m (ovl 1α, 23)	50.6 d	1.24 m (ovl 15α, 23, 27)
6 α	20.0 t	1.47 m	20.0 t	1.47 m (ovl 6*β*)	20.0 t	1.48 m (ovl 5′)	20.0 t	1.51 m
β		1.54 m (ovl 4′)		1.53 dddd 13.0/13.0/ 13.0/2.5 (ovl 6α)		1.53 dddd 13.0/13.0/ 13.0/3.0		1.55 dddd 13.0/13.0/ 13.0/2.5
7 α	31.8 t	1.38 m (ovl 19*β*, 27)	31.7 t	1.36 m (ovl 19*β*, 27)	31.7 t	1.36 m (ovl 19*β*, 27)	31.6 t	1.39 m (ovl 3′b)
β		1.31 m (ovl 30)		1.32 m (ovl 27, 30)		1.33 m (ovl 27, 30)		1.31 br d 13.0
8	38.7 s		38.7 s		38.7 s		38.8 s	
9	42.5 d	1.82 m (ovl 11*β*)	42.5 d	1.79 dd 12.5/4.5 (ovl 11*β*)	42.5 d	1.80 m (ovl 4′)	42.5 d	1.79 m (ovl 11*β*, 3′a)
10	35.2 s		35.2 s		35.2 s		35.2 s	
11 α	24.3 t	2.08 m (ovl 2*β*)	24.3 t	2.05 dd 12.5/3.5 (ovl 2*β*)	24.3 t	2.08 m (ovl 2*β*)	24.2 t	2.04 m (ovl 16*β*, 2*β*)
β		1.86 m (ovl 9,15*β*)		1.80 m (ovl 9, 15*β*)		1.85 m (ovl 9, 15*β*)		1.83 m (ovl 9, 15*β*, 21 *β*)
12	122.6 d	5.67 br s	122.6 d	5.62 br s	122.7 d	5.65 br s	122.7 d	5.57 br s
13	144.3 s		144.3 s		144.3 s		144.0 s	
14	42.8 s		42.7 s		42.8 s		42.4 s	
15 α	28.6 t	1.29 m (ovl 29)	28.5 t	1.26 m (ovl 5, 23)	28.6 t	1.28 m (ovl 23, 24)	28.2 t	1.22 m (ovl 1α, 5, 30)
β		1.87 m (ovl 11*β*)		1.86 m (ovl 11*β*)		1.88 m (ovl 11*β*)		1.84 m (ovl, 11*β*, 21*β*)
16 α	26.2 t	3.03 br dd 13.7/13.7	26.3 t	3.03 dd 13.4/13.4	26.2 t	3.04 dd 13.5/13.5	24.8 t	1.96 m (ovl 19α)
β		2.41 br d 13.7		2.38 dd 13.4/4.0		2.39 br d 13.5		2.02 m (ovl 2*β*, 11α)
17	51.5 s		51.4 s		51.5 s		50.9 s	
18	40.7 d	3.66 dd 14.5/3.5	40.6 d	3.61 dd 14.1/3.2	40.6 d	3.67 dd 14.0/3.3	40.0 d	3.47 dd 13.8/3.6
19 α	42.0 t	2.65 t 13.9	41.8 t	2.64 t 14.0	41.8 t	2.66 t 14.0	46.2 t	1.92 m (ovl 16α)
β		1.39 m (ovl 7α)		1.37 m (ovl 7α)		1.38 m (ovl 7α)		1.43 m (ovl 3′b, 7α)
20	35.1 s		35.0 s		34.9 s		30.4 s	
21	74.3 d	4.03 br s (ovl 25b)	74.2 d	4.03 br s (ovl 25b)	74.1 d	4.07 br s	38.6 t	1.66 dd 14.9/3.5 1.87 m (ovl 11*β*, 15*β*)
22	79.0 d	5.89 d 3.2	79.7 d	5.92 d 2.9	80.1 d	5.89 d 2.9	76.5 d	5.50 br s
23	27.8 q	1.267 s (ovl 5)	27.8 q	1.27 s	27.8 q	1.27 s	27.8 q	1.26 s
24	19.4 q	1.273 s	19.4 q	1.28 s	19.4 q	1.28 s	19.4 q	1.28 s
25 a	67.6 t	4.42 dd 8.5/2.5	67.5 t	4.42 dd 8.5/2.0	67.5 t	4.43 dd 8.5/2.5	67.5 t	4.40 br d 7.2
b		4.02 d 8.5 (ovl 21)		4.02 m (ovl 21)		4.03 d 8.2		3.99 br d 8.7
26	17.8 q	1.00 s	17.6 q	0.98 s	17.7 q	1.00 s	17.5 q	0.97 s
27	25.2 q	1.35 s	25.2 q	1.34 s	25.2 q	1.35 s	25.5 q	1.25 s
28	176.6 s		176.6 s		176.6 s		176.2 s	
29	29.1 q	1.29 s (ovl 15)	29.1 q	1.30 s	29.1 q	1.30 s	33.8 q	0.96 s
30	25.2 q	1.33 s	25.1 q	1.31 s	25.0 q	1.32 s	26.8 q	1.20 s (ovl 1α, 15α)
1′	165.5 s		166.7 s		166.9 s		175.1 s	
2′	116.8 d	5.78 t 1.1	128.5 s		129.3 s		42.0 d	2.38 m (ovl 2α)
3′ a	157.0 s		138.1 d	5.84 q 7.0	137.6 d	7.06 dq 7.0/1.5	26.7 t	1.78 m (ovl 9, 11*β*)
b								1.40 m (ovl 7α, 19*β*)
4′	26.9 q	1.54 br s	20.7 q	1.93 s	12.2 q	1.81 s	12.0 q	0.83 t 7.2
5′	20.1 q	2.12 br s	15.8 q	2.00 d 7.0	14.1 q	1.44 d 7.0	16.9 q	1.10 d 6.9

[a] Measured in pyridine-d_6; ^{13}C multiplicities from DEPT and edited HSQC spectra; ^{1}H shifts from 1D ^{1}H NMR or HSQC; ^{1}H multiplicities and J values in Hz from 1D ^{1}H NMR. [b] ovl: designates overlapping signals.

HRFABMS. DEPT and edited HSQC spectra revealed the presence of seven methine, nine methylene, and eight methyl groups. In the ^{13}C NMR (Table 1) two signals at δ_C 176.6 and 165.5 were typical for a carboxylic acid and an ester moiety, respectively. Of the four double-bond carbons, the position of two at δ_C 122.6 (d) and 144.3 (s) pointed toward an olean-12-ene skeleton, while the other two at δ_C 116.8 (d) and δ_C 157.0 (s) were in agreement with a 3-methylbut-2-en-1-oyloxy group. Two tertiary and one secondary carbon signal appeared at positions typical for oxygen substitution (δ_C 74.3, 79.0, and 67.6). A quaternary carbon at δ_C 97.8 suggested the presence of a carbon substituted with two oxygens. A pair of geminal methyl groups (Me-23, Me-24) showed HMBC cross-peaks to this carbon and to a tertiary carbon at δ_C 50.6 (Table 2). Assuming an oleanene skeleton, the quaternary carbon was assigned as C-3 and the latter as C-5. A methylene proton at δ_H 4.02 (H-25b), a chemical shift typical for oxygen substitution, also showed HMBC correlations with C-3 and C-5, which can be explained by the presence of a 3*β*,25-epoxy moiety. This additional ring is in agreement with a degree of unsaturation of 10, calculated from the molecular formula determined by HRFABMS. The geminal methyl Me-29 and Me-30 and the methine proton corresponding to the oxygen-substituted tertiary carbon at δ_C 79.0 showed HMBC correlations with the oxygen-substituted carbon at δ_C 74.3. This places both oxygen substituents at positions 21 and 22. An HMBC correlation between H-22 and the carbonyl carbon of the 3-methylbut-2-en-1-oyloxy substituent showed that this side chain had to be located at position 22. The coupling constant for protons H-21 and H-22 is typical for trans-diequatorial coupling ($J_{21/22}$ = 3.2 Hz). ROESY spectra (Table 2) and the coupling constant observed for H-18 showed that H-18 and Me-30 are in axial

Table 2. HMBC and ROESY Data of **1** and **4**

	1		**4**	
position	HMBC (H to C)	ROESY	HMBC (H to C)	ROESY
1 α	2, 5, 10, 25	1*β*, 2α*β*, 9, 11α		1*β*, 2α*β*, 5, 9, 11α,
β	2, 5, 25	1α, 2*β*, 25b	2, 5, 10, 25	1α, 25b
2 α	1, 3	1α, 2*β*, 23	1, 3	1α, 2*β*, 23
β	1, 3, 4, 10	1α*β*, 2α, 25b	1, 3, 4	2α, 25b
5	4, 6, 10, 23, 25	9		
6 α	8, 10	24	7, 10	24, 26
β		24, 25a, 26	5	24, 25a, 26
7 α	8, 14			26, 27
β	5, 8, 26	26	5, 9	26
9	8, 10, 11, 14, 25, 26	1α, 5, 27	8, 10, 11	
11 α	8, 9, 12, 13	1α, 9, 11*β*, 12	9, 12, 13	
β	10, 12, 13	11*β*, 12, 25b, 26	10, 12	25b
12	9, 14, 18	11α*β*,18, 19*β*, 26	11, 14, 18	11α*β*, 18, 19α*β*, 26, 27
15 α		15α, 16*β*		
β	13, 17	15*β*,16*β*, 26		
16 α	28	16*β*, 19α, 27	15, 17, 28	26, 27
β		15α*β*,16α, 22	15, 17	22, 26, 27
18	12, 13, 14, 17	12, 19*β*, 30	9, 12, 13, 16, 17, 19, 28	12, 19*β*, 26, 27, 29, 30, 5′
19 α	18, 20, 29, 30	16α, 19*β*, 27, 29	13, 18, 20, 29, 30	12
β	17, 18, 20, 21	12, 18, 19α	17, 18, 20, 21	12, 18
21 α		22, 29, 30	20, 29, 30	22
β			17, 19, 22	
22	16, 17, 18, 20, 21, 28, 1′	16*β*, 21	16, 17, 18, 20	16α*β*, 21α*β*
23	3, 4, 5, 24	2α	3, 4, 5, 24	2α
24	3, 4, 5, 23	25a	3, 4, 5, 23	6α*β*, 25a
25 a	1, 5	6*β*, 24, 25b, 26	1, 3, 5	6*β*, 24, 25b, 26
b	1, 3, 5, 10	1*β*, 2*β*, 11*β*, 25a, 26	1, 3, 5, 10	1*β*, 2*β*, 11*β*, 25a, 26
26	7, 8, 9, 14	6*β*, 7*β*, 11*β*, 12, 15*β*, 25ab	7, 8, 9	6α*β*, 7α*β*, 11*β*, 12, 15*β*, 18, 25ab
27	8, 13, 14, 15	9, 16α, 19α	8, 13, 14, 15	7α, 9, 16α*β*, 19α
29	19, 20, 21, 30	19α, 21	19, 20, 21, 30	19α*β*, 21α*β*
30	19, 20, 21, 29	18, 21, 2′	19, 20, 21, 29	19*β*, 2′
2′	1′, 4′, 5′	30, 4′	1′, 3′, 4′, 5′	30, 4′, 5′
3′ a			1′, 2′, 4′, 5′	3′b, 4′, 5′
b				3′a, 4′, 5′
4′	1′, 2′, 3′, 5′	2′, 5′	2′, 3′	2′, 3′ab
5′	1′, 2′, 3′, 4′	4′	1′, 2′, 3′	2′, 3′ab

positions at an E ring in a chair conformation and excluded the possibility of an axial−equatorial coupling between H-21 and H-22 from which similar coupling constants $J_{21/22}$ could result. In ROESY spectra, proton H-21 showed cross-peaks of about equal intensity with methyl groups at C-29 and C-30, confirming its equatorial position. The equatorial position of proton H-22 follows from the absence of cross-peaks with H-18 or the methyl group Me-30, while cross-peaks to H-16*β* and H-21 could be observed. Based on these observations, we assigned compound **1** the structure of 3*β*,25-epoxy-3α,21α-dihydroxy-22*β*-(3-methylbut-2-en-1-oyl-oxy)olean-12-ene-28-oic acid.

In compounds **2** and **3**, angeloyloxy and tigloyloxy moieties replace the 3-methylbut-2-en-1-oyloxy side chain of compound **1**. All other NMR signals of these three compounds are virtually identical. Therefore, we assigned the structures of 3*β*,25-epoxy-3α,21α-dihydroxy-22*β*-angeloyloxyolean-12-ene-28-oic acid and 3*β*,25-epoxy-3α,21α-dihydroxy-22*β*-tigloyloxyolean-12-ene-28-oic acid to compounds **2** and **3**, respectively. HSQC, HMBC, and ROESY spectra allowed the complete assignment of proton and carbon signals of compounds **1**−**3** (Tables 1 and 2).

From the ^{1}H and ^{13}C NMR of **4** it was obvious that this compound was very similar to triterpenoids **1**−**3**. Its molecular composition was determined as $C_{35}H_{54}O_6$ by HRFABMS. In the ^{13}C NMR spectrum only one oxygen-substituted tertiary carbon appeared. HMBC spectra showed that, when compared to compounds **1**−**3**, the hydroxy group at position 21 was missing in **4**. Also, only one vinylic proton appeared in the ^{1}H NMR spectrum, suggesting a saturated ester side chain. A carbonyl carbon at δ_C 175.1 and a shift of the carbonyl band in the IR spectrum of **4** to higher wavenumbers (1732 cm^{-1} in **4** vs 1712 cm^{-1} in **1** and **2** and 1714 cm^{-1} in **3**), when compared to **1**−**3**, were in agreement with this assumption. In the ^{1}H NMR spectrum, a triplet (3H, $\delta_H = 0.83$, $J = 7.2$) and a doublet (3H, $\delta_H = 1.10$, $J = 6.9$) suggested a 2-methylbutyrate moiety. The structure of **4** was confirmed by HMBC and ROESY spectra (Table 2). The signal of H-22 appeared as a broad singlet ($w_{1/2} = 6.8$) suggesting an equatorial position of H-22 and an axial position of the ester substituent at this position at ring E of **4**. Because H-18 of oleanenes is *β*-configurated and axial with respect to ring E, as evident from a coupling constant typical for trans-diaxial coupling (H-18, $\delta_H = 3.47$, dd, $J = 13.8/3.6$), the axial ester substituent in position 22 had to be *β*-configured, as well. Based on these observations, **4** was assigned the structure of 3*β*,25-epoxy-3α-hydroxy-22*β*-(2-methylbutan-1-oyloxy)olean-12-ene-28-oic acid.

Compounds **5**−**7** were closely related to **4**. The ester side chain in position 22 is a 3-methylbut-2-en-1-oyloxy moiety in **5**, an angeloyloxy in **6**, and completely missing from **7**. These compounds were, therefore, identified as the known compounds lantanilic, camaric, and lantanolic acids previously isolated from *Lantana camara*.[17−19]

Compound **8** is the only one of the isolated triterpenoids that lacks a 3*β*,25-epoxy bridge. Instead, its spectra showed the presence of a ketone in position 3. Comparison of the ^{13}C NMR data with published data showed that **8** is rehmannic acid, a compound previously isolated from *Lippia rehmanni*[16] and *Lantana camara*.[20]

The MIC values of the eight triterpenoids **1**−**8** isolated from *L. turbinata* range from 32 to greater than 128 *μ*M (compare to 0.3 *μ*M determined for the antitubercular drug

rifampin). These activities are comparable to those of a number of common triterpenoids such as oleanolic acid, lupeol, or betulin.[4] Compound **8** (MIC 32 μM) is the most active compound of the series. The more active compounds (**2**, **6**, and **8**; MICs of 64, 64, and 32 μM, respectively) all contain an angelate in position 22. The least active compounds **1**, **3**, **4**, **5**, and **7** contain methylbutenoyl (**1** and **5**; MICs >128 μM and 128 μM, respectively), tigloyl (**3**; MIC 128 μM), a saturated ester side chain (**4**; MIC 128 μM), or no side chain (**7**; MIC >128 μM). Although the presence of a keto group in position 3 may be responsible for increased activity (compound **8**, MIC 32 μM), additional hydroxy groups in position 21 appear to reduce the activity or have no influence, as can be seen when comparing the MIC values of compounds **5** and **1** (MICs 128 μM and >128 μM) or **6** and **2** (both MICs 64 μM). The MICs of the described compounds **1**−**8** in μg/mL units are >75, 38, 75, 73, 73, 36, >60, and 18, respectively. In the Vero cell assay,[6] the most active compound of the series (**8**, MIC 32 μM) showed toxicity, with an IC_{50} value of 15 μM. The antitubercular activity and toxicity of this compound are comparable to those of previously reported tetracyclic triterpenoids.[5,6]

Experimental Section

General Experimental Procedures. NMR spectra were recorded on Varian Unity 300 and Bruker DRX 500 spectrometers in deuterated pyridine. Solvent signals at δ_H 7.19 and δ_C 123.5 were used to reference the spectra. Carbon multiplicities were established by DEPT and edited HSQC experiments. Gradient-selected HSQC and HMBC experiments were used. ROESY spectra were recorded with 200-ms delays. Optical rotations were determined on a JASCO P1020 polarimeter. IR spectra were recorded with a Buck Scientific 500 spectrometer. HRFABMS were recorded on a JEOL HX 110 spectrometer with a resolution of 10 000, using a mixed matrix consisting of glycerol, thioglycerol, and *m*-NBA. Reversed-phase HPLC was carried out using a 10 × 250 mm, 10-μm Reliasil C_{18} column with 75% CH_3CN, 5% MeOH, and 20% aqueous 0.1% HCOOH at 4 mL/min. Normal-phase HPLC was carried out using a 10 × 250 mm, 10-μm Econosil Si gel column at 5 mL/min. TLCs were sprayed with 0.5% anisaldehyde, 10% HOAc, and 5% H_2SO_4 in MeOH and heated until colored spots appeared.

Plant Material. Aerial parts of *L. turbinata* Griseb. (Verbenaceae) were collected and identified by Renée H. Fortunato in November 1995, in the district Ojo del Agua of the Argentinean province Santiago del Estero. A specimen has been deposited in the herbarium of the INTA, Castelar, Buenos Aires, Argentina (coll. no. RF 5064). Intellectual Property Rights Agreements for plant collections and collaborative research have been fully executed between The University of Arizona and INTA.

Biological Assays. Antitubercular activity against the $H_{37}Rv$ strain (ATCC 27294) of *M. tuberculosis* using the radiorespirometric BACTEC 460 system and toxicity against Vero cells (ATCC CCL-81) was determined as described earlier.[2,6]

Extraction and Isolation. Air-dried and ground aerial parts of *L. turbinata* (1.5 kg) were extracted three times with MeOH−CH_2Cl_2 (1:1) at room temperature. The dried extract (45 g) was subjected to column chromatography on Si gel with hexane−Me_2CO mixtures of increasing polarity. The resulting fractions were assayed in the BACTEC system for inhibition of mycobacterial growth. Activity was found to be concentrated in fractions eluting with 20−60% Me_2CO. A column fraction (1.2 g) eluting with 60% Me_2CO was dissolved in CH_3CN with heating. After cooling the solution, an amorphous powder precipitated (210 mg). HPLC of this material on a reversed-phase column gave two major fractions of which the one eluting earlier (42 mg) gave a characteristic green spot on TLC after spraying with anisaldehyde reagent and subsequent heating. From this fraction compounds **1** (6 mg), **2** (12 mg), and **3** (3 mg) were isolated by normal-phase HPLC (20% Me_2CO in hexane). The second fraction gave a blue spot under the same TLC conditions, similar to a fraction from the initial Si gel column, which eluted with 50% Me_2CO. From the latter fraction (2.2 g) compound **5** (400 mg) precipitated after treatment with Me_2CO. The column fraction eluting with 40% Me_2CO contained the largest amount (10.4 g) and also gave a blue spot under the above-mentioned TLC conditions. A portion of this material (500 mg) was dissolved in hot CH_3CN. From the precipitate obtained after cooling (145 mg), compounds **4** (31 mg), **6** (25 mg), and **7** (33 mg) were isolated by normal-phase HPLC (15% Me_2CO, 0.5% HCOOH, 84.5% hexane). From a column fraction eluting with 20−30% Me_2CO (660 mg), compound **8** (110 mg) precipitated after treatment with EtOAc.

3*β*,25-Epoxy-3α,21α-dihydroxy-22*β*-(3-methylbut-2-en-1-oyloxy)olean-12-ene-28-oic acid (1): colorless solid; $[\alpha]^{25}_D$ +49.6° (*c* 0.3; MeOH); IR (neat) 1712 cm^{-1}; ^1H, ^{13}C, and 2D NMR data, Tables 1 and 2; HRFABMS obsd *m*/*z* 585.3784 $[M + H]^+$ calcd for $C_{35}H_{53}O_7$ 585.3791.

3*β*,25-Epoxy-3α,21α-dihydroxy-22*β*-angeloyloxyolean-12-ene-28-oic acid (2): colorless solid; $[\alpha]^{25}_D$ +157.2° (*c* 0.25; MeOH); IR (neat) 1712 cm^{-1}; ^1H, ^{13}C, and 2D NMR data, Table 1; HRFABMS obsd *m*/*z* 585.3807 $[M + H]^+$, calcd for $C_{35}H_{53}O_7$ 585.3791.

3*β*,25-Epoxy-3α,21α-dihydroxy-22*β*-tigloyloxyolean-12-ene-28-oic acid (3): colorless solid; $[\alpha]^{25}_D$ +124.6° (*c* 0.15; MeOH); IR (neat) 1714 cm^{-1}; ^1H, ^{13}C, and 2D NMR data, Table 1; HRFABMS obsd *m*/*z* 585.3802 $[M + H]^+$, calcd for $C_{35}H_{53}O_7$ 585.3791.

3*β*,25-Epoxy-3α-hydroxy-22*β*-(2-methylbutan-1-oyloxy)-olean-12-ene-28-oic acid (4): colorless solid; $[\alpha]^{25}_D$ +132.3° (*c* 1.5; MeOH); IR (neat) 1732 cm^{-1}; ^1H, ^{13}C, and 2D NMR data, Tables 1 and 2; HRFABMS obsd *m*/*z* 571.3999 $[M + H]^+$, calcd for $C_{35}H_{55}O_6$ 571.3999.

Acknowledgment. This study was supported by the ICBG "Bioactive Agents from Dryland Biodiversity of Latin America" grant 2 UO1 TW 00316 from the National Institutes of Health (NIH), the National Science Foundation (NSF), and the U.S. Department of Agriculture (USDA) (to B.N.T.). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH, NSF, or USDA. The authors thank Renée H. Fortunato (INTA, Buenos Aires), Julian Greppi (Universidad de Moron, Buenos Aires), Alfredo D'Agostini (INTA, Chaco), and Edgardo Saavedra (Universidad Nacional de la Patagonia, Chubut) for collecting and identifying the plant material and producing the extract for chemical work at The University of Arizona.

References and Notes

(1) Timmermann, B. N.; Wächter, G. A.; Valcic, S.; Hutchinson, B.; Henzel, J.; Casler, C.; Ram, S.; Currim, F.; Manak, R.; Franzblau, S.; Maiese, W.; Galinis, D.; Suarez, E.; Fortunato, R.; Saavedra, E.; Bye, R.; Mata, R.; Montenegro, G. *Pharm. Biol.* **1999**, *37* (Suppl., J. Rosenthal, Ed.), 35−54.
(2) Collins, L. A. and Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004−1009.
(3) Wächter, G. A.; Franzblau, S. G.; Montenegro, G.; Suarez, E.; Fortunato, R. H.; Saavedra E.; Timmermann, B. N. *J. Nat Prod.* **1998**, *61*, 965−968.
(4) Wächter, G. A.; Valcic, S.; Flagg, M. L.; Franzblau, S. G.; Suarez E.; Montenegro G.; Timmermann, B. N. *Phytomedicine* **1999**, *6*, 341−346.
(5) Cantrell, C. L.; Rajab, M. S.; Franzblau, S. G.; Fischer, N. H. *J. Nat. Prod.* **1999**, *62*, 546−548.
(6) Cantrell, C. L.; Lu, T.; Fronczek, F. R.; Fischer, N. H.; Adams, L. B.; Franzblau, S. G. *J. Nat. Prod.* **1996**, *59*, 1131−1136.
(7) Grundy, D. L.; Still, C. C. *Pestic. Biochem. Physiol.* **1985**, *23*, 378−382.
(8) Valentin, A.; Pélissier, Y.; Benoit, F.; Marion, C.; Kone, D.; Mallie, M.; Bastide, J.-M.; Bessière, J.-M. *Phytochemistry* **1995**, *40*, 1439−1442.
(9) Mwangi, J. W.; Njonge, E. W.; Addea-Mensah, I.; Munavu, R. W.; Lwande, W. *Discuss. Innovat.* **1994**, *6*, 58−60.
(10) Chanh, P. H.; Koffi, Y.; Chanh, A. P. H. *Planta Med.* **1988**, *54*, 294−296.

(11) Noamesi, B. K.; Adebayo, G. I.; Bamgbose, S. O. A. *Planta Med*. **1985**, *51*, 253−255.
(12) Souto-Bachiller, F. A.; De Jesus-Echevarría, M.; Cárdenas-González, O. E.; Acuña-Rodriguez, M. F.; Meléndez, P. A.; Romero-Ramsey, L. *Phytochemistry* **1997**, *44*, 1077−1086.
(13) Taoubi, K.; Fauvel, M. T.; Gleye, J.; Moulis, C.; Fourasté, I. *Planta Med*. **1997**, *63*, 192−193.
(14) (a) Tomás-Barberán, F. A.; Harborne, J. B.; Self, R. *Phytochemistry* **1987**, *26*, 2281- 2284. (b) Nair, A. G. R.; Ramesh, P.; Nagarajan, S.; Subramanian, S. S. *Indian J. Chem*. **1973**, *11*, 1316−1317. (c) Skaltsa, H.; Shammas, G. *Planta Med*. **1988**, *54*, 465−465.
(15) Macambira, L. M. A.; Andrade, C. H. S.; Matos, F. J. A.; Craveiro, A. A.; Braz Filho, R. *J. Nat. Prod*. **1986**, *49*, 310−312.
(16) Barton, D. H. R.; De Mayo, P. *J. Chem. Soc.* **1954**, 887−900, 900−903.
(17) Barua, A. K.; Chakrabarti, P.; Chowdhury, M. K.; Basak, A.; Basu, K. *Phytochemistry* **1976**, *15*, 987−989.
(18) Siddiqui, B. S.; Raza, S. M.; Begum, S.; Siddiqui, S.; Firdous, S. *Phytochemistry* **1995**, *38*, 681−685.
(19) Barua, A. K.; Chakrabarti, P.; Dutta, S. P.; Mukherjee, D. K.; Das, B. C. *Tedrahedron* **1971**, *27*, 1141−1147.
(20) Barton, D. H. R.; De Mayo, P.; Orr, J. C. *J. Chem. Soc.* **1956**, 4160−4162.

NP000267B