Characterization of an Anti-tuberculosis Resin Glycoside from the Prairie Medicinal Plant *Ipomoea leptophylla*

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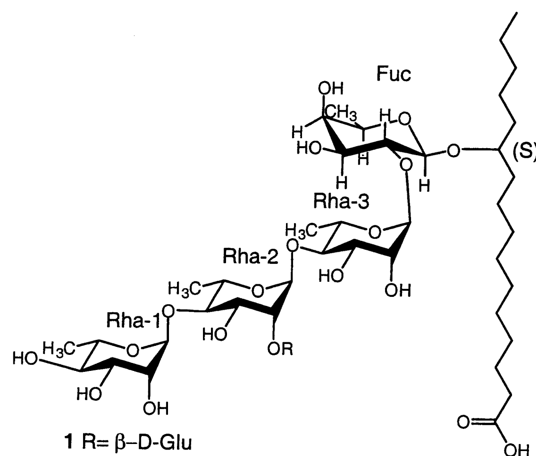
The organic soluble extract from the leaves of the native North American prairie plant *Ipomoea leptophylla* (big root morning glory) showed in vitro activity against *M. tuberculosis.* Bioassay-guided fractionation of this extract resulted in the identification of two new resin glycosides (**6**, **7**). Base-catalyzed hydrolysis of these glycosides gave operculinic acid (**1**) as the glycosidic acid component as well as *trans*-cinnamic acid, propanoic acid, and lauric acid. The complete structure elucidation was accomplished through derivatization, 1D and 2D NMR spectroscopy (TOCSY, ROESY, HSQC, HMBC), and MS/MS experiments on **6** and **7** as well as the permethylated derivative **8**.

For the past 10 years this laboratory has been investigating the chemistry of native North American prairie plants with a history of medicinal uses by Native Americans and early European immigrants to the plains. Prairie plants with documented medicinal uses have been collected and bioassayed for activity against HIV-1, human tumor cell lines, and *Mycobacterium tuberculosis*.[1] The bioassays have been performed in collaboration with the U.S. National Institutes of Health (NIH) pilot program for the development of AIDS-related therapeutics.[2] Plant collection has been guided by examination of the ethnobotanical literature regarding the uses of plants by indigenous North Americans.[3] Forty different plant species have been assayed for activity against *M. tuberculosis*, with four having been found to be active. Among the active species was *Ipomoea leptophylla*, which showed 92% inhibition at an initial test concentration of 150 μg/mL for the crude organic extract. This communication describes the isolation and structure elucidation of a new resin glycoside from *I. leptophylla* which appears to be the major anti-tuberculosis compound.

I. leptophylla, a member of the Convolvulaceae family (morning glory family),[4] is a perennial herb with a large woody root the size of a man's leg. This explains two of its common names, man root and big root morning glory. Its stems lie on the ground or are erect with lengths from 0.2 to 1.2 m. Its narrow leaves grow to 3−15 cm. The plant flowers from May to September with long purple-red funnel-shaped flowers. Its habitat is open prairies, often sandy, in central North America from south central South Dakota and eastern Colorado to north central Texas and east central Kansas.

Native Americans primarily used the enormous root for medicinal purposes. The Pawnee dried the root, burned it, and inhaled the smoke for treatment of nervousness.[5] The Lakota people ate portions of the root for stomach ailments.[6] Early European settlers reported the root to have exceptional aperient and tonic properties.[7] In contrast to the ethnomedicinal reports of this plant, all of the antimicrobial activity reported here is associated with the leaves and stems of the plant. Scientific research on the chemistry of *I. leptophylla* is nonexistent in the literature, but there has been extensive research on related species *I. operculata* (Brazil),[8] *I. stans* (Mexico),[9] *I. orzabensis* (Mexico),[10] and *I. tricolor* (Mexico).[11] These species have an extensive history of use in Mexican folk medicine.[12]

The active ingredients isolated from these species have been a series of related "resin glycosides" which can be loosely defined as ether-soluble (jalapin) and ether-insoluble glycosides (convolvulin) that are isolated from the alcoholic extracts of the roots of various species of *Ipomoea*.[13] A common feature of all resin glycosides is that a glycosidic acid is isolated after mild alkaline hydrolysis. Ono et al. identified operculinic acids as the glycosidic acids from *I. operculata*, with operculinic acid A (**1**) being the



major acid isolated.[8] Minor acids were also isolated with the main variability being the substitution of xylose and glucose for fucose.[14] In a later publication, four different resin glycosides (operculins, **2**−**5**) derived from the parent operculinic acid A (**1**) were reported (Figure 1).[15] The same paper reported another operculin identical to **2** in which the macrocyclic lactone was bonded to Rha-3 at C-3. Common to all these resin glycosides is the macrocyclic ester formed between the anomeric carbon on fucose and C-2 or C-3 on rhamnose-3. The acid responsible for this esterification is (*S*)-11-hydroxypalmitic acid (jalapinolic

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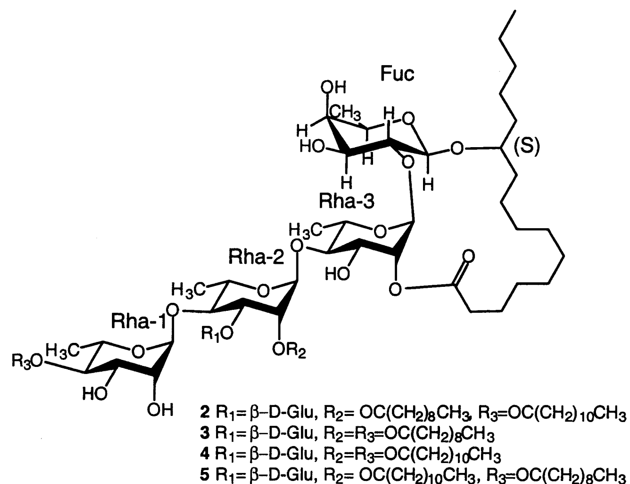
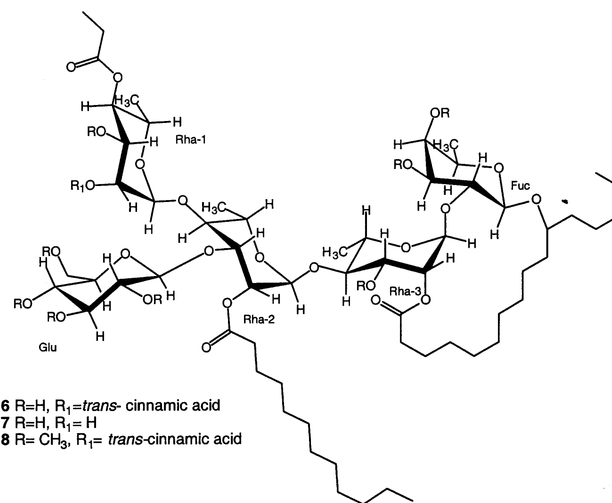


Figure 1. Resin glycosides from *I. operculata*.



acid). A second common feature is the presence of C-10 and C-12 acids esterified to Rha-1 and Rha-2. To date, a total of 18 operculins have been isolated from *I. operculata*.[16,17] In addition to the operculins isolated from *I. operculata*, similar compounds have been isolated from *I. stans*, containing quinovose in place of fucose, as well as different acid esters (methyl propanoic, 2-methylbutanoic, and 3-hydroxy-2-methylbutanoic acid).[18] A number of other resin glycosides related to those from *I. operculata* have also been reported from other genera within the Convolvulaceae family.[19]

Results and Discussion

The dried leaves and stems of *I. leptophylla* were extracted with MeOH−CH_2Cl_2. An in vitro bioassay for activity against *Mycobacterium tuberculosis* was performed by the Tuberculosis Antimicrobial and Acquisition Coordinating Facility (TAACF). The crude extract showed 92% growth inhibition at a concentration of 150 μg/mL. The crude extract was subsequently chromatographed on lipophilic Sephadex-LH-20. Like fractions were combined and bioassayed. An early-eluting fraction showed an increase in activity of approximately 3-fold (89% inhibition at 50 μg/mL). This fraction was further separated by first using flash silica chromatography followed by HPLC on silica. Two pure compounds were isolated. The first eluting compound (**6**) contained a chromophore that had a λ_{max}(EtOH) at 280 nm. The second eluting compound (**7**) had no accessible UV absorbance but could be detected by thin-layer chromatography charring with sulfuric acid.

The ^{1}H NMR spectrum of **6** showed numerous signals from 4.20 to 5.50 ppm, which was consistent for both anomeric protons associated with reducing sugars and protons attached to carbons on ester oxygens. A ^{13}C−^{1}H HETCOR experiment indicated five carbons between 98.0 and 104.2 ppm which showed correlations to signals between 4.20 and 5.40 ppm. These results suggested that part of the molecule was a pentasaccharide. The presence of a *trans* coupled olfenic pair of protons at 6.69 and 7.80 ppm (J = 15 Hz) suggested an α,β-unsaturated carbonyl. These signals along with a two-proton multiplet at 7.70 ppm and a three-proton multiplet at 7.55 ppm suggested that the observed chromophore may be attributed to a cinnamic acid residue. A high-resolution FABMS analysis of **6** produced a molecular ion at m/z = 1501.6372, which supported a chemical formula of $[C_{70}H_{112}O_{26}Cs]^+$.

When **6** was hydrolyzed in acid followed by pertrimethylsilylation, GC/MS analysis of the products identified glucose, rhamnose, and fucose in a ratio of 1:3:1. The absolute stereochemistry of these sugars was determined by reacting the acid hydrolysis product of **6** with (*S*)-2-butanol prior to persilylation. The retention times of the C-1 (*S*)-2-butyl glycosides were identical to those observed from D-glucose, L-rhamnose, and D-fucose. Other products identified from their GC/MS were the trimethylsilylated derivatives of cinnamic acid, lauric acid, propanoic acid, and 11-hydroxypalmitic acid.

The results of the hydrolysis experiments coupled with the NMR data suggested that **6** may be a derivative of operculinic acid (**1**). This was confirmed by hydrolyzing **6** in base and converting the resultant free acid to its methyl ester. Comparison of the optical rotation and ^{13}C and ^{1}H NMR spectra of the hydrolyzed methyl ester of **6** matched, within experimental error, that reported for **1**.[8] Mass spectral linkage analysis of the base-hydrolyzed product of **6** further confirmed that sugar linkage positions were consistent with the structure of operculinic acid (**1**).

On the basis of the initial GC analysis of the acid-hydrolyzed products of **6** and the identification of operculinic acid from its base hydrolysis, it was clear that **6** was related to the operculins from *I. operculata*. The location of the three acid residues (lauric, cinnamic, and propanoic) and point of lactonization of the palmitate remained to be determined. This was accomplished through derivatization of **6** to its permethylated derivative (**8**) followed by a series of mass spectral and ^{1}H and ^{13}C NMR experiments.

Various attempts at methylating **6** under basic conditions consistently resulted in methylation of the hydroxyl groups and the hydrolysis of the four ester bonds. Utilizing a neutral methylation technique, developed by Prehm,[20] the permethylated derivative (**8**) could be isolated with all esters intact. The isolation of the octamethyl derivative was confirmed from high-resolution MALDI-TOF-MS, which resulted in a molecular ion at m/z = 1503.8555, which corresponded to a formula of $[C_{78}H_{128}O_{26}Na]^+$. To determine the location of the ester groups, the permethylated derivative was subjected to base hydrolysis to cleave all ester functionalities. The product gave a molecular ion at m/z = 1175, which corresponded to $[C_{54}H_{97}O_{24}Na]Na^+$. The MS^2 electrospray ionization of the peak at m/z = 1175 resulted in major fragment ions at m/z = 1015, 957, 899, 707, 651, 547, and 491 (Figure 2). The peak at m/z = 491 was attributed to the fucose glycosidically bonded to palmitic acid, thus indicating that fucose had no attached esters. This was further verified by an MS^3 of the 491 peak, which resulted in ions at m/z = 317 (Na hydroxypalmitate),

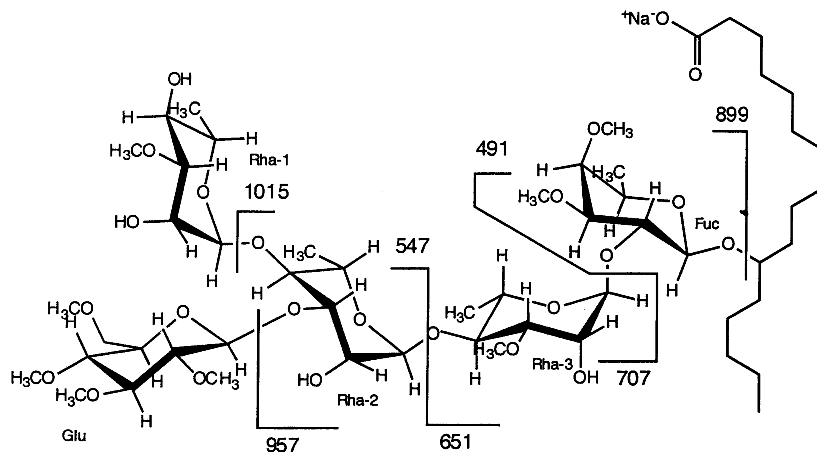


Figure 2. Observed fragmentation of **6** after neutral permethylation followed by base hydrolysis.

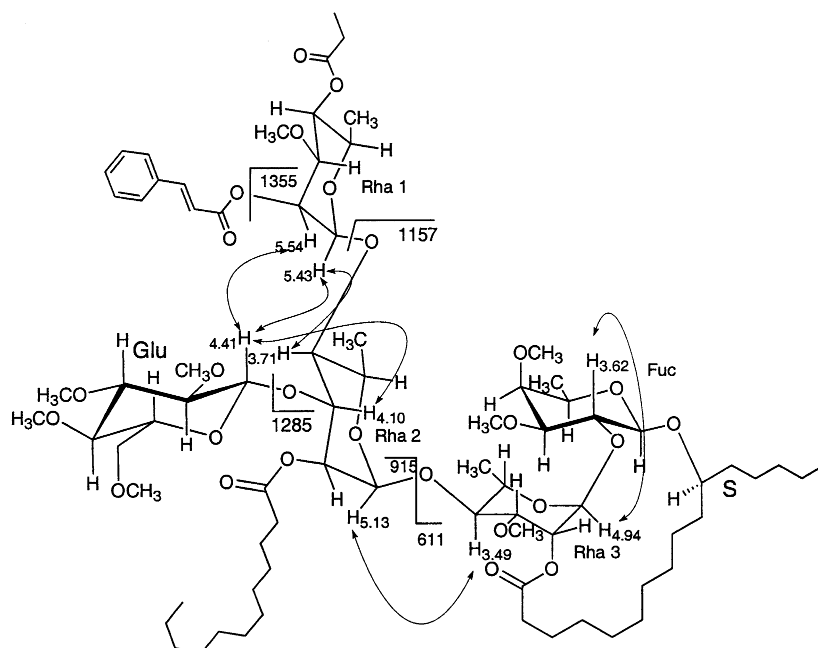


Figure 3. Observed fragmentation from electrospray ion trap MS and key ROE enhancements for **8**.

$m/z = 299$ (Na palmitate), and $m/z = 175$ (fucose + 2 OMe). The peak at $m/z = 651$ was attributed to cleavage between Rha-3 and Rha-2. The presence of three OMe groups indicated that Rha-2 was bonded only to the palmitic acid at either C-2 or C-3. The peak at $m/z = 1015$ was attributed to the loss of Rha-1 with one OMe group, indicating that Rha-1 had two esters bonded to C-2, C-3, or C-4. The peak at $m/z = 957$ was attributed to the loss of a permethylated glucose, indicating that it was not substituted, thus implying that an ester and the glucose were bonded to C-2 and C-3 of Rha-2.

Identifying the specific ester associated with the sugars was determined by MS/MS of the $(M + Na)^+$ molecular ion of the intact permethylated derivative at m/z 1504. (A peak at $m/z = 1532$ was observed with an intensity of approximately 10% of that of the $m/z = 1504$ peak. This was attributed to some substitution of mysteric acid for lauric acid at C-2. This variability in acid structure has been previously observed.[8]) The key fragment was at $m/z = 1157$, which was attributed to the loss of Rha-1 containing cinnamic and propanoic acids. The lauric acid was therefore bonded to Rha-2 (Figure 3).

With the sequence of the substituted carbohydrates established through mass spectral experiments, the final structural dilemma to be resolved was to determine the regiochemistry of each ester on their appropriate sugar. To accomplish this, it was necessary to unequivocally assign the important ^{1}H and ^{13}C chemical shifts of each sugar unit. This was accomplished through a series of HSQC, HMBC, COSY, ROESY, and TOCSY experiments.

Key to identifying all of the spin systems within each sugar was to identify all nine of the anomeric and ester protons. Even at 600 MHz, these signals were not resolved in the ^{1}H NMR spectrum of **6**. However, using the permethylated derivative (**8**) resulted in excellent resolution of all relevant signals (even at 300 MHz). Consequently **8** was used to determine the regiochemistry. Given in Table 1 are the NMR spectral chemical shift assignments for **8**. The anomeric protons on fucose and glucose were determined from their upfield chemical shifts relative to the rhamnose anomeric signals, due to their axial orientation and larger coupling constants (7.7 Hz). The glucose anomeric proton (4.41 ppm) was distinguished from the fucose anomeric proton (4.21 ppm) from the TOCSY correlation to the C-6 primary alcohol protons at 3.50 and 3.68 ppm. The ester protons from cinnamic acid (5.54 ppm) and propanoic acid (5.04 ppm) on Rha-1 were identified through their HMBC correlations to their respective carbonyls (165.5 and 175.9

Table 1. ^{13}C and ^{1}H NMR Chemical Shifts for **8**[a]

carbon	^{13}C	^{1}H
Rha-1		
1	98.8	5.43
2	68.0	5.54
3	77.3	3.53
4	71.8	5.04
5	67.4	3.94
6	17.0	1.22
Rha-2		
1	98.6	5.13
2	72.8	5.16
3	76.5	4.10
4	77.3	3.77
5	67.7	3.83
6	18.8	1.27
Rha-3		4.94
1	98.4	
2	67.0	5.38
3	79.5	3.57
4	78.8	3.48
5	67.7	3.83
6	18.4	1.32
Fuc		
1	104.0	4.21
2	77.7	3.62
3	83.2	3.11
4	77.2	3.38
5	69.5	3.45
6	17.3	1.25
Glu		
1	102.5	4.41
2	84.1	2.74
3	85.9	3.21
4	79.7	2.92
5	75.8	3.37
6	71.8	3.50, 3.68
C-11 palmitic	83.2	3.57
OMe	61.4, 60.6(2), 60.3	3.58, 3.49(3), 3.45
	60.2, 57.6, 57.5, 57.0	3.39, 3.89, 3.38
carbonyl		
PR	175.9	
LA, PA	172.6, 172.5	
CA	165.5	
acid methyl		
PR	16.7	1.25
LA	11.5	0.91
PA	14.1	0.88

[a] 500 MHz ^{1}H, 125 MHz ^{13}C, $CDCl_3$.

ppm). TOCSY and COSY correlations established the cinnamic acid at C-2 and the propanoic acid at C-4 on Rha-1. Establishing the substitution position of the glucose on C-3 of Rha-2 was determined through the ROESY correlation of the glucose anomeric proton (4.41 ppm) to the anomeric and cinnamic acid ester protons on Rha-1 and the signal at 4.10 ppm on C-3 of Rha-2. On the basis of model building, the ROESY correlation from the glucose to the C-1 and C-2 signals on Rha-1 are only possible if the glucose is on C-3 of Rha-2. Establishing the proton at 4.10 ppm as the C-3 proton on Rha-2 allowed identification of the rest of the spin system through TOCSY experiments and established the C-2 ester proton associated with lauric acid to be at 5.16 ppm and the anomeric proton on Rha-2 at 5.13 ppm. The final regiochemistry to be determined was the location of the palmitic acid on Rha-3 (C-2 or C-3). COSY analysis showed a three-bond correlation from the palmitic acid ester proton at 5.38 ppm to the anomeric proton at Rha-3 at 4.94 ppm, thus establishing that the palmitic acid was bonded to C-2, and C-3 was a free hydroxyl. A ROESY correlation from the anomeric proton on Rha-3 to the C-2 proton on fucose at 3.62 ppm confirmed that Rha-3 and fucose were bonded at C-2 on the fucose. Figure 3 gives the important ROESY correlation used to establish the regiochemistry.

In addition to **6**, compound **7** was also isolated. Its spectral characteristics were very similar to **6** with the notable exception of the lack of a cinnamic acid residue. Acid hydrolysis of this compound produced the same sugars as **6** along with propanoic, lauric, and palmitic acids. Base hydrolysis resulted in the isolation of operculinic acid (**1**). On the basis of these observations it was concluded that **7** was structurally identical with **6** with an H in place of the cinnamate on Rha-1.

Conclusion

The crude organic extract of *I. leptophylla* showed 92% inhibition at 150 μg/mL against *M. tuberculosis* in vitro in the anti-tuberculosis assay performed by TAACF. Operculinic acid (**1**), **8**, and **7** showed no activity when tested in vitro. Compound **6** showed 13% inhibition when tested at 6.25 μg/mL. The potency of **6** is insufficient to warrant further biological assessment by TAACF (100% protection at 6.25 μg/mL is required for pure a compound). The bioassay results indicate that the cinnamic acid residue is required for the observed antimicrobial activity. Since **8** has a cinnamic acid and is inactive in the bioassay, we would hypothesize that the activity is also related to the solubility properties of the resin glycoside. Compound **8** is only slightly soluble in methanol, while **6** is very soluble. Upon the basis of the activity of the crude organic extract, it appears that there may be other minor metabolites that contribute to the extract's antimicrobial activity. Work on these compounds continues in this lab.

Both compounds **6** and **7** can be considered derivatives of the parent glycosidic acid operculinic acid (**1**), which has been previously isolated from *I. operculata*. Since **6** and **7** appear to come only from *I. leptophylla* and possess acids (cinnamic and propanoic) not previously identified in resin glycosides isolated from Convolvulaceae, we propose to refer to these compounds as leptophyllins A and B, respectively. This is consistent with the nomenclature previously established for resin glycosides isolated from Convolvulaceae.

Experimental Section

General Experimental Procedures. NMR experiments (UNI) were carried out on a Tecmag Aquerius (GE QE 300) at 300 MHz (^{1}H) or 75 MHz (^{13}C). HSQC and HMBC experiments were performed on a Varian VXR 500 MHz at MTDDP at the U.S. NCI Frederick, Maryland. TOCSY and ROESY experiments were performed on a Varian UnityInova 600 MHz NMR at the University of Kansas. MS/MS experiments were performed on a Micromass Q-TOF or Finnigan LCQ using nanoelectrospray for ionization at NIH, Bethesda, MD. Glycosides were dissolved in 1 mM sodium acetate in 70:30 methanol−water for nanoelectrospray, and spectra were acquired under conditions as previously described.[21] For linkage analysis, methylated samples were hydrolyzed in TFA, reduced with sodium borohydride, acetylated, and analyzed by GC−MS. Basic permethylation was by the method of Ciucanu and Kerek.[22]

High-resolution MS experiments were performed on a JEOL SX 102 (FAB) or a Bruker Biflex-lll MALDI/TOF mass spectrometer operating in reflectron mode. The matrix for laser desorption was α-cyano-4-hydroxycinnamic acid with 0.001 M sodium iodide in 1:1 acetonitrile−water. Accurate mass was determined from the sodium adduct of the primary isotope using bradykinin, angiotensin, and somatotropin as reference masses. GC/CI-MS were performed on a Finnigan 700 ion trap mass spectrometer using isobutane as the CI gas. IR data were obtained on a Galaxy 2020 FT-IR spectrometer. UV spectra

were obtained using a Shimadzu 2101 UV spectrometer. Optical rotations were obtained using a Rudolph DP781 polarimeter. HPLC chromatography was performed on an ISCO gradient HPLC with an ISCO V-4 UV detector or a Waters R401 RI detector. HPLC columns were Rainin Dynamax. All solvents were HPLC grade purchased from Fisher Scientific. Chemicals for permethylation were purchased from Aldrich. Flash silica was purchased from Fisher Scientific, and TLC plates were purchased from EM Science.

Biological Testing. Antimicrobial assays were performed by the Tuberculosis Anti-microbial Acquisition & Coordinating Facility in Frederick, MD. The crude plant extract was assayed against *Mycobacterium tuberculosis* H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system at an initial concentration of 150 μg/mL. Purified compounds were subsequently tested at an initial concentration of 6.25 μg/mL.

Plant Material. *I. leptophylla* was collected and identified by Hillary Loring at a location in western Kansas. Plant identification follows the Flora of the Great Plains (Great Plains Flora Association, 1986). A voucher specimen is archived at the R. L. McGregor Herbarium at the University of Kansas.

Extraction and Isolation. The air-dried leaves and stems of *I. leptophylla* (360 g) were extracted overnight with CH_2Cl_2−MeOH (1:1). The solvent was removed and the plant material extracted with MeOH for 12 h. The MeOH extract was combined with the CH_2Cl_2 extract and the solvents removed at reduced pressure to yield 17.0 g of crude organic extract. A small sample of this extract was assayed against *M. tuberculosis* through TAACF. This extract showed 92% growth inhibition at a test concentration of 150 μg/mL. The remaining plant material was then extracted with H_2O. This aqueous extract was freeze-dried and showed no activity against *M. tuberculosis*. The organic extract (7.5 g) was dissolved/suspended in 25 mL of CH_2Cl_2−MeOH (1:1) and filtered. This filtrate was placed on an LH-20 column (100 cm × 2.5 cm) packed and eluted in CH_2Cl_2−MeOH (1:1). Fractions were collected every 500 drops. Fractions showing similar composition (vide TLC) were combined and submitted for assay against *M. tuberculosis*. The most active fraction showed a 3-fold increase in activity (89% inhibition at 50 μg/mL). This fraction was adsorbed onto 2 g of silica and placed on top of a small flash silica column (50 mL syringe with 40 mL of dry silica). This column was eluted with 80 mL portions of $CHCl_3$; $CHCl_3$−MtBE (1:1); $CHCl_3$−MtBE−MeOH (50:45:5); and $CHCl_3$−MtBE−MeOH (50:25:25). The last fraction (0.2456 g) showed two major compounds when analyzed by TLC (silica; $CHCl_3$−MtBE−MeOH, 50:40:10). A UV-active spot (R_f = 0.50) and a UV-inactive spot (R_f = 0.42) were observed. Both spots turned a light brown when sprayed with sulfuric acid. The sample was dissolved in a minimal amount (300 μL) of $CHCl_3$−MtBE−MeOH (48:38:14), filtered (0.45 μm), injected onto the HPLC (50 μL/injection; 12 mL/min; Rainin Silica Dynamax 25 cm × 2.5 cm), and eluted with the dissolving solvent system. The eluent was monitored by both UV (λ = 254 nm) and RI. Along with minor peaks, a major peak showing a large UV response was collected at 10.5 min (**6**, 0.1133 g) and a second peak emerged after 15 min which gave only an RI response (**7**, 0.0592 g).

Spectral characterization of 6: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.79 (1H, d J = 15.5 Hz), 7.64 (2H, m), 7.40 (3H, m), 6.64 (1H, d J = 15.5), 5.52 (1H, t, J = 1.9), 5.36 (1H dd, J = 1.6, 3.2), 5.31 (1H s br), 5.11 (2H m), 5.02 (2H, m), 4.49 (1H d, J = 7.8), 4.37 (1H d, J = 7.5), 4.27 (1H dd, J = 9.1, 3.0), 4.17 (1H dd, J = 9.2, 3.2), 4.07 (1H dd, J = 9.7, 3.2), 3.9(5H m), 3.75 (1H t, J = 9.2), 3.62 (4H, m), 3.48 (2H, m), 3.40 (1H, t, J = 9.5), 3.25 (2H, m), 2.43 (6H, m), 1.80−1.45 (8H, m), 1.48−1.00 (50H, m), 0.98 (3H, t, J = 7.4), 0.93 (3H, t, J = 7.4); ^{13}C NMR (CD_3OD, 75 MHz) 177.8, 175.2, 175.1, 168.6, 147.4, 135.8, 131.6, 130.0 (2), 129.4 (2), 118.7, 105.3, 105.2, 100.5, 100.5, 98.9, 84.2, 82.0, 80.2, 79.89, 79.86, 78.21, 78.17, 75.1, 74.9, 74.7, 74.1, 73.9, 73.47, 73.3, 71.6, 71.4, 70.2, 69.4, 69.2, 68.7, 68.6, 63.2, 42.5, 35.2, 35.0, 34.9, 34.0, 33.3, 32.97, 30.74, 30.67, 30.56, 30.51, 30.38, 30.00, 29.0, 28.6, 28.5, 28.21, 28.1, 27.7, 26.1, 25.9, 25.7, 24.2, 23.62, 19.14, 18.92, 17.85, 17.1, 16.9, 14.4, 11.87; IR ($CDCl_3$) ν_{max} 3445, 2929, 2857, 1725, 1712 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 216(5.0), 223(4.9), 280(4.9) nm; $[\alpha]_D$ −6.6° ($CDCl_3$ c 0.18); HR-MS (FAB) found 1501.6372, calcd for $[C_{70}H_{112}O_{26}Cs]^+$ 1501.6464.

Hydrolysis and Derivatization of 6. Approximately 1 mg of **6** was dissolved in 0.5 mL of freshly distilled THF and 200 μL of 1.0 M NaOH. The solution was refluxed for 60 min while the reaction progress was monitored using silica TLC. Once it was established that no starting material remained, the reaction was cooled to room temperature and acidified with 1.0 M HCl. This solution was then extracted three times with 0.5 mL aliquots on MtBE. The MtBE extracts were combined and evaporated to dryness. The dried residue was dissolved in 180 μL of pyridine, 20 μL of trimethylchlorosilane, and 60 μL of hexamethyldisilazane and heated for 1 h at 70 °C. A GC/CI-MS analysis identified three acids, cinnamic, lauric, and propanoic, as their TMS esters. To verify the identities, standard samples of the three acids were purchased and treated as above.

The aqueous-THF layer from the initial base hydrolysis was evaporated to dryness and dissolved in 1.0 mL of fresh THF containing 150 μL of 1 N HCl. The sample was refluxed for 2 h, after which the solvent was removed and then treated as above to make the TMS ethers. A GC/CI-MS analysis identified glucose, fucose, and rhamnose as their TMS derivatives in a ratio of 1:1:3. The monosaccharide identities were determined by derivatizing purchased standards of the glucose, fucose, and rhamnose, treating as above, and comparing retention times. 11-Hydroxypalmitic acid was identified on the basis of its mass spectrum of the di-TMS derivative ($m/z - H^+ = 429$).

Butanolysis of 6. Following a procedure similar to that of Vliegenthart,[23] 2.5 mg of **6** was dissolved in 200 μL of (*S*)-(+)-2-butanol (Aldrich) and made acidic by bubbling HCl gas into the solution. The solution was heated for 7 h to 70 °C under nitrogen with continuous stirring. After cooling to room temperature, 20 mg of $Ag_2CO_3 \cdot 2H_2O$ was added to neutralize the solution. This mixture was then triturated and centrifuged. The supernatant was placed in a vial and stored over P_2O_5 for 14 h. The remaining solution was removed from the drying agent and treated with 100 μL of Sylon HTP (Supelco) to make the TMS ethers. The same procedure was repeated for D-glucose, D-fucose, and L-rhamnose. The butanolysis-TMS derivatives of **6** were run through a capillary GC (HP-1; 5:00 min at 200−270 °C at 30 °C/min; 1.25 mL/min). These retention times were compared to those for the (*S*)-(+)-2-butanol derivatives synthesized from D-glucose, D-fucose, and L-rhamnose, thus confirming the absolute stereochemistry of the carbohydrates.

Base Hydrolysis of 6. A sample of **6** (31.1 mg) was dissolved in a 1:1 mixture of H_2O−EtOH, treated with 10 μL of 0.5 M NaOH, and stirred at room temperature for 30 min. The solution was then acidified with acetic acid. The solvent was removed at reduced pressure and the remaining residue placed on a small C_{18} flash column, which was first eluted with two column volumes of H_2O followed by excess methanol. The methanol was removed at reduced pressure and the remaining residue filtered and further separated on an HPLC using a C_{18} (Rainin 25 cm × 1.0 cm; 3.5 mL/min; RI) semipreparative column eluted with methanol−water (75:25). A peak eluting at 7.2 min corresponding to the glycosidic acid was isolated (7.6 mg). This compound was then treated with diazomethane to make the methyl ester of operculinic acid, **1**: (HR-MALDI-TOF-MS) m/z 1055.5243 (calcd for $C_{47}H_{84}O_{24}-Na^+$ 1055.5226; mp 165−167 (dec); $[\alpha]_D$ −57° (MeOH; c 0.18). All other spectral data were consistent with that reported for **1**.[8]

Spectral characterization of 7: ^{1}H NMR (300 MHz, CD_3OD) δ 5.55 (1H t, J = 2.0), 5.30 (1H d, J = 1.5), 5.14 (1H dd, J = 2.0, 3.0), 5.11 (1H d, J = 1.4), 5.05 (1H d, J = 1.8), 4.97 (1H t J = 9.0), 4.48 (1H d, J = 7.4), 4.35 (1H d, J = 7.0), 4.25 (1H dd J = 3.2, 8.45), 4.10 (2H m), 3.89 (5H m), 3.76 (2H dd, J = 10.7, 3.2), 4.10 (2H m), 3.90 (5H m), 3.76 (1H dd J = 9.4, 3.3), 3.7−3.30 (11H, unresolved), 3.22 (2H m), 2.43 (6H, m), 1.80−1.45 (8H, m), 1.48−1.00 (50H, unresolved), 0.98 (3H, t, J = 7.4), 0.93 (3H, t, J = 7.4); ^{13}C NMR (75 MHz, CD_3OD) δ 178.0, 175.1 (2), 105.5, 105.2, 103.2, 102.8, 100.3, 98.9, 84.1,

82.2, 80.8, 80.2, 79.3, 78.4, 78.1, 75.4, 74.2, 74.0, 73.8, 73.3, 72.4, 71.8, 71.4, 70.6, 70.2, 69.4, 69.2, 68.6, 63.2,42.6, 35.2, 35.1, 35.0, 34.1, 33.2, 33.0, 30.7, 30.6, 30.54, 30.50, 30.3, 29.0, 28.7, 28.6, 28.3, 28.2, 27.7, 26.1, 25.9, 25.7, 24.3, 23.62, 23.60, 19.1, 18.8, 17.8, 17.0, 16.9, 14.3, 11.8; HRMS (MALDI-TOF) 1261.6858 calcd for $[C_{61}H_{106}O_{25}Na]^+$ 1261.6891; $[\alpha]_D$ −5.5° (MeOH; *c* 0.18); IR (neat NaCl) ν_{max} 3443, 2972, 2856, 1725 cm^{-1}.

Synthesis of 8. Following a procedure reported by Prehm,[20] a sample of **6** (10.1 mg) was dissolved in 1 mL of trimethyl phosphite, 150 μL of 1,5-di-*tert*-butylpyridine, and 100 μL of methyl trifluoromethanesulfonate. The sample was stirred for 2 h at 55 °C. The entire sample was then placed on an LH-20 lipophilic Sephadex column (25 cm × 2.5 cm) and eluted with 1:1 CH_2Cl_2−MeOH. The column eluent was monitored with a UV detector. The first eluting peak contained the permethylated product as well as unreacted starting material. This peak was then eluted on an HPLC using an amino-bonded silica column (Rainin) using an eluent of 1:1 hexane−$CHCl_3$. The peak eluting at 5.2 min with a flow rate of 4.7 mL/min was identified as the permethyl product (2 mg). The process was repeated until 15 mg of the permethylated product was isolated and purified. HR-MS-(MALDI-TOF) 1503.8349, calcd for $[C_{78}H_{128}O_{26}Na]^+$ 1503.8555. All ^{1}H and ^{13}C NMR data are reported in Table 1.

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