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Application of molecular mechanics in the total stereochemical elucidation of spicigerolide, a cytotoxic 6-tetraacetyl-oxyheptenyl-5,6-dihydro-α-pyrone from *Hyptis spicigera*☆

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Abstract—Bioactivity-directed fractionation of the crude extract prepared from the medicinal Mexican plant *Hyptis spicigera* (Lamiaceae) tested on KB cells led to the isolation of spicigerolide (**1**). The structure for this novel cytotoxic compound was elucidated as 6*R*-[3*S*,4*S*,5*S*,6*S*-tetraacetyloxy-1*Z*-heptenyl]-5,6-dihydro-2*H*-pyran-2-one. The relative stereochemistry of this flexible molecule was determined by a combination of molecular mechanics calculations and $^{1}H-^{1}H$ coupling constant data, while the absolute configuration was established according to CD measurements. The MM/$^{3}J_{H-H}$ calculations, as applied to **1**, was validated with model linear compounds prepared from L-rhamnose: 2,3,4,5-tetra-*O*-acetyl-6-deoxy-L-mannose (**5**) and tetra-*O*-acetyl-1,6-dideoxy-L-mannitol (**8**). Both compounds possess the same stereochemistry predicted to be present in the acyclic moiety of spicigerolide (**1**) but lacking the stereochemical influence of the chiral pyrone.

1. Introduction

Polyoxygenated 6-heptenyl-5,6-dihydro-α-pyrones occur in several members of the plant family Lamiaceae[1,2] although their distribution among the genus *Hyptis* seems to be restricted[3–6] to species belonging to the *Mesosphaeria* section.[7] Many of these compounds have displayed antimicrobial, antifungal and phytotoxic activities,[1,2] as well as cytotoxicity[8] against human tumor cells.

Hyptis spicigera, a herbaceous member of the *Mesosphaeria* section with a pantropical distribution,[7] is used in traditional Mexican medicine for the treatment of gastrointestinal disturbances, skin infections, as well as wounds and insect bites. The insecticidal efficacy of this weed has also been demonstrated in agriculture.[9] Preceding this report, a 1993 chemical study on the aerial parts of this plant[10] described the isolation of a 5,6-dihydro-α-pyrone named spicigera lactone whose stereochemistry was not established. After comparing the NMR data and the melting point evidence for 5-deacetoxy-5′-epiolguine, the cytotoxic constituent of *H. oblongifolia*,[3] it would seem that the two compounds are apparently the same. Recently, the chemical investigation of the aerial parts of *H. spicigera* guided by a bioassay that tested for toxicity on the European corn borer larvae allowed us to trace the insecticidal activity to a fraction containing a rich mixture of new labdane diterpenes.[9]

In our ongoing investigations directed toward the discovery of bioactive constituents from traditionally used Mexican plants,[8] it was found that the crude extract of *H. spicigera* was also cytotoxic (ED_{50}=18.5 μg/ml) when tested in the in vitro human nasopharyngeal carcinoma (KB) assay system. Subsequent bioactivity-directed fractionation of the active extract tested on KB cells led to the isolation of a new 6-heptenyl-5,6-dihydro-α-pyrone as the major cytotoxic principle (ED_{50}=1.5 μg/mL), which was named spicigerolide (**1**). In this paper, we describe the application of molecular mechanics calculations and $^{1}H-^{1}H$ coupling constant analysis (MM/$^{3}J_{H-H}$) used in the stereochemical elucidation of spicigerolide (**1**), which resulted in the prediction of the relative configuration for the five stereogenic centers. Two chiral compounds, 2,3,4,5-tetra-*O*-acetyl-6-deoxy-L-mannose (**5**) and tetra-*O*-acetyl-1,6-dideoxy-L-mannitol (**8**), were prepared from L-rhamnose in order to obtain simple flexible models with the same relative stereochemistry predicted to be present in the acyclic moiety of spicigerolide (**1**) but lacking the stereochemical influence of the chiral 5,6-dihydro-α-pyrone nucleus. The remarkable correlation

☆ Taken in part from the PhD thesis of Mabel Fragoso-Serrano.

Keywords: pyrones; molecular mechanics; NMR; stereochemistry; configuration; biologically active compounds; plants; *Hyptis spicigera*.

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Table 1. J_{H-H} values for the minimized conformers of **1** vs **2**

Conformer[a]	**1**					**2**				
	E_{MMX}[b]	$n\times10^3$	$n\,J_{3'-4'}$[c]	$n\,J_{4'-5'}$[c]	$n\,J_{5'-6'}$[c]	E_{MMX}[b]	$n\times10^3$	$n\,J_{3'-4'}$[c]	$n\,J_{4'-5'}$[c]	$n\,J_{5'-6'}$[c]
PPP	20.34	0.002	0.000002	0.000017	0.000003	21.80	0.082	0.000116	0.000630	0.000174
PPA	18.95	0.018	0.000032	0.000181	0.000148	22.34	0.034	0.000060	0.000344	0.000292
PPM	17.22	0.326	0.000388	0.003110	0.000767	20.48	0.764	0.000817	0.007250	0.001612
PAP	18.19	0.064	0.000191	0.000217	0.000173	21.81	0.082	0.000226	0.000299	0.000241
PAA	16.01	2.520	0.008250	0.007830	0.023400	19.67	3.050	0.009028	0.010523	0.028243
PAM	19.76	0.005	0.000013	0.000019	0.000004	23.44	0.005	0.000014	0.000023	0.000005
APP	18.30	0.053	0.000484	0.000498	0.000048	21.85	0.077	0.000674	0.000725	0.000070
APA	15.82	3.470	0.030800	0.028400	0.030500	18.80	13.244	0.115885	0.107806	0.116150
APM	16.38	1.350	0.012600	0.012500	0.003100	19.70	2.900	0.026651	0.027086	0.006496
AAP	15.40	7.050	0.066400	0.024700	0.025000	19.95	1.900	0.017879	0.007296	0.006270
AAA	13.16	309.104	2.910000	1.070000	2.870000	18.47	23.118	0.208987	0.076058	0.214535
AAM	17.71	0.143	0.001340	0.000601	0.000126	21.78	0.087	0.000816	0.000344	0.000079
AMA	12.78	587.056	5.530000	0.704000	5.330000	16.82	374.563	3.520892	0.644248	3.438488
AMM	14.65	24.993	0.235000	0.006500	0.058200	18.85	12.172	0.105554	0.007067	0.021649
MPP	18.44	0.042	0.000124	0.000418	0.000052	18.82	12.805	0.031243	0.120106	0.011524
MPA	15.11	11.500	0.025600	0.113000	0.106000	17.82	69.254	0.136430	0.700157	0.642676
MPM	15.17	10.400	0.023000	0.103000	0.019800	20.72	0.518	0.000098	0.000529	0.000099
MAP	20.20	0.002	0.000003	0.000016	0.000003	23.76	0.003	0.000004	0.000024	0.000004
MAA	15.82	3.470	0.005830	0.015700	0.032200	18.76	14.169	0.015586	0.055402	0.131348
MAM	20.18	0.002	0.000004	0.000018	0.000005	24.58	0.001	0.000001	0.000006	0.000002
MMA	14.44	35.600	0.098300	0.028100	0.325000	16.71	450.975	0.820776	0.423917	4.144466
MMM	15.94	2.830	0.008040	0.000680	0.008190	18.55	20.197	0.045241	0.003433	0.046857
Total	–	1000.000	8.956401	2.119505	8.832719	–	1000.000	5.056978	2.193273	8.811280

[a] Descriptors are based on initial dihedral angles +60°(*P*), 180°(*A*) and −60°(*M*) for the C(3′)–C(4′)–C(5′)–C(6′) fragment.
[b] In kcal/mol.
[c] Calculated $^3J_{H-H}$ values in Hz.

between the molecular mechanics calculated coupling constants and the experimentally registered values obtained for synthesized models was used to further support the application of this methodology for the stereochemical elucidation of polyoxygenated linear compounds, as **1**.

2. Results and discussion

Spicigerolide (**1**) exhibited a molecular formula of $C_{20}H_{26}O_{10}$ based on its HREIMS data. The UV (λ_{max} 208 nm) and IR (ν_{max} 1740 cm^{-1}) spectra were in accord with the presence of an α,β-unsaturated-δ-lactone.[1] The NMR data indicated that compound **1** has a structure similar to those of synrotolide[11] and related 6-heptenyl-5,6-dihydro-α-pyrones from the Lamiaceae family.[1,2] In particular, the ^{13}C NMR spectrum, assisted by $^1H,^1H$- and $^1H,^{13}C$-COSY techniques, was in full agreement with the presence of a 3,4,5,6-(tetraacetyloxy)-1-heptenyl moiety at C-6. The discernible 10.5 Hz coupling constant for the two olefinic protons at C-1′ and C-2′ demonstrated the *cis* configuration of the side chain double bond.[1,6] The characteristic coupling constant values between the methylene protons at C-5 and H-6 ($J_{5ax\text{-}6}$=11.0 Hz; $J_{5eq\text{-}6}$=4.5 Hz) indicated the pseudo-equatorial orientation of the side chain.[1,12] The stereochemical elucidation of spicigerolide (**1**) was determined by taking into account the following considerations: (1) The positive sign of the CD curve ($\Delta\epsilon_{256}$=+2.8) provided evidence for an (*R*)-configuration in the stereogenic center of the pyrone nucleus (C-6)[1,2] according to Snatzke's rule;[13] (2) The application of molecular mechanics and vicinal coupling constant calculations (MM/$^3J_{H-H}$) to establish the relative configuration in the acyclic portion.[14,15]

Due to the fact that the proton spin system attached to C(3′)–C(4′)–C(5′)–C(6′) of spicigerolide (**1**) was not of first order, the spectral simulation method was employed to obtain accurate values for the observed coupling constants with a root mean square error of 0.17 Hz for the fitting of experimental and simulated spectra. The experimental coupling constant data (J_{obs}) for the antiparallel ($J_{3'-4'}$=9.0 Hz, $J_{5'-6'}$=8.7 Hz) and gauche oriented protons ($J_{4'-5'}$=2.1 Hz) were in accord with a planar, zigzag arrangement[16] of the side chain. The comparison of vicinal coupling constants for the side chain (H-3′ through H-6′) with peracetylated hexa-alditol models was used as the initial criterion to select the proper diastereoisomer from among the eight possible hexose configurations.[17] This approach revealed the close similarity for the $^3J_{H-H}$ values of spicigerolide (**1**) and model peracetyl derivatives of mannose,[15,17] e.g. 2*R*,3*R*,4*R*,5*R*-hexitol hexaacetate (J_{2-3}=J_{4-5}=9.2 Hz; J_{3-4}=2.4 Hz), which suggested that the 6-deoxy-mannose (rhamnose) configuration must be present in the side chain of spicigerolide. The establishment of an (*R*)-configuration for the C-6 in the pyrone nucleus indicated that the stereochemistry for the side chain in the natural product could be either diastereoisomer **1** (6*R*,3′*S*,4′*S*,5′*S*,6′*S*) or **2** (6*R*,3′*R*,4′*R*,5′*R*,6′*R*). These structures were analyzed by MM calculations and their minimum energy conformers were calculated after considering the following premises:

1. The number of possible conformers were established using a systematic search procedure by varying 120° each of the torsion angles around the C(3′)–C(4′)–C(5′)–C(6′) fragment in the side chain, and all possible combinations of staggered arrangements[18] were considered, i.e. *gauche*^{P} (dihedral angle of +60°), *anti*^{A} (180°) and *gauche*^{M} (−60°) (Table 1). Conformers with at least one forbidden *gauche*^{P}–*gauche*^{M} sequence[18] which

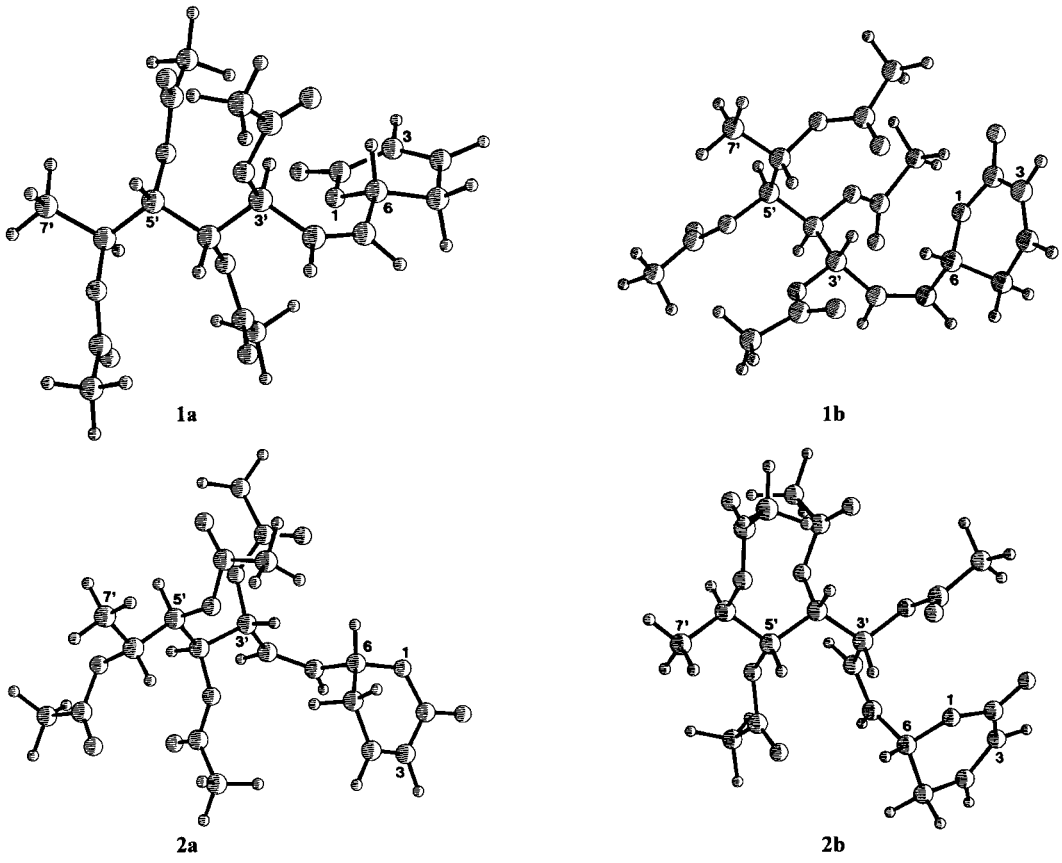
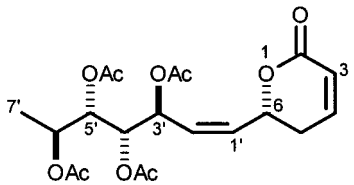


Figure 1. Major molecular mechanics minimum energy conformers of spicigerolide (**1**) vs its hypothetical diastereoisomer (**2**).

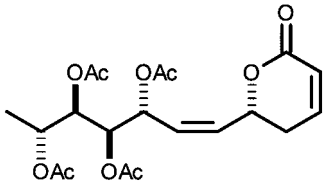
resulted in O//O 1,3 interactions were excluded,[15] i.e. *P–M* for the C(3′)–C(4′)–C(5′) fragment and/or *M–P* for the C(4′)–C(5′)–C(6′).

2. The conformation for the acetyloxy moieties was adjusted to the most favorable *anticlinal*[15,19] geometry prior to the minimization procedure but was left without any geometry restriction during the calculations.
3. The initial dihedral angles H(6)–C(6)–C(1′)–H(1′) and H(2′)–C(2′)–C(3′)–H(3′) were set at −152° and +147° in agreement with the observed coupling constants $J_{6\text{-}1'}$=9.5 Hz and $J_{2'-3'}$=9.0 Hz, respectively.
4. The pseudo-chair conformation with C-6 at the flap was the starting geometry for the 5,6-dihydro-α-pyrone moiety according to X-ray analysis of related compounds.[12]

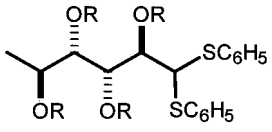
The conformation analysis using the above mentioned systematic search procedure[20] allowed a total number of 22 conformers to be geometry-optimized (Table 1). For diastereoisomer **1** (6*R*,3′*S*,4′*S*,5′*S*,6′*S*), two minimum



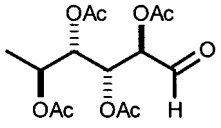
1



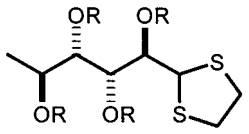
2



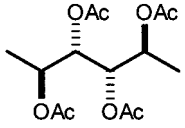
3 : R = H
4 : R = Ac



5



6 : R = H
7 : R = Ac



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energy conformers (Fig. 1) represented 90% of the total population in contrast with the previously reported even conformational distribution associated to flexible molecules in solution.[15] The most stable conformation corresponded to **1a** (*AMA*, 58.7%) with an E_{MMX}=12.78 kcal/mol. The second minimum conformation was found to be **1b** (*AAA*, 30.9%) and its calculated E_{MMX}=13.16 kcal/mol. Both conformers defined extreme values for the calculated vicinal couplings (among all the protons on the chiral centers of the side chain) which resulted from the antiperiplanar disposition for $H_{3'}$–$H_{4'}$ (J_{calc}=9.0 Hz) and $H_{5'}$–$H_{6'}$ (8.8 Hz), as well as the synclinal arrangement for $H_{4'}$–H5′ (2.1 Hz). In contrast, the 6*R*,3′*R*,4′*R*,5′*R*,6′*R* configuration in diastereoisomer **2** induced an increment in the conformational dispersion of this molecule (Table 1). In this case, the contribution of the two minimum energy conformers stood only for 82% of the whole population (Fig. 1). Furthermore, *AMA* conformation (**2a**, 37.4%, E_{MMX}=16.82 kcal/mol) did not represent the major contributor as calculated for **1** since it shared an almost even distribution with the *MMA* conformer (**2b**, 45%, E_{MMX}=16.71 kcal/mol). This situation provoked a decrement in the calculated value for the $H_{3'}$–$H_{4'}$ coupling constant (J_{calc}=5.1 Hz) which deviated significantly from the value obtained for **1** (J_{obs}=9.0 Hz) as expected from the stereochemical influence exerted by the chiral pyrone nucleus over the equilibrium among the three C(3)–C(4) rotamers. The biogenetic consideration that all 6-heptenyl-5,6-dihydro-α-pyrones isolated from the Lamiaceae possess an (*S*)-configuration[21] for the stereogenic center at C-6′ was in agreement with the correspondence between the molecular mechanics calculated coupling constant for diastereoisomer **1** and the measured values for spicigerolide. Therefore, the structure for this biodynamic compound was elucidated as 6*R*-[3*S*,4*S*,5*S*,6*S*-tetraacetyloxy-1*Z*-heptenyl]-5,6-dihydro-2*H*-pyran-2-one (**1**).

In order to validate this approach, the MM/$^3J_{H-H}$ calculations were applied to the synthesized compounds **5** and **8**. These linear substances were prepared following previously reported procedures[16,22,23] and both represented new derivatives of L-rhamnose. The conformational analysis revealed that the minimum energy conformer for **5** and **8** corresponded to *AMA* which represented 80% of the total population and the same planar zigzag conformation adopted by the side chain of spicigerolide (**1**) was confirmed from the experimentally registered $^3J_{H-H}$ values for both flexible acyclic compounds. Extreme values were recorded for the antiperiplanar oriented H_2–H_3, (J_{obs}=8.0 Hz; J_{calc}=8.5 Hz) and H_4–H_5 (J_{obs}=8.5 Hz; J_{calc}=8.7 Hz), as well as the synclinal disposed H_3–H_4 (J_{obs}=2.5 Hz; J_{calc}=1.9 Hz) in model aldehyde **5** (E_{MMX}=−2.91 kcal/mol). The same trend was observed for compound **8** (E_{MMX}=−0.51 kcal/mol) with calculated values of J_{2-3}=J_{4-5}=7.9 Hz (J_{obs}=7.7 Hz) and J_{3-4}=3.0 Hz (J_{obs}=3.4 Hz). The experimental coupling constant values for this symmetrical linear substance were measured under irradiation of the methyl group signal and employing the spectral simulation method. The high preference for the *AMA* rotamer in the whole population of **8** as opposed to the conformational dispersion previously described for peracetyl alditols (e.g. 2*R*,3*R*,4*R*,5*R*-hexitol hexaacetate)[15] is a result of the conformer equivalence obtained by substitution of the terminal acetyloxy functionality by a methyl group. The correlation between the molecular mechanics calculated vicinal coupling constants and the observed ones for the synthesized models was remarkable, as it was found for the 6-tetraacetyloxyheptenyl-5,6-dihydro-α-pyrone **1**. In this way, the application of this approach to the stereochemical elucidation of spicigerolide (**1**) was validated. Finally, this study represents a relevant example of the potentiality associated with this approach for determining the stereochemistry in polyhydroxylated linear natural products where a limited amount of material for an isolated bioactive principle might preclude the availability of suitable crystals for X-ray analysis,[11,12] or the alternative use of chiral chemical methods (e.g. preparation of Moshers' esters)[24,25] and degradative correlations.[6,11]

3. Experimental

3.1. General methods

Melting point determinations were performed on a Fisher-Johns apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-360 digital polarimeter. CD spectrum was registered on a JASCO 720 spectropolarimeter at 25°C. NMR spectra including COSY and HMQC experiments[26] were recorded on a Varian Unity Plus 500, a Varian XL300GS, or a Bruker DMX500. Spectral simulation was achieved using the Varian spectrometer software as implemented by the manufacturer. FABMS were recorded on a JEOL DX300 mass spectrometer in the positive mode using NBA as the matrix. EIMS data were obtained on a JEOL JMS-AX505HA mass spectrometer. Open column chromatography: Si gel 60 (70–230 mesh, Merck). TLC: Si gel 60 F_{254} (Merck).

3.2. Molecular modeling calculations

An exhaustive minimization procedure using molecular mechanics[27] was achieved for each conformer using the MMX force field as implemented in the PCMODEL program V 6.00 (Serena Software, Bloomington, IN 47402-3076). A cyclic equilibrium at 298 K between the 22 selected conformers included in Table 1 was assumed,

which yielded $K_{1,2}=n_2/n_1$, $K_{2,3}=n_3/n_2,\ldots K_{22,1}=n_1/n_{22}$ and $n_1+n_2+n_3+\cdots+n_{22}=1$.

Taking the Gibbs free energy equation $\Delta G = -RT\ ln\ K$ and considering $\Delta S\cong 0$ and $\Delta G\cong\Delta H_f\cong\Delta E_{MMX}$,[28] the molecular mechanic energy E_{MMX} was used to obtain the population for each conformer n_i by solving the following set of equations:

$$n_1 = n_2/\exp[(E_2 - E_1)/-RT]$$

$$n_3 = n_2/\exp[(E_2 - E_3)/-RT]$$

$$n_4 = n_2/\exp\{[(E_2 - E_3)/-RT][(E_3 - E_4)/-RT]\}$$

$$n_5 = n_2/\exp\{[(E_2 - E_3)/-RT][(E_3 - E_4)/-RT] \times[(E_4 - E_5)/-RT]\}$$

$$\vdots$$

$$n_{22} = n_2/\exp\{[(E_2 - E_3)/-RT][(E_3 - E_4)/-RT][(E_4 - E_5)/-RT]\ldots[(E_{21} - E_{22})/-RT]\}$$

Conversions from dihedral angles to vicinal coupling constants ($^3J_{H-H}$) for each conformer were done using the Altona equation.[29] The population-weighted average coupling constant for each H–C–C–H dihedral fragment was calculated with $^3J_{calc}=n_1J_1+n_2J_2+\cdots+n_{22}J_{22}$ (Table 1).

3.3. Cytotoxicity assay

Human nasopharyngeal carcinoma (KB) cells were maintained in RMPI 1640 (10×) medium with 10% fetal bovine serum and cultured at 37°C in an atmosphere of 5% CO_2 in air (100% humidity). The cells at log phase of their growth cycle were treated in triplicate at various concentrations of the test samples (0.16–20.0 μg/mL), and incubated for 72 h at 37°C in a humidified atmosphere of 5% CO_2. The cell concentration was determined by the sulforhodamine B method.[30] Results were expressed as the dose that inhibits 50% control growth after the incubation period (ED_{50}). Ellipticine was included as a positive drug control: ED_{50} 0.4 μg/mL.

3.4. Isolation and purification of spicigerolide (1)

Defatted aerial parts of *H. spicigera* (788 g) were extracted[9] by maceration with $CHCl_3$–MeOH (1:1) at room temperature. After filtration, the solvent was removed under vacuum to yield 55 g of a dark-green residue (KB, ED_{50} 18.5 μg/mL). The extract was fractionated by Si gel column chromatography (450 g) using a gradient of Me_2CO–MeOH in $CHCl_3$. Seventy fractions (200 mL each) were collected. Combined fractions 23–37 (6 g), eluted from the original column with $CHCl_3$–Me_2CO (2:3), were found to concentrate the cytotoxic activity (KB, ED_{50} 10.5 μg/mL). The active eluates were further rechromatographed over Si gel (200 g) using the same solvent system and collecting fractions of 50 mL each. Cytotoxic activity was identified with a single compound in subfractions 78–82 (200 mg; KB, ED_{50} 2.3 μg/mL) which was purified by preparative TLC on Si gel, using *n*-hexane–EtOAc (3:2) as eluent (R_f=0.48). This major TLC band afforded 12 mg of **1** (KB, ED_{50} 1.5 μg/mL).

3.4.1. Spicigerolide (1). Oil: CD (*c* 0.06, MeOH) $\Delta\epsilon$ (nm) 0 (310)+2.8 (256), +1.2 (246), 0 (239), −1.2 (230), +17.1 (204). IR ($CHCl_3$) ν_{max} 1740, 1720, 1630, 1374, 1266, 1238, 1026, 820 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.90 (ddd, *J*=9.7, 5.2, 2.5 Hz, H-4), 6.06 (ddd, *J*=9.7, 2.5, 1.3 Hz, H-3), 5.79 (ddd, *J*=10.5, 9.5, 0.5 Hz, H-1′), 5.49 (ddd, *J*=10.5, 9.0, 1.0 Hz, H-2′), 5.40 (ddd, *J*=9.0, 9.0, 0.5 Hz, H-3′), 5.37 (dd, *J*=9.0, 2.1 Hz, H-4′), 5.35 (dddd, *J*=11.0, 9.5, 4.5, 1.0 Hz, H-6), 5.30 (dd, *J*=8.7, 2.1 Hz, H-5′), 4.96 (dq, *J*=8.7, 6.2 Hz, H-6′), 2.52 (dddd, *J*=18.5, 5.2, 4.5, 1.3 Hz, H-5eq), 2.35 (dddd, *J*=18.5, 11.0, 2.5, 2.5 Hz, H-5ax) 2.12 (s, 6H), 2.04 (s, 3H), 2.03 (s, 3H), 1.19 (d, *J*=6.2 Hz, CH_3-7′); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.0, 169.9 (×2), 169.8, 163.7 (C-2), 144.8 (C-4), 132.8 (C-1′), 128.6 (C-2′), 121.5, (C-3), 73.7 (C-6), 71.0 (C-5′), 69.2 (C-4′), 66.9 (C-6′), 66.3 (C-3′), 29.2 (C-5), 21.0 (×2), 20.9, 20.7; EIMS (20 eV) *m/z* (rel. int.) $[M]^+$ 426 (7.5), $[M-C_2H_4O_2]^+$ 366 (27.1), $[M-C_2H_4O_2-C_2H_2O]^+$ 324 (3.6), $[M-2C_2H_4O_2-C_2H_2O]^+$ 264 (8.5), $[M-3C_2H_4O_2-C_2H_2O]^+$ 204 (18.0), $[M-C_{10}H_{15}O_6]^+$ 195 (5.0), 178 (30.3), 154 (28.2), 153 (57.7), $[M-C_{10}H_{15}O_6-C_2H_4O_2]^+$ 135 (48.0), 134 (39.0), 128 (30.8), 107 (24.5), $[M-C_{15}H_{21}O_8]^+$ 97 (30.0), 85 (35.0), 81 (29.3), 71 (37.3), $[M-C_{15}H_{21}O_8-CO]^+$ 69 (28.1), $[C_4H_4O]^+$ 68 (27.3), 58 (40.4), 57 (44.1), 43 (100.0); HREIMS *m/z* 426.1528 (calcd for $C_{20}H_{26}O_{10}$, 426.1526).

3.4.2. 6-Deoxy-L-mannose diphenyldithioacetal (3). A mixture of L-rhamnose (500 mg) and benzenethiol (1.5 mL) in 90% trifluoroacetic acid (5 mL) was refluxed at 55°C for 1 h.[22] The reaction mixture was evaporated to dryness under an Ar flow and the residue was purified by column chromatography on Si gel (100 g). Elution with CH_2Cl_2–MeOH (9:1) afforded the diphenyldithioacetal derivative **3**[16] (R_f=0.43), as the major product (515 mg, 51%). Colorless solid; mp 124–126°C; ORD (*c* 2.91, MeOH) $[\alpha]_{589}$= +48.8°, $[\alpha]_{578}$=+51.6°, $[\alpha]_{546}$=+59.8°, $[\alpha]_{436}$=+119.2°, $[\alpha]_{365}$=+228.9°; ^1H NMR (300 MHz, C_5D_5N) δ 7.81–7.78, 7.56–7.54, 7.22–7.09, 6.04 (brs, H-1), 5.27 (brd, *J*=9.3 Hz, H-3), 5.07 (brd, *J*=9.3 Hz, H-2), 4.55 (m, H-4 and H-5); 1.70 (d, *J*=6.1 Hz, H-6); ^{13}C NMR (75.5 Hz, C_5D_5N) δ 136.1, 135.6, 130.0 (×2), 129.6 (×2), 129.0 (×2), 128.9 (×2), 126.5, 126.4, 73.3 (C-4), 72.3 (C-2), 69.4 (C-3), 66.4 (C-5), 61.5 (C-1), 21.0 (C-6); EIMS (20 eV) *m/z* (rel. int.) $[M]^+$ 366 (0.7), $[M-C_6H_5S]^+$ 257(44.6), $[M-C_6H_6S]^+$ 256 (60.4), $[M-C_6H_5S-H_2O]^+$ 239 (19.6), 231 (27.9), 177 (19.4), 153 (66.8), 152 (17.2), $[M-C_6H_5S-C_6H_6S]^+$ 147 (100.0), $[C_{13}H_{11}S_2]^+$ 135 (36.2), 129 (26.2), 123 (37.9), 119 (29.4), 110 (20.6), 109 (12.3), 91 (17.2), 85 (20.0), 79 (23.3), 75 (26.6), 61 (13.4), 45 (37.3). HREIMS (70 eV) *m/z* 366.0960 (calcd for $C_{18}H_{22}O_4S_2$, 366.0960).

3.4.3. Tetra-*O*-acetyl-6-deoxy-L-mannose diphenyldithioacetal (4). Compound **3** (440 mg) was dissolved in AcCl (10 mL), stirred at room temperature for 2 h and evaporated under a N_2 flow. Column chromatography on Si gel (75 g;

n-hexane–EtOAc, 9:1) yielded 206 mg (32%) of product **4** (R_f=0.46),[16] as the major product. Oil; ORD (*c* 1.65, $CHCl_3$) $[\alpha]_{589}$=+20.0°, $[\alpha]_{578}$=+21.2°, $[\alpha]_{546}$=+25.5°, $[\alpha]_{436}$=+53.9°, $[\alpha]_{365}$=+104.2° (365); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58–7.56, 7.35–7.26, 5.88 (dd, *J*=8.5, 2.0 Hz, H-3), 5.34 (dd, *J*=8.5, 3.0 Hz, H-2), 5.21 (dd, *J*=8.5, 2.0 Hz, H-4), 4.85 (dq, 8.5, 6.5 Hz, H-5), 4.38 (d, *J*=3.0 Hz, H-1), 2.06 (s, 3H), 2.01 (s, 3H), 1.98 (s, 6H), 1.17 (d, *J*=6.5 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.2, 170.0, 169.6, 169.4, 134.0, 133.7, 133.2 (×4), 129.0 (×4), 128.2 (×2), 71.3 (C-2), 71.2 (C-4), 68.8 (C-3), 67.2 (C-5), 61.4 (C-1), 21.1, 20.7, 20.6, 20.5, 16.4 (C-6); EIMS (20 eV) *m/z* (rel. int.) $[M]^+$ 534 (0.9), $[M-C_6H_5S]^+$ 425 (77.3), $[M-C_6H_5S-C_2H_4O_2]^+$ 365 (26.0), 111 (15.3), $[C_6H_6S]^+$ 110 (47.0), $[C_6H_5S]^+$ 109 (12.4), 87 (14.5), 85 (95.9), 71 (100.0), 59 (43.9), 58 (11.1), 57 (46.9), 55 (20.5), 45 (28.0), 43 (44.7), 41 (26.2), 31 (12.1), 29 (17.2); positive FAB-MS *m/z* (rel. int.) [M+H] 535 (10.0), 534 (8.0), [M+H−60]+ 475 (4.0), 425 (100), 365 (40), 323 (25), 263 (20), 221 (90), 203 (55); positive HRFAB-MS *m/z* 535.1468 $[M+H]^+$ (calcd for $C_{26}H_{31}O_8S_2$, 535.1460).

3.4.4. 2,3,4,5-Tetra-*O*-acetyl-6-deoxy-L-mannose (5). Derivative **4** (28 mg) dissolved in acetone (2 mL) was added to a solution of *N*-bromosuccinimide (140 mg) in ice-cold 97% aqueous acetone (10 mL), and the mixture was stirred for 90 min at −2°C.[23] Finely ground $Na_2S_2O_3{\cdot}5H_2O$ (96 mg) and $NaHCO_3$ (33 mg) were added, and stirring continued for 30 min at room temperature. Salts were filtered off and the filtrate was evaporated under an Ar flow. The residue was dissolved in $CHCl_3$, and the solution washed with H_2O, dried with Na_2SO_4, and evaporated to dryness, The crude reaction mixture was submitted to column chromatography on Si gel (5 g; *n*-hexane–EtOAc, 7:3) impregnated with 25% H_2O (w/w), collecting fractions of 5 mL. The reaction product was recovered from fractions 9–14 and further purified by column chromatography on Si gel (CH_2Cl_2–MeOH, 99:1) to afford 7.7 mg (44%) of aldehyde **5** (R_f=0.34; CH_2Cl_2–MeOH, 19:1). Oil; ORD (*c* 0.47, $CHCl_3$) $[\alpha]_{589}$= −1.9, $[\alpha]_{578}$=−2.1, $[\alpha]_{546}$=−2.3, $[\alpha]_{436}$=−4.0, $[\alpha]_{365}$= −5.5; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (d, *J*=1.2 Hz, H-1), 5.54 (dd, *J*=8.0, 2.5 Hz, H-3), 5.29 (dd, *J*=8.5, 2.5 Hz, H-4), 5.03 (dd, *J*=8.0, 1.2 Hz, H-2), 5.00 (dq, *J*=8.5, 6.2 Hz, H-5), 2.18 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.03 (s, 3H), 1.21 (d, *J*=6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 195.2 (C-1), 169.9 (×2), 169.8, 169.6, 74.2 (C-2), 71.3 (C-4), 67.3 (C-3), 66.6 (C-5), 21.0, 20.6, 20.5, 20.4, 16.5 (C-6); EIMS (20 eV) *m/z* (rel. int.) $[M]^+$ 332 (0.3), 272 (5.3), 201 (19.9), 184 (17.9), 157 (88.0), 142 (32.6), 129 (14.1), 115 (66.8), 99 (39.2), 73 (10.5), 43 (100); positive HRFAB-MS *m/z* 333.1195 $[M+H]^+$ (calcd for $C_{14}H_{21}O_9$, 333.1186).

3.4.5. 6-Deoxy-L-mannose ethylenedithioacetal (6). L-Rhamnose (1 g) in AcOH (7.5 mL) was treated with a solution of 1,2-ethandithiol (2.5 mL) and $Et_2O{\cdot}BF_3$ (0.3 mL) and stirred during 60 min. The reaction mixture was left overnight at room temperature affording 476 mg of **6** (36%). White solid; mp 166–168°C; ORD (*c* 1.35, MeOH) $[\alpha]_{589}$=−4.4, $[\alpha]_{578}$=−4.4, $[\alpha]_{546}$=−5.2, $[\alpha]_{436}$=−9.6, $[\alpha]_{365}$=−19.3; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.66 (d, *J*=3.4 Hz, H-1), 4.78 (dd, *J*=8.4, 1.1 Hz, H-3), 4.61 (dd, *J*=8.4, 3.4 Hz, H-2), 4.53 (dq, *J*=7.4, 6.2 Hz, H-5), 4.43 (dd, *J*=7.4, 4.4 Hz, H-4), 3.48–3.34 (m, 2H), 3.19–3.07 (m, 2H), 1.67 (d, *J*=6.2 Hz, H-6); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 74.9 (C-2), 73.5 (C-4), 71.4 (C-3), 66.3 (C-5), 56.0 (C-1), 38.3, 37.9, 20.9 (C-6); EIMS (20 eV) *m/z* (rel. int.) $[M]^+$ 240 (0.4), 147 (68.0), 117 (10.3), 107 (19.7), 106 (74.2), 105 (100.0), 73 (14.8), 61 (25.5), 57 (13.9) 45 (15.3).

3.4.6. Tetra-*O*-acetyl-6-deoxy-L-mannose ethylenedithioacetal (7). The procedure used for peracetylation of compound **4** was applied for derivatization of product **6** (370 mg). The crude reaction mixture was purified by column chromatography on Si gel (*n*-hexane–EtOAc, 4:1) to yield 365 mg of **7** (58%), as the major reaction product (R_f=0.27). Oil; ORD (*c* 3.0, $CHCl_3$) $[\alpha]_{589}$=−26.7, $[\alpha]_{578}$=−27.7, $[\alpha]_{546}$=−32.0, $[\alpha]_{436}$=−57.1, $[\alpha]_{365}$=−97.7; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.53 (dd, *J*=7.7, 2.2 Hz, H-3), 5.24 (dd, *J*=8.3, 2.2 Hz, H-4), 5.14 (dd, *J*=7.7, 6.0 Hz, H-2), 4.92 (dq, *J*=8.3, 6.4 Hz, H-5), 4.62 (d, *J*=6.0 Hz, H-1), 3.22–3.09 (m, 4H), 2.11 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 1.18 (d, *J*=6.4 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.2, 170.0, 169.8, 169.7, 72.3 (C-2), 71.1 (C-4), 70.3 (C-3), 67.0 (C-5), 53.1 (C-1), 39.3, 37.9, 21.0, 20.9 (×2), 20.7, 16.4 (C-6); EI-MS (20 eV) *m/z* (rel. int.) $[M-C_2H_4O_2]^+$ 348 (0.4), $[M-2C_2H_4O_2]^+$ 288 (35.2), 200 (32.9), 189 (27.7), 186 (15.4), 147 (40.4), $[C_3H_5S_2]^+$ 105 (100.0), 99 (10.7), 43 (33.8); positive FAB-MS *m/z* (rel. int) $[M+H]^+$ 409 (2.5), 349 (20), 331 (45), 289 (75), 187 (100), 105 (50); positive HRFAB-MS *m/z* 409.0999 $[M+H]^+$ (calcd for $C_{16}H_{25}O_8S_2$, 409.0991).

3.4.7. Tetra-*O*-acetyl-1,6-dideoxy-L-mannitol (8). A solution of **7** (50 mg) in EtOH (2 mL) was treated with Raney-Ni (1.5 g) in EtOH (6 mL). The reaction mixture was refluxed for 10 h and filtered under Celite. The solvent was removed at reduced pressure. Then, the crude product was purified by column chromatography on Si gel (*n*-hexane–EtOAc, 9:1) to afford 10 mg (26%) of **8** (R_f=0.51). Oil; ORD (*c* 1.08, $CHCl_3$) $[\alpha]_{589}$=−30.5, $[\alpha]_{578}$=−32.4, $[\alpha]_{546}$=−37.0, $[\alpha]_{436}$=−63.9, $[\alpha]_{365}$=−103.7; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.27 (dd, *J*=7.7, 3.4 Hz, H-3 and H-4), 4.93 (dq, *J*=7.7, 6.5 Hz, H-2 and H-5), 2.09 (s, 6H), 2.03 (s, 6H), 1.21 (d, *J*=6.5 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.1 (×2), 170.0 (×2), 71.2 (C-3 and C-4), 67.1 (C-2 and C-5), 21.0 (×2), 20.7 (×2), 16.1 (C-1 and C-6); EI-MS (20 eV) *m/z* (rel int.) 317 (4), 231 (10.0), 189 (5.0), 172 (26.0), 149 (14.0), 130 (61), 129 (74), 97 (23), 95 (22.0), 83 (64.0), 69 (37.0), 57 (29.0), 43 (100); positive FAB-MS *m/z* (rel. int.) $[M+H]^+$ 319 (15.0), $[M+H-C_2H_4O_2]^+$ 259 (100); HRFAB-MS *m/z* 319.1397 $[M+H]^+$ (calcd for $C_{14}H_{23}O_8$, 319.1393).

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