Anti-HCV Bioactivity of Pseudoguaianolides from *Parthenium hispitum*

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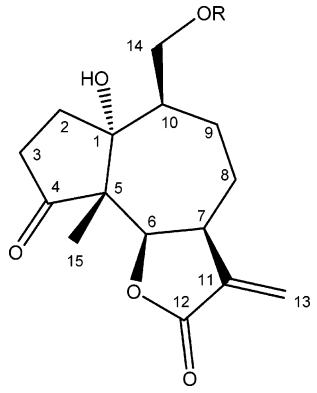
Received November 12, 2006

Five new (**1**−**5**) and four known (**6**−**9**) C_{14}-oxygenated 1α-hydroxy-11(13)-pseudoguaien-6β,12-olides with potent inhibition of hepatitis C virus (HCV) replication were obtained from *Parthenium hispitum* via high-throughput natural product chemistry methods. A semipreparative HPLC system was used to purify these compounds. The miniaturization of the structure elucidation and dereplication for the mass-limited samples were performed primarily utilizing a capillary-scale NMR probe. Compounds **2**−**4** were found to possess in vitro anti-HCV activity in the subgenomic HCV replicon system containing luciferase reporter with significant inhibition above 90% at 2 μM concentration.

The hepatitis C virus (HCV) infects about 170 million people worldwide.[1,2] Millions of chronically infected patients develop cirrhosis requiring liver transplantation.[3] Although treatments for HCV infection have been improved over the past several years, many infected people with genotype 1 virus have poor response to standard therapy and side effects remain a significant problem. Therefore, development of small molecular inhibitors of HCV replication would meet a largely unmet medical need. An HCV subgenomic replicon assay was recently introduced providing a critical system for identifying inhibitors of HCV.[2] In order to discover new anti-HCV agents, this replicon system was used to screen our compound libraries generated from American and African plants via the high-throughput natural product chemistry procedures.[4] The test results revealed that the samples from the plant *Parthenium hispitum* Raf. (Asteraceae) showed inhibition of HCV replication. The genus *Parthenium* is an herbaceous annual weed with noxiousness to other plants, humans, and animals.[5,6] *P. hispitum* has been found to yield two 1α-hydroxy-11(13)-pseudoguaien-6β,12-olides, tetraneurin-C and tetraneurin-E.[7] In this paper, we report the semipreparative HPLC isolation (Figure 1) of five new (**1**−**5**) and four known (**6**−**9**) C_{14}-oxygenated 1α-hydroxy-11(13)-pseudoguaien-6β,12-olides and their inhibition of HCV replication. The miniaturization of the structure elucidation and dereplication of these mass-limited pseudoguaianolides were performed primarily using a capillary-scale NMR probe and MS spectroscopic methods.



1: R = *n*-propanoyl
2: R = *n*-butanoyl
3: R = tigloyl
4: R = 3-methyl-2-butenoyl
5: R = angeloyl
6: R = acetyl
7: R = isopropanoyl
8: R = 2-methylbutanoyl
9: R = 3-methylbutanoyl

Results and Discussion

During the anti-HCV biological screening, the sample in the preparative HPLC fraction 3 generated from the leaves of *P. hispitum* via our high-throughput procedures (Experimental Section)[4] exhibited primarily inhibition of HCV replicon, which attracted our attention to the further purification and reconfirmation of the biological activity of each single compound from this sample. A semipreparative HPLC method was then successfully performed to the isolation of nine pure compounds (**1**−**9**) from this HPLC fraction (Figure 1). The ^{1}H NMR data of all the compounds showed that they have the same core structure with general features similar to those of known C14-oxygenated 1α-hydroxy-4-oxo-11(13)-pseudoguaien-6β,12-olides.[7−9] The IR, specific rotation, and NMR data of the major component **6** are identical with those of tetraneurin-A, a common metabolite from *Parthenium* spp.[7−9] The molecular weights of the new compounds **1**−**5** and their chemical formula were deduced from either positive or negative mode HRESIMS (Experimental Section). ^{1}H NMR data (Table 1) indicated that all five new compounds were esterified analogues at the C-14 hydroxy group of compound **6**: *n*-propanoyl [1.13 (3H, t, J = 7.3 Hz), 2.37 (2H, q, J = 7.3 Hz)] in **1**, *n*-butanoyl [0.96 (3H, t, J = 7.4 Hz), 1.66 (2H, m), 2.32 (2H, t, J = 7.3 Hz)] in **2**, tigloyl [1.82 (3H, d, J = 7.1 Hz), 1.84 (3H, brs), 6.88 (1H, q, J = 7.1 Hz)] in **3**, 3-methyl-2-butenoyl [1.92 (3H, brs), 2.17 (3H, brs), 5.71 (1H, brs)] in **4**, and angeloyl [1.89 (3H, brs), 1.97 (3H, d, J = 7.2 Hz), 6.14 (1H, q, J = 7.2 Hz)] in **5**. The COSY NMR, ^{13}C NMR (Table 2), and MS data were in full agreement with those moieties and the assignments.

A different NMR solvent has been applied to compounds **1** (acetone-d_6), **3** (acetone-d_6), and **5** ($CDCl_3$). The correlations in their NOESY spectra recorded in acetone-d_6/$CDCl_3$ were still ambiguous. However, on the basis of the biogenetic consideration and the observed coupling constants, we assume that all of the new compounds **1**−**5** have the same relative configuration (1α,5β,6β,-10β) as that in the major component, tetraneurin-A (**6**).[7−9] The ambrosanolides (**8** and **9**) were previously obtained as a mixture.[9] We herein named the pure compounds **8** and **9** as ambrosanolide-A and ambrosanolide-B, respectively. Only partial NMR data were reported for the known pseudoguaianolides (**6**−**9**).[7−9] Therefore,

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10.1021/np060567e CCC: $37.00
Published on Web 02/10/2007

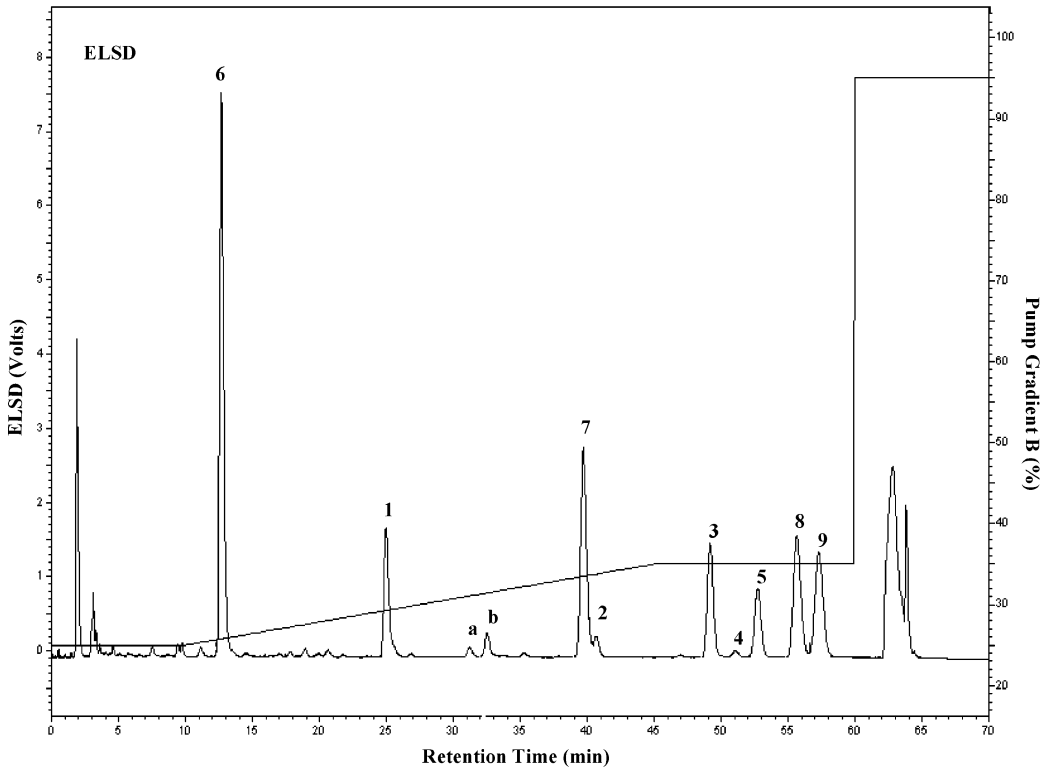

Figure 1. Semipreparative HPLC purification of the C_{14}-oxygenated pseudoguaianolides (**1**−**9**). The ELSD chromatogram is represented for only one of the three collections. The other two ELSD chromatograms are very similar. Quantities of each compound were estimated by ELSD peak integration areas. The total amount of each compound resulted from the combination of the corresponding ELSD peaks from all three collections.[4c] Peaks a and b: inactive minor components, NMR spectra not acquired.

all chemical shifts for the protons and carbons were assigned in the Experimental Section.

Pseudoguaianolides are widely distributed in the genus *Parthenium* (Asteraceae),[7−10] and some of them have been found to show bioactivities against chronic inflammation[11] and human cancer cell lines.[12] Testing of our purified C_{14}-oxygenated pseudoguaianolides (the major component **6** was not tested) in an HCV subgenomic replicon system showed that the majority of the compounds had antiviral activities (Table 3). At 2 μM concentration, compounds **2**−**4** had ≥90% inhibition of the reporter replicon, indicating potent activities, and compounds **1**, **5**, and **9** also had significant inhibition above 50%. These compounds were confirmed not to inhibit luciferase enzyme in an in vitro enzyme assay and were not cytotoxic to Huh-7 replicon cells at the testing concentrations, suggesting that the inhibition is likely the result of inhibiting the HCV replicon system.[13] Furthermore, compounds **1** and **3** were also shown to inhibit HCV RNA level using the same cells and a branched DNA detection method for HCV NS3 RNA.[13] Therefore, these compounds appear to be HCV replication inhibitors and may serve as hits for further optimization and drug development.

It is worthy to note that a series of sesquiterpene lactones including some pseudoguaianolides such as helenalin and ambrosin have just recently been studied for their anti-HCV activities in the subgenomic HCV replicon system.[14] Thus, identification of compounds **1**−**9**, with the majority showing anti-HCV activities, further adds to the natural products that are potential antiviral lead molecules to develop effective therapies.

Experimental Section

General Experimental Procedures. For instrumentation and general automated flash chromatography, solvent evaporation, and capillary-scale NMR see the preceding papers.[4]

Plant Material. The leaves of *P. hispitum* were collected from Missouri in the Spring of 2000. Plant samples were first stored at −20 °C and then shipped to Sequoia Sciences, where they were lyophilized. The plant was identified by Adam Bradley (Missouri Botanical Garden Herbarium, St. Louis, MO). A voucher specimen (No. 418) was deposited at the Herbarium of Missouri Botanical Garden.

Extraction and Isolation. The aerial parts of the plant (88 g) were extracted with EtOH/EtOAc (50:50) followed by H_2O/MeOH (30:70) to obtain 4.5 and 8.0 g of dry organic and aqueous extracts, respectively. One gram of the organic extract was loaded on the Flash Master II automated chromatographic system using our standard elution gradient to generate the Flash Fractions.[4] The Flash Fraction 5 (50% EtOAc, 50% hexane) totaled 172 mg; a 50 mg aliquot was fractionated by preparative C_{18} HPLC from 45% to 100% acetonitrile in H_2O, collecting 40 1-min fractions. Compounds **1**−**9** resided in preparative HPLC fraction 3, which exhibited primarily inhibition of HCV replicon. Review of the HPLC-ELSD-MS data acquired on all of the preparative fractions from the Flash Fraction 5 suggested that preparative HPLC fraction 3 contained compounds with molecular masses less than 500 Da, which could readily be isolated using reversed-phase chromatography. The initial mobile phase gradient applied to isolating compounds **1**−**9** from HPLC fraction 3 was based on the elution profile observed during the preparative HPLC separation that created the fraction. A semipreparative HPLC method was developed that resulted in an isocratic gradient of 25% acetonitrile in H_2O acidified with 0.05% TFA for 10 min, followed by a linear gradient of acetonitrile from 25% to 35% over 35 min, then followed by an isocratic gradient of 35% acetonitrile for 15 min, and finally followed by an isocratic gradient of 95% acetonitrile for 10 min. The corresponding ELSD peaks (Figure 1) from three collections were combined to yield pure compounds **1** (250 μg, t_R = 25.0 min), **2** (35 μg, t_R = 40.7 min), **3** (220 μg, t_R = 49.2 min), **4** (20 μg, t_R = 51.1 min), **5** (160 μg, t_R = 52.8 min), **6** (1500 μg, t_R = 12.7 min), **7** (450 μg, t_R = 39.8 min), **8** (200 μg, t_R = 55.6 min), and **9** (200 μg, t_R = 57.4 min).

Hispitolide-A (1): gummy solid; ^{1}H and ^{13}C NMR (CD_3OD) data, see Tables 1 and 2; ^{1}H (in acetone-d_6, 600 MHz) δ 2.40 (1H, m, H-2α), 1.84 (1H, m, H-2β), 2.72 (1H, H-3α), 2.39 (1H, H-3β), 4.78 (1H, d, J = 7.9 Hz, H-6α), 3.39 (1H, m, H-7α), 1.99 (1H, m, H-8α), 1.71 (1H, m, H-8β), 2.17 (1H, m, H-9α), 1.86 (1H, m, H-9β), 2.28 (1H, m, H-10α), 4.44 (1 H, brd, J = 10.6 Hz, H-14a), 4.22 (1H, dd, J = 10.6, 10.1 Hz, H-14b), 6.09 (1H, d, J = 2.3 Hz, H-13a), 5.67 (1H, d, J =

Table 1. ^{1}H NMR Data for **1**−**5** (in CD_3OD at 600 MHz, *J* values in Hz)[a]

H #	1	2	3	4	5
2	2.43 (1H, m, H-2β)	2.43 (1H, m, H-2β)	2.43 (1H, m, H-2β)	2.42 (1H, m, H-2β)	2.43 (1H, m, H-2β)
	1.80 (1H, m, H-2α)	1.81 (1H, m, H-2α)	1.81 (1H, m, H-2α)	1.81 (1H, m, H-2α)	1.82 (1H, m, H-2α)
3	2.67 (1H, m, H-3β)	2.68 (1H, m, H-3β)	2.68 (1H, m, H-3β)	2.68 (1H, m, H-3β)	2.69 (1H, m, H-3β)
	2.44 (1H, m, H-3α)	2.44 1H, m, H-3α)	2.44 1H, m, H-3α)	2.43 1H, m, H-3α)	2.44 1H, m, H-3α)
6	4.85 (1H, d, 7.8)[b]	4.85 (1H, d, 7.9)[b]	4.85 (1H, d, 7.8)[b]	4.86 (1H, d, 7.8)[b]	4.87 (1H, d, 7.8)[b]
7	3.42 (1H, m)	3.41 (1H, m)	3.42 (1H, m)	3.42 (1H, m)	3.43 (1H, m)
8	1.97 (1H, m, H-8β)	1.97 (1H, m, H-8β)	1.98 (1H, m, H-8β)	1.98 (1H, m, H-8β)	1.99 (1H, m, H-8β)
	1.74 (1H, m, H-8α)	1.75 (1H, m, H-8α)	1.77 (1H, m, H-8α)	1.75 (1H, m, H-8α)	1.76 (1H, m, H-8α)
9	2.16 (1H, m, H-9β)	2.16 (1H, m, H-9β)	2.18 (1H, m, H-9β)	2.16 (1H, m, H-9β)	2.19 (1H, m, H-9β)
	1.87 (1H, m, H-9α)	1.88 (1H, m, H-9α)	1.90 (1H, m, H-9α)	1.88 (1H, m, H-9α)	1.91 (1H, m, H-9α)
10	2.23 (1H, m)	2.22 (1H, m)	2.26 (1H, m)	2.24 (1H, m)	2.27 (1H, m)
13	6.19 (1H, d, 2.3)	6.19 (1H, d, 2.5)	6.19 (1H, d, 2.4)	6.19 (1H, d, 2.4)	6.19 (1H, d, 2.4)
	5.71 (1H, d, 2.1)	5.71 (1H, d, 2.1)	5.71 (1H, d, 2.1)	5.71 (1H, d, 2.1)	5.72 (1H, d, 2.1)
14	4.45 (1H, dd, 11.1, 2.1)	4.45 (1H, dd, 10.9, 2.1)	4.49 (1H, dd, 11.4, 2.2)	4.45 (1H, dd, 10.8, 2.0)	4.54 (1H, dd, 11.0, 2.1)
	4.23 (1H, dd, 11.1, 10.3)	4.23 (1H, dd, 10.9, 10.4)	4.27 (1H, dd, 11.4, 10.6)	4.23 (1H, dd, 10.8, 10.2)	4.29 (1H, dd, 11.0, 10.4)
15	1.04 (3H, s)	1.04 (3H, s)	1.06 (3H, s)	1.06 (3H, s)	1.07 (3H, s)
	n-propanoyl	*n*-butanoyl	tigloyl	3-methyl-2-butenoyl	angeloyl
	1.13 (3H, t, 7.3)	0.96 (3H, t, 7.4)	1.82 (3H, d, 7.1)	1.92 (3H, brs)	1.97 (3H, d, 7.2)
	2.37 (2H, q, 7.3)	1.66 (2H, m)	6.88 (1H, q, 7.1)	5.71 (1H, brs)	6.14 (1H, q, 7.2)
		2.32 (2H, t, 7.3)	1.84 (3H, brs)	2.17 (3H, brs)	1.89 (3H, brs)

[a] Assignments were made by a combination of 1D- and 2D-NMR techniques (^{1}H−^{1}H COSY, HSQC, and HMBC). [b] Proton resonances were overlapped within the residual HOD signal in the NMR solvent of CD_3OD.

Table 2. ^{13}C NMR Data for **1**−**3** and **5** (in CD_3OD at 150 MHz)[a]

C #	1	2	3	5
1	83.6	83.4	83.0	83.3
2	32.6	32.8	32.4	33.0
3	32.9	33.1	33.2	33.4
4	220.5	220.9	221.1	220.9
5	59.2	59.3	59.5	59.2
6	81.8	81.8	81.9	81.8
7	46.3	46.2	46.3	46.4
8	29.1	29.1	29.1	29.0
9	26.5	26.4	26.4	26.6
10	49.2	49.3	49.1	49.1
11	141.9	142.0	142.1	141.8
12	172.2	172.2	172.2	171.9
13	123.1	123.1	123.3	123.1
14	64.8	64.8	64.8	64.5
15	13.9	14.0	14.2	14.1
	n-propanoyl	*n*-butanoyl	tigloyl	angeloyl
	9.3	13.8	11.9	16.1
	28.2	19.2	14.0	20.7
	171.5	37.3	129.3	129.2
		171.0	139.1	139.6
			168.6	168.2

[a] Assignments were made by a combination of 1D- and 2D-NMR techniques (^{1}H−^{1}H COSY, HSQC, and HMBC).

2.0 Hz, H-13b), 1.04 (3H, s, H-15); *n*-propanoyl, 1.08 (3H, t, J = 7.6 Hz) and 2.34 (2H, q, J = 7.6 Hz); ESIMS *m/z* 335 [M − H]$^-$, 381 [M + HCOO$^-$]$^-$, 337 [M + H]$^+$, 673 [2M + H]$^+$, 695 [2M + Na]$^+$; HRESIMS *m/z* 337.1653 ($C_{18}H_{25}O_6$ requires 337.1651).

Table 3. HCV Replicon Assay[15] of Compounds **1**−**9**

compound	inhibition (%) of the replication of HCV at 2 μM
1	55
2	90
3	98
4	98
5	65
6	NT[a]
7	26
8	25
9	63

[a] NT = not tested.

Hispitolide-B (2): ^{1}H and ^{13}C NMR (CD_3OD) data, see Tables 1 and 2; ESIMS *m/z* 395 [M + HCOO$^-$]$^-$, 373 [M + Na]$^+$, 723 [2M + Na]$^+$, 1073 [3M + Na]$^+$; HRESIMS *m/z* 373.1625 ($C_{19}H_{26}O_6Na$ requires 373.1627).

Hispitolide-C (3): gummy solid; ^{1}H and ^{13}C NMR (CD_3OD) data, see Tables 1 and 2; ^{1}H (in acetone-d_6, 600 MHz) δ 4.80 (1H, d, J = 7.9 Hz, H-6α), 3.41 (1H, m, H-7α), 4.53 (1 H, brd, J = 10.7 Hz, H-14a), 4.29 (1H, dd, J = 10.7, 10.4 Hz, H-14b), 6.10 (1H, d, J = 2.3 Hz, H-13a), 5.68 (1H, brs, H-13b), 1.08 (3H, s, H-15); tigloyl, 1.79 (3H, d, J = 7.0 Hz), 1.82 (3H, brs), and 6.85 (1H, q, J = 7.0 Hz); ESIMS *m/z* 407 [M + HCOO$^-$]$^-$, 363 [M + H]$^+$, 725 [2M + H]$^+$, 747 [2M + Na]$^+$; HRESIMS *m/z* 363.1810 ($C_{20}H_{27}O_6$ requires 363.1807).

Hispitolide-D (4): ^{1}H NMR (CD_3OD) data, see Table 1; ESIMS *m/z* 407 [M + HCOO$^-$]$^-$, 385 [M + Na]$^+$, 747 [2M + Na]$^+$; HRESIMS *m/z* 385.1625 ($C_{20}H_{26}O_6Na$ requires 385.1627).

Hispitolide-E (5): gummy solid; ^{1}H and ^{13}C NMR (CD_3OD) data, see Tables 1 and 2; ^{1}H (in $CDCl_3$, 600 MHz) δ 4.87 (1H, d, J = 7.8

Hz, H-6α), 3.38 (1H, m, H-7α), 4.51 (1 H, brd, $J = 10.6$ Hz, H-14a), 4.15 (1H, dd, $J = 10.6$, 10.1 Hz, H-14b), 6.27 (1H, brs, H-13a), 5.60 (1H, brs, H-13b), 1.08 (3H, s, H-15); angeloyl, 1.89 (3H, brs), 1.99 (3H, d, $J = 7.1$ Hz), and 6.11 (1H, q, $J = 7.1$ Hz); ESIMS *m/z* 407 $[M + HCOO^-]^-$, 385 $[M + Na]^+$, 747 $[2M + Na]^+$.

Tetraneurin-A (6):[8] gummy solid; $[\alpha]_D^{20}$ +4.0 (*c* 0.15, MeOH); IR ν_{max} (film) 3515 (brs), 1737 and 1642 cm^{-1}; ^1H (in CD_3OD, 600 MHz) δ 2.43 (1H, m, H-2α), 1.80 (1H, m, H-2*β*), 2.64 (1H, H-3α), 2.43 (1H, H-3*β*), 4.84 (1H, d, $J = 7.9$ Hz, H-6α), 3.41 (1H, m, H-7α), 1.95 (1H, m, H-8α), 1.73 (1H, m, H-8*β*), 2.16 (1H, m, H-9α), 1.87 (1H, m, H-9*β*), 2.21 (1H, m, H-10α), 4.42 (1 H, dd, $J = 11.1$, 1.8 Hz, H-14a), 4.21 (1H, dd, $J = 11.1$, 10.6 Hz, H-14b), 6.18 (1H, d, $J = 2.6$ Hz, H-13a), 5.71 (1H, d, $J = 2.4$ Hz, H-13b), 1.03 (3H, s, H-15), and 2.05 (3H, s, acetyl); ^{13}C NMR (in CD_3OD, 150 MHz) δ 83.6 (s, C-1), 32.9 (t, C-2), 33.2 (t, C-3), 220.5 (s, C-4), 59.2 (s, C-5), 81.6 (d, C-6), 46.1 (d, C-7), 29.1 (t, C-8), 26.2 (t, C-9), 49.0 (d, C-10), 141.9 (s, C-11), 172.2 (s, C-12), 122.7 (t, C-13), 64.4 (t, C-14), 14.4 (q, C-15); acetyl, 21.0 (q) and 171.9 (s); ESIMS *m/z* 367 $[M + HCOO^-]^-$, 345 $[M + Na]^+$.

Chiapin B (7):[7] gummy solid; ^1H (in CD_3OD, 600 MHz) δ 2.43 (1H, m, H-2α), 1.81 (1H, m, H-2*β*), 2.67 (1H, H-3α), 2.43 (1H, H-3*β*), 4.84 (1H, d, $J = 7.9$ Hz, H-6α), 3.41 (1H, m, H-7α), 1.97 (1H, m, H-8α), 1.74 (1H, m, H-8*β*), 2.17 (1H, m, H-9α), 1.86 (1H, m, H-9*β*), 2.21 (1H, m, H-10α), 4.44 (1 H, dd, $J = 10.6$, 2.1 Hz, H-14a), 4.22 (1H, dd, $J = 10.6$, 10.3 Hz, H-14b), 6.18 (1H, d, $J = 2.4$ Hz, H-13a), 5.70 (1H, d, $J = 2.3$ Hz, H-13b), 1.04 (3H, s, H-15); isopropanoyl, 1.16 (3H, d, $J = 6.8$ Hz), 1.16 (3H, d, $J = 6.8$ Hz), and 2.57 (1H, m); ^{13}C NMR (in CD_3OD, 150 MHz) δ 83.1 (s, C-1), 32.9 (t, C-2), 33.2 (t, C-3), 220.8 (s, C-4), 59.5 (s, C-5), 81.8 (d, C-6), 46.5 (d, C-7), 29.1 (t, C-8), 26.4 (t, C-9), 49.6 (d, C-10), 142.3 (s, C-11), 172.2 (s, C-12), 122.8 (t, C-13), 64.8 (t, C-14), 14.4 (q, C-15); isopropanoyl, 19.6 (q), 19.6 (q), 35.2 (d), and 177.6 (s); ESIMS *m/z* 395 $[M + HCOO^-]^-$, 373 $[M + Na]^+$.

Ambrosanolide-A (8):[9] gummy solid; ^1H (in CD_3OD, 600 MHz) δ 2.43 (1H, m, H-2α), 1.81 (1H, m, H-2*β*), 2.67 (1H, H-3α), 2.43 (1H, H-3*β*), 4.84 (1H, d, $J = 7.9$ Hz, H-6α), 3.42 (1H, m, H-7α), 1.97 (1H, m, H-8α), 1.75 (1H, m, H-8*β*), 2.17 (1H, m, H-9α), 1.88 (1H, m, H-9*β*), 2.23 (1H, m, H-10α), 4.46 (1 H, dd, $J = 10.8$, 2.1 Hz, H-14a), 4.24 (1H, dd, $J = 10.8$, 10.2 Hz, H-14b), 6.19 (1H, d, $J = 2.6$ Hz, H-13a), 5.71 (1H, d, $J = 2.1$ Hz, H-13b), 1.05 (3H, s, H-15); 2-methylbutanoyl, 0.92 (3H, t, $J = 7.3$ Hz), 1.15 (3H, d, $J = 7.0$ Hz), 1.50 (1H, m), 1.67 (1H, m), 2.40 (1H, m); ^{13}C NMR (in CD_3OD, 150 MHz) δ 84.1 (s, C-1), 32.8 (t, C-2), 33.2 (t, C-3), 220.5 (s, C-4), 59.4 (s, C-5), 81.7 (d, C-6), 45.9 (d, C-7), 29.1 (t, C-8), 26.4 (t, C-9), 49.2 (d, C-10), 141.4 (s, C-11), 172.1 (s, C-12), 122.8 (t, C-13), 64.8 (t, C-14), 14.4 (q, C-15); 2-methylbutanoyl, 12.1 (q), 17.2 (q), 28.0 (t), 42.6 (d), and 177.6 (s); ESIMS *m/z* 409 $[M + HCOO^-]^-$, 365 $[M + H]^+$.

Ambrosanolide-B (9):[9] gummy solid; ^1H (in CD_3OD, 600 MHz) δ 2.43 (1H, m, H-2α), 1.81 (1H, m, H-2*β*), 2.67 (1H, H-3α), 2.43 (1H, H-3*β*), 4.85 (1H, d, $J = 7.9$ Hz, H-6α), 3.41 (1H, m, H-7α), 1.99 (1H, m, H-8α), 1.74 (1H, m, H-8*β*), 2.18 (1H, m, H-9α), 1.87 (1H, m, H-9*β*), 2.23 (1H, m, H-10α, overlapped), 4.46 (1 H, dd, $J = 10.9$, 2.1 Hz, H-14a), 4.24 (1H, dd, $J = 10.9$, 10.3 Hz, H-14b), 6.19 (1H, d, $J = 2.2$ Hz, H-13a), 5.71 (1H, d, $J = 1.9$ Hz, H-13b), 1.04 (3H, s, H-15); 3-methylbutanoyl, 0.97 (3H, d, $J = 6.5$ Hz), 0.97 (3H, d, $J = 6.5$ Hz), 2.07 (1H, m), and 2.23 (1H, m, overlapped); ^{13}C NMR (in CD3OD, 150 MHz) δ 84.1 (s, C-1), 32.8 (t, C-2), 33.2 (t, C-3), 220.5 (s, C-4), 59.4 (s, C-5), 81.7 (d, C-6), 45.9 (d, C-7), 29.1 (t, C-8), 26.4 (t, C-9), 49.2 (d, C-10), 141.4 (s, C-11), 172.1 (s, C-12), 122.8 (t, C-13), 64.8 (t, C-14), 14.4 (q, C-15); 3-methylbutanoyl, 22.7 (q), 22.7 (q), 27.3 (d), 44.4 (t), and 177.4 (s); ESIMS *m/z* 409 $[M + HCOO^-]^-$, 365 $[M + H]^+$, 387 $[M + Na]^+$.

Antiviral Activity. Compounds **1**−**5** and **7**−**9** were assayed for their antiviral activities using an HCV subgenomic replicon system containing luciferase reporter, which was obtained from Dr. R. Batenschlager's laboratory.[2] Huh-7 replicon cells were grown to 85% confluency and had more than 95% viability before assay. Cells were seeded at a density of 11 000 cells per well in 135 μL of assay volume in 96-well microplates. Compounds in 15 μL volume were added to the plates, which were incubated for 48 h at 37 °C in a tissue culture incubator. Before luciferase detection, the plates were cooled to room temperature for 30 min and 75 uL of SteadyLite luciferase reagent (Promega, Madison, WI) was added. After 3 min of incubation at room temperature, the plates were read for luminescence on a Victor 2 plate reader (Perkin-Elmer, Wellesley, MA). Assay wells without compounds served as controls[15] for calculation of compound inhibition. Compounds were tested in duplicate.

Acknowledgment. Sequoia Sciences gratefully acknowledges J. Stone, A. Bradley, G. Walters, and Dr. J. Miller from Missouri Botanical Garden for the plant collections and identifications, and Dr. J.-N. Peng from Department of Pharmcognosy, University of Mississippi (Olemiss), for the technical assistance with the measurement of optical rotation data. The authors would like to acknowledge T. Peck, D. Olson, and J. Norcross from Magnetic Resonance Microsensors (Savoy, IL) for making the first 5 μL proton indirect carbon gradient CapNMR probe available to Sequoia Sciences.

References and Notes

(1) Fanning, L. J. *Lett. Drug Des. Discovery* **2005**, *2*, 150−161.
(2) (a) Lohmann, V.; Korner, F.; Koch, J. O.; Herian, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999**, *285*, 110−113. (b) Vrolijk, J. M.; Kaul, A.; Hansen, B. E.; Lohmann, V.; Haagmans, B. L.; Schalm, S. W.; Bartenschlager, R. *J. Virol. Methods* **2003**, *110*, 201−209.
(3) Hughes, C. A.; Shafran, S. D. *Ann. Pharmacother.* **2006**, *40*, 74−82.
(4) (a) Hu, J.-F.; Garo, E.; Hough, G. W.; Goering, M. G.; O'Neil-Johnson, M.; Eldridge, G. R. *J. Nat. Prod.* **2006**, *69*, 585−590. (b) Hu, J.-F.; Yoo, H.-D.; Williams, C. T.; Garo, E.; Cremin, P. A.; Zeng, L.; Vervoort, H. C.; Lee, C. M.; Hart, S. M.; Goering, M. G.; O'Neil-Johnson, M.; Eldridge, G. R. *Planta Med.* **2005**, *71*, 176−180. (c) Hu, J.-F.; Garo, E.; Yoo, H.-D.; Cremin, P. A.; Zeng, L.; Goering, M. G.; O'Neil-Johnson, M.; Eldridge, G. R. *Phytochem. Anal.* **2005**, *16*, 127−133.
(5) Tamado, T.; Milberg, P. *Weed Technol.* **2004**, *18*, 100−105.
(6) Tamado, T.; Schütz, W.; Milberg, P. *Ann. Appl. Biol.* **2002**, *140*, 263−270.
(7) Rodriguez, E.; Yoshioka, H.; Mabry, T. J. *Phytochemistry* **1971**, *10*, 1145−1154.
(8) Büesch, H.; Mabry, T. J. *Tetrahedron* **1969**, *25*, 805−811.
(9) De La Fuente, J. R.; Novara, L.; Alarcón, S. R.; Díaz, O. J.; Uriburu, M. L.; Sosa, V. E. *Phytochemistry* **1997**, *45*, 1185−1188.
(10) (a) Yoshioka, H.; Rodriguze, E.; Mabry, T. J. *J. Org. Chem.* **1970**, *35*, 2888−2891. (b) Romo De Vivar, A.; Aguilar, M. *Tetrahedron* **1970**, *26*, 2775−2780. (c) De La Fuente, J. R.; Uriburu, M. L.; Burton, G.; Sosa, V. E. *Phytochemistry* **2000**, *55*, 769−772. (d) Venkataiah, B.; Ramesh, C.; Ravindranath, N.; Das, B. *Phytochemistry* **2003**, *63*, 383−386. (e) Das, R.; Geethangili, M.; Majhi, A.; Das, B.; Rao, Y. K.; Tzeng, Y. M. *Chem. Pharm. Bull.* **2005**, *53*, 861−862. (f) Das, B.; Mahender, G.; Rao, Y. K.; Ramesh, C.; Venkateswarlu, K.; Ravikumar, K.; Geethangili, M.; Tzeng, Y. M. *Helv. Chim. Acta* **2006**, *89*, 285−290.
(11) Recio, M. C.; Giner, R. M.; Uriburu, L.; Manez, S.; Cerda, M.; De La Fuente, J. R.; Rios, J. L. *Life Sci.* **2000**, *66*, 2509−2518.
(12) Wu, C. Q.; Chen, F.; Rushing, J. W.; Wang, X.; Kim, H. J.; Huang, G.; Haley-Zitlin, V.; He, G. Q. *J. Med. Food* **2006**, *9*, 55−61.
(13) Data were shared only between the two companies of Anadys Pharmaceuticals and Sequoia Sciences.
(14) Hwang, D.-R.; Wu, Y.-S.; Chang, C.-W.; Lien, T.-W.; Chen, W.-C.; Tan, U.-K.; Hsu, J. T. A.; Hsieh, H.-P. *Bioorg. Med. Chem.* **2006**, *14*, 83−91.
(15) For anti-HCV assay, a GSK polymerase inhibitor was used as a positive control (compound **4** in Dhanak, D. et al. *J. Biol. Chem.* **2002**, *277*, 38322−38327) to check the cells periodically. Complete inhibition resulted in a low signal equal to that of no cells. In calculation of compound inhibition, a low signal of the assay was from wells without cells and a high signal came from cells without compounds.

NP060567E