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Activity-Guided Isolation of Novel Norwithanolides from *Deprea subtriflora* with Potential Cancer Chemopreventive Activity

Bao-Ning Su,[†] Eun Jung Park,[†] Dejan Nikolic,[†] Bernard D. Santarsiero,[‡] Andrew D. Mesecar,[‡] Jose Schunke Vigo,[§] James G. Graham,[†] Fernando Cabieses,[§] Richard B. van Breemen,[†] Harry H. S. Fong,[†] Norman R. Farnsworth,[†] John M. Pezzuto,[†] and A. Douglas Kinghorn*[,†]

Program for Collaborative Research in the Pharmaceutical Sciences and Center for Pharmaceutical Biotechnology, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, and Instituto Nacional de Medicina Tradicional (INMETRA), Minesterio de Salud, Jesus Maria, Lima, Peru

kinghorn@uic.edu

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Activity-monitored fractionation of a $CHCl_3$-soluble extract of *Deprea subtriflora* using a quinone reductase induction assay led to the purification of subtrifloralactones A−J (**1**−**10**), 10 novel C-18 norwithanolides based on a new C_{27} skeleton. These compounds were characterized by spectroscopic and chemical studies, and single-crystal X-ray diffraction analysis was used to confirm the structures of **1** and **4**. Compounds **1**−**10** were evaluated for their cancer chemopreventive activity in terms of their ability to induce quinone reductase activity with cultured murine hepatoma cells, and compounds **1** and **6** were found to be highly effective.

Introduction

Cancer chemoprevention is a strategy for reducing cancer mortality and involves the prevention, delay, or reversal of cancer by the ingestion of dietary or pharmaceutical agents capable of modulating the process of carcinogenesis.[1] Induction of phase II drug-metabolizing enzymes, such as quinone reductase, is considered a relevant mechanism for achieving protection against the toxic and neoplastic effects of many carcinogens.[2] As part of our ongoing project directed toward the search for novel, plant-derived cancer chemopreventive agents,[3] the whole plant of *Deprea subtriflora* (Ruiz & Pavon) D'arcy (Solanaceae) collected in Peru was chosen for detailed investigation, since its $CHCl_3$-soluble extract significantly induced quinone reductase activity with cultured Hepa 1c1c7 (mouse hepatoma) cells.[2b,4] There have been no previous laboratory investigations on *D. subtriflora* to date.

Withanolides are a group of naturally occurring C_{28} steroid derivatives built on an ergostane skeleton, in which a six-membered-ring 22-hydroxy-26-oic acid lactone or a five-membered-ring 23-hydroxy-26-oic acid lactone is generally present in the side chain. Withanolides are restricted in distribution but are characteristic of the genera *Acnistus*, *Datura*, *Discopodium*, *Dunalia*, *Jaborosa*, *Lycium*, *Nicandra*, *Physalis*, *Solanum*, and *Withania*, belonging to the plant family Solanaceae.[5] The isolation and synthesis of withanolides have attracted great interest, due to their diverse antitumor,[6] antifeedant,[7] antiulcer,[8] antistress,[9] cytotoxic,[10] immunosuppres-

* To whom correspondence should be addressed. Phone: (312) 996-0914. Fax: (312) 996-7107.

[†] Program for Collaborative Research in the Pharmaceutical Sciences, University of Illinois at Chicago.

[‡] Center for Pharmaceutical Biotechnology, University of Illinois at Chicago.

[§] Instituto Nacional de Medicina Tradicional.

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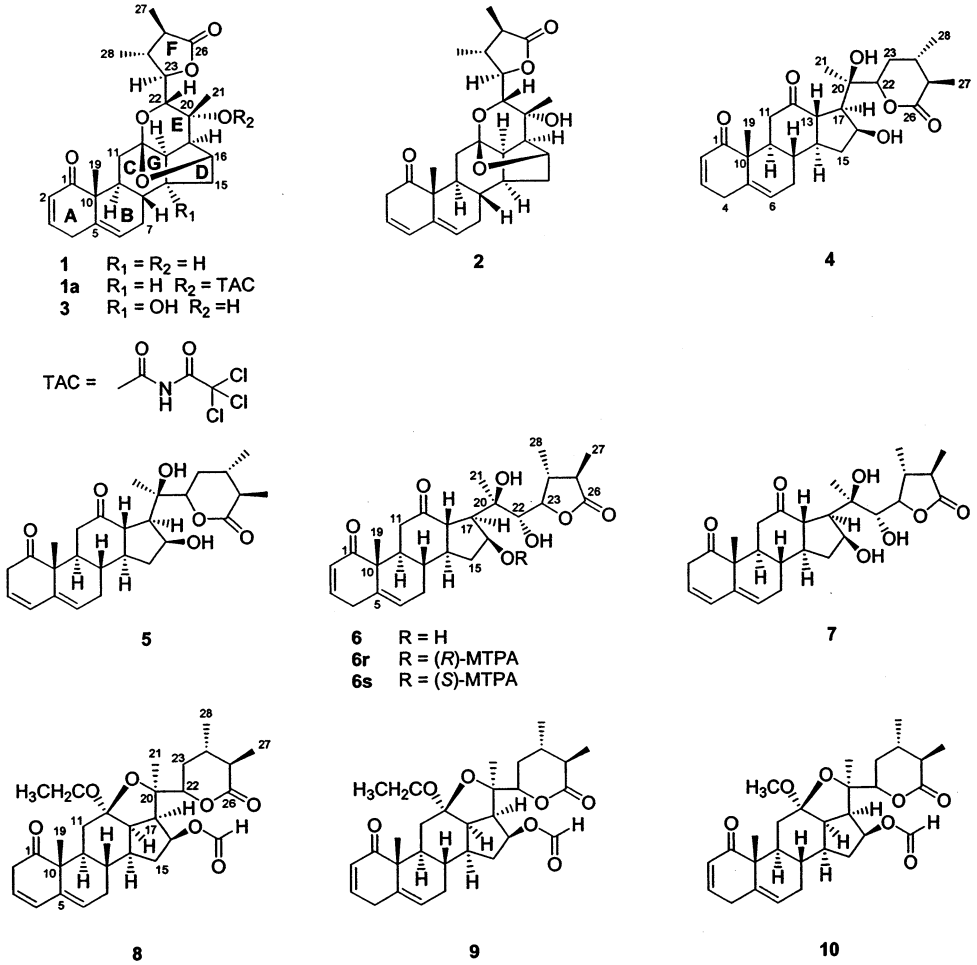

sive,[10,11] antimicrobial,[12] and antiinflammatory[13] activities. In our previous work, several withanolides isolated from *Physalis philadelphica* Lam. (tomatillo) have shown potential in vitro activity as inducers of quinone reductase (QR).[14] Structural requirements for inducing activity and cytotoxicity in the cell-based QR assay have been investigated for several subclasses of withanolides isolated from a number of plants of the Solanaceae.[15]

A literature survey has revealed that all previously reported withanolides have 28 carbons in their skeleton. In the current study, we have obtained a series of highly oxygenated C-18 norwithanolides (**1**−**10**) with a new C_{27} skeleton from the $CHCl_3$-soluble extract of *D. subtriflora*.[16] All isolates were evaluated for their potential cancer chemopreventive properties utilizing in vitro assay to determine quinone reductase induction.[2b,4,15] In this paper we describe the isolation, structure elucidation, and biological evaluation of **1**−**10**.

Results and Discussion

Structure Elucidation of Subtrifloralactones A−C (1−3). Subtrifloralactone A (**1**) was obtained as colorless needles, mp 221−222 °C, $[\alpha]^{23}_D$ −13.3° (*c* 0.15, MeOH). A molecular formula of $C_{27}H_{34}O_6$, indicating 11 degrees of unsaturation, was assigned to **1** on the basis of its HRTOFMS (*m*/*z* found 455.2425, calcd for $C_{27}H_{35}O_6$ 455.2434 $[M + H]^+$). Twenty-seven carbon signals, including four methyl groups, four methylenes, thirteen methines, and six quaternary carbons, were evident from the ^{13}C NMR and DEPT spectral data of **1** (Table 1). On the basis of the chemical shifts of these carbon signals, it was apparent that an α,β-unsaturated ketone (δ_C 203.6, C-1; 146.2, C-3; 127.7, C-2), a lactone carbonyl carbon (δ_C 178.9, C-26), a trisubstituted double bond (δ_C 135.8, C-5; 124.9, C-6), three oxygenated methine carbons (δ_C 79.3, C-16; 79.8, C-22; 82.1, C-23), and two oxygenated quaternary carbons (δ_C 105.4, C-12; 69.3, C-20) were

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(16) Part of the present study was presented at the 43rd Annual Meeting of the American Society of Pharmacognosy and Third Monroe Wall Symposium, New Brunswick, NJ, July 27−31, 2002; Abstract O-6. After our initial taxonomic identification, the species name for the plant material worked on was changed from *Solanum altissimum* to *D. subtriflora*. We are accordingly changing the trivial names of altissimumlactones A−J presented earlier to subtrifloralactones A−J herein.

TABLE 1. ^{13}C NMR Spectral Data of Compounds 1−3 and 8−10[a]

position	1[b]	1[c]	2[b]	2[c]	3[b]	3[c]	8[b]	8[c]	9[c]	10[c]
1	203.6 s	203.8 s	209.9 s	210.2 s	203.5 s	203.8 s	209.6 s	209.9 s	203.4 s	203.4 s
2	127.7 d	127.8 d	39.8 t	39.5 t	128.0 d	127.9 d	39.8 t	39.4 t	127.8 d	127.8 d
3	146.2 d	145.4 d	122.4 d	121.8 d	146.1 d	145.6 d	122.3 d	121.6 d	145.5 d	145.6 d
4	33.3 t	33.28 t[d]	129.3 d	129.2 d	33.4 t	33.3 t	129.3 d	129.0 d	33.1 t	33.0 t
5	135.8 s	135.7 s	140.9 s	140.7 s	136.4 s	135.7 s	140.7 s	140.3 s	135.7 s	135.7 s
6	124.9 d	124.7 d	127.2 d	126.7 d	124.9 d	124.7 d	126.9 d	126.3 d	124.1 d	124.1 d
7	35.1 t	35.0 t	35.3 t	35.2 t	36.1 t	35.6 t	35.0 t	34.6 t	34.5 t	34.6 t
8	37.8 d	37.5 d	36.5 d	36.3 d[d]	38.1 d	37.6 d	37.7 d	37.5 d	38.7 d	38.5 d
9	39.1 d	38.5 d	36.9 d	36.4 d[d]	37.4 d	36.8 d	34.2 d	33.8 d	36.0 d	36.1 d
10	51.2 s	51.0 s	52.7 s	52.5 s	51.5 s	51.1 s	52.5 s	52.1 s	50.3 s	50.2 s
11	34.5 t	33.31 t[d]	33.0 t	31.9 t	32.5 t	31.5 t	33.3 t	32.9 t	33.3 t	32.3 t
12	105.4 s	104.9 s	105.3 s	104.9 s	102.5 s	101.6 s	110.0 s	109.6 s	110.1 s	110.3 s
13	39.8 d	39.2 d	40.0 d	39.4 d	44.2 d	43.1 d	53.3 d	52.7 d	52.3 d	52.1 d
14	38.4 d	38.0 d	38.7 d	38.3 d	79.7 s	79.2 s	42.7 d	42.2 d	41.5 d	41.5 d
15	42.5 t	42.0 t	42.7 t	42.29 t	42.9 t	42.5 t	42.5 t	42.1 t	42.2 t	42.0 t
16	79.3 d	79.1 d	79.4 d	79.2 d	77.3 d	77.1 d	76.9 d	76.8 d	76.8 d	76.7 d
17	60.3 d	59.1 d	60.3 d	59.1 d	59.3 d	58.7 d	58.2 d	57.9 d	57.9 d	57.8 d
19	19.1 q	19.0 q	20.4 q	20.4 q	18.7 q	18.6 q	20.0 q	20.1 q	18.8 q	18.7 q
20	69.3 s	70.0 s	69.2 s	70.0 s	71.3 s	71.7 s	84.5 s	84.2 s	84.3 s	84.5 s
21	26.3 q	26.1 q	26.3 q	26.1 q	26.1 q	26.6 q	22.0 q	21.9 q	22.0 q	21.9 q
22	79.8 d	78.8 d	79.7 d	78.8 d	79.4 d	78.6 d	77.3 d	77.1 d	77.1 d	77.1 d
23	82.1 d	81.7 d	82.1 d	81.1 d	81.9 d	81.5 d	32.4 t	32.3 t	32.4 t	32.4 d
24	43.0 d	43.5 d	42.3 d	43.4 d	42.9 d	43.5 d	31.4 d	31.1 d	31.1 d	31.1 d
25	42.3 d	42.3 d	42.9 d	42.32 d	42.4 d	42.3 d	40.6 d	40.8 d	40.8 d	40.9 d
26	178.9 s	179.0 s	179.0 s	179.0 s	178.9 s	178.9 s	175.6 s	175.5 s	175.5 s	175.5 s
27	13.8 q	13.4 q	13.8 q	13.5 q	13.7 q	13.4 q	14.5 q	14.2 q	14.2 q	14.2 q
28	18.1 q	17.8 q	18.0 q	17.8 q	17.9 q	17.8 q	21.1 q	21.2 q	21.2 q	21.2 q
OCOH							161.5 d	160.2 d	160.3 d	160.3 d
O*C*H_2CH$_3$							56.7 t	56.6 t	56.7 t	
OCH$_2$*C*H_3							16.1 q	15.5 q	15.6 q	
OMe										48.9 q

[a] Spectra taken at 125 MHz, with chemical shift values assigned on the basis of observed 2D NMR correlations and presented in parts per million. [b] In pyridine-d_5. [c] In $CDCl_3$. [d] Data in the same column are interchangeable.

present in the molecule of **1**. The most notable feature in the ^{13}C NMR spectrum of **1** was the observation of a resonance signal of a doubly oxygenated quaternary carbon in a downfield region (δ_C 105.4, C-12), which suggested the presence of a hemiketal or ketal functionality.[17] Consistent with the ^{13}C NMR spectral data analysis, the ^{1}H NMR spectrum of **1** (Table 2) displayed four methyl signals, two singlets at δ_H 1.15 (3H, s, CH$_3$-19) and 1.60 (3H, s, CH$_3$-21), two doublets at δ_H 1.18 (3H, d, J = 6.2 Hz, CH$_3$-28) and 1.20 (3H, d, J = 6.8 Hz, CH$_3$-27), three oxygenated methine protons at δ_H 4.41 (1H, s, H-16), 4.10 (1H, d, J = 7.9 Hz, H-22) and 4.74 (1H, t, J = 8.2 Hz, H-23), and three olefinic protons at δ_H 5.99 (1H, br dd, J = 10.0, 2.1 Hz, H-2), 5.38 (1H, br d, J = 5.6 Hz, H-6), and 6.70 (1H, ddd, J = 10.0, 4.9, 2.4 Hz, H-3), as well as other aliphatic proton signals.

Analysis of the ^{1}H−^{1}H COSY, HMBC, and NOESY spectra of **1**, after assignment of all protons to their directly bonded carbons from the HMQC correlations observed, enabled the structural units of rings A and B and the γ-lactone ring F to be determined. These three partial structural units and a consideration of the chemotaxonomy of the genus *Deprea* suggested that compound **1** is a withanolide derivative.[5,14,18] Further inspection of the chemical shifts of the remaining carbon signals indicated that four carbons (δ_C 105.4, C-12; 79.3, C-16; 69.3, C-20; 79.8, C-22) were linked to oxygen atoms. However, it was clear that only three oxygen atoms were attached to these four oxygenated carbons, since of the six oxygen atoms in the molecular formula of **1**, three were already assigned in the α,β-unsaturated ketone and the γ-lactone ring. Therefore, the presence of two oxygen ether bridges and only one hydroxyl group in the molecule of **1** could be deduced. Compound **1** did not undergo acetylation with acetic anhydride in pyridine, suggesting that the hydroxyl group was attached to a quaternary carbon, either C-12 or C-20. However, compound **1** reacted with trichloroacetyl isocyanate to afford a monocarbamate (**1a**),[19] which was characterized in its ^{1}H NMR spectrum (Experimental Section) by a singlet in the undisturbed downfield area at δ_H 8.40. Furthermore, both the CH$_3$-21 and H-17 signals of the monocarbamate **1a** showed significant downfield shifts with values of 0.40 and 1.16 ppm, respectively, when compared with analogous data for **1**. This permitted the placement of the only hydroxyl group in compound **1** at C-20.[19] Thus, two oxygen ether bridges could be proposed for **1** between C-12 and C-16, and between C-12 and C-22. Accordingly, the signal at δ_C 105.4 represented a ketal rather than a hemiketal carbon. In the HMBC spectrum of **1**, the correlations from H-16 and H-22 to C-12 and the correlations from H-9, H$_2$-11, H-13, H-14, and H-17 to C-12 confirmed the presence and the position of this ketal

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TABLE 2. ^{1}H NMR Spectral Data of 1−3[a]

position	**1**[b]	**1**[c]	**2**[b]	**2**[c]	**3**[b]	**3**[c]
2	5.99, br dd (10.0, 2.1)	5.88, ddd (10.0, 2.0, 0.9)	3.32, br d (20.0) 2.79, dd (20.0, 4.7)	3.27, br d (20.0) 2.75, br dd (20.0, 4.4)	5.96, dd (10.0, 2.2)	5.88, dd (10.0, 2.1)
3	6.70, ddd (10.0, 4.9, 2.4)	6.79, ddd (10.0, 5.0, 2.5)	5.58, br dt (9.7, 4.8)	5.61 (overlapped with H-6)	6.67, ddd (10.0, 4.9, 2.4)	6.79, ddd (10.0, 5.0, 2.5)
4	3.15, br d (21.3) 2.70, br dd (21.3, 4.9)	3.27, br dd (21.4, 2.2) 2.85, br dd (21.4, 4.9)	6.03, br d (9.7)	6.04, br d (9.3)	3.15, br d (21.3) 2.70, br dd (21.3, 4.9)	3.27, br dd (21.3, 2.3) 2.85, br dd (21.3, 5.0)
6	5.38, br d (5.6)	5.54, br d (5.9)	5.49, br d (3.7)	5.61 (overlapped with H-3)	5.40, br d (5.7)	5.54, br d (5.8)
7	2.05−2.13, m 1.60−1.64, m	2.18−2.27, m 1.77−1.83, m	2.27, m 1.64, m	2.34−2.47, m 1.78−1.89, m	2.12−2.18, m 1.90, m	2.13−2.26, m 1.87−1.92, m
8	1.44, m	1.46−1.48, m	1.58−1.65, m	1.46−1.57, m	1.63, m	1.49, m
9	2.41, dd (12.8, 5.6)	2.18−2.27, m	2.60, m	2.08−2.18, m	3.22−3.27, m	2.61−2.68, m
11	3.10, dd (14.0, 5.6) 2.33, t (12.8)	2.60, dd (14.6, 6.2) 1.91−2.00, m	2.67, dd (14.0, 6.1) 2.08−2.15, m	2.24, dd (14.7, 6.3) 1.74, dd (14.7, 12.5)	3.22−3.27, m 2.43, dd (16.7, 14.1)	2.61−2.68, m 1.99, dd (16.5, 13.6)
13	3.07, br d (4.7)	2.58, br d (6.0)	3.09, br d (5.1)	2.62, br d (5.2)	2.09, m	1.87−1.92, m
14	1.70, m	1.77−1.83, m	1.64−1.72, m	1.78−1.89, m		
15	1.93, br t (11.4) 1.50, br d (12.3)	1.91−2.00, m 1.46−1.48, m	1.93, br t (11.4) 1.51, br d (12.2)	1.78−1.89, m 1.46−1.57, m	2.12−2.18, m 1.70, br d (12.3)	2.13−2.26, m 1.63, br d (12.6)
16	4.41, s	4.28, s	4.41, s	4.29, s	4.30, br s	4.14, br s
17	2.25, br s	2.06, br s	2.25, br s	2.06, br s	2.24, br s	2.02, br s
19	1.15, s	1.19, s	1.28, s	1.31, s	1.21, s	1.20, s
21	1.60, s	1.41, s	1.60, s	1.41, s	1.54, s	1.40, s
22	4.10, d (7.9)	3.76, d (8.3)	4.09, d (8.2)	3.74, d (8.3)	4.18, d (8.1)	3.84, d (8.1)
23	4.74, t (8.2)	4.30, t (8.3)	4.73, t (8.2)	4.30, t (8.3)	4.76, t (8.1)	4.39, t (8.1)
24	2.21, m	2.07−2.18, m	2.17−2.22, m	2.08−2.18, m	2.26, m	2.13−2.26, m
25	2.05−2.13, m	2.07−2.18, m	2.08−2.15, m	2.08−2.18, m	2.12−2.18, m	2.13−2.26, m
27	1.20, d (6.8)	1.24, d (6.8)	1.20, d (7.0)	1.23, d (6.8)	1.20, d (6.8)	1.25, d (6.6)
28	1.18, d (6.2)	1.29, d (6.2)	1.17, d (6.4)	1.25, d (6.2)	1.20, d (6.8)	1.32, d (6.0)

[a] Spectra taken at 500 MHz, with chemical shift values assigned on the basis of observed 2D NMR correlations and presented in parts per million. *J* values are given in hertz in parentheses. [b] In pyridine-d_5. [c] In $CDCl_3$.

group. The methyl group (CH_3-21) was assigned at C-20 on the basis of the observed HMBC correlations from δ_H 1.60 (CH_3-21) to δ_C 60.3 (C-17), 69.3 (C-20), and 79.8 (C-22). Thus, compound **1** was tentatively assigned as a C-18 norwithanolide with a novel C_{27} skeleton, and having a ketal functionality at C-12. The ^1H and ^{13}C NMR data were assigned from the interpretation of its $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra.

The HRTOFMS (*m*/*z* 455.2439 $[M + H]^+$) of subtrifloralactone B (**2**) provided a molecular formula of $C_{27}H_{34}O_6$, the same as that of **1**. The MS−MS data of **2** were almost identical to those of **1**. The ^1H (Table 2) and ^{13}C (Table 1) NMR data of **2** were very close to those of **1**, and suggested compound **2** to also be a C-18 norwithanolide with the same skeleton as that of **1**. Differences between these two compounds were apparent only in the NMR chemical shifts of the α,β-unsaturated ketone unit of ring A. When compared to that of **1**, the ketone group signal (δ_C 209.9, C-1) of **2** showed a significant downfield shift (6.3 ppm), which suggested that this functionality was not conjugated to a double bond. The observed HMBC correlations from δ_H 3.32 (1H, br d, J = 20.0, Hz, H-2a) and 2.79 (1H, dd, J = 20.0, 4.7 Hz, H-2b) to δ_C 209.9 (s, C-1), 52.5 (s, C-10), 122.4 (d, C-3), and 129.3 (d, C-4), respectively, and from δ_H 6.03 (1H, br d, J = 9.7, Hz, H-4) to δ_C 39.8 (t, C-2), 52.7 (s, C-10), and 127.2 (d, C-6) permitted the placement of the double bond of ring A between C-3 and C-4 in **2**. The structure assigned to subtrifloralactone B (**2**) was further confirmed by the observed 2D NMR ($^1H-^1H$ COSY, HMQC, HMBC, and NOESY) correlations in both pyridine-d_5 and $CDCl_3$.

Both the ^1H (Table 2) and ^{13}C (Table 1) NMR data of compound **3** were also closely comparable to those of **1**. The ^{13}C NMR and DEPT spectra of **3** displayed an additional oxygenated quaternary carbon (δ_C 79.7, s, C-14) and one methine less than in **1**. These observations, in combination with the molecular formula of $C_{27}H_{34}O_7$ determined by HRTOFMS at *m*/*z* 471.2363 $[M + H]^+$ (calcd for $C_{27}H_{35}O_7$ 471.2383), suggested the presence of one more hydroxyl group in the molecule of **3** compared to **1**. In the HMBC spectrum of **3**, correlations were observed from H-6, CH_3-19, and CH_3-21, respectively, to the C-8, C-9, and C-17 methine carbons. The signals of CH_3-27 and CH_3-28 appeared as doublets in the ^1H NMR spectrum of **3**, which suggested that both C-24 and C-25 were methines. Thus, the second hydroxyl group of **3** could only be located at either C-13 or C-14. Clear correlations from one of the H_2-11 resonances (δ_H 2.43 in pyridine-d_5, and δ_H 1.99 in $CDCl_3$) to C-8, C-9, C-10, C-12, and C-13 were observed in the HMBC spectrum of **3**. This permitted the assignment of the second hydroxyl group at C-14 in subtrifloralactone C (**3**). In a manner similar to that of compound **1**, a strong HMBC correlation from H-16 to C-12 was also observed for both compounds **2** and **3**.

Structure Elucidation of Subtrifloralactones D−G (4−7). Subtrifloralactone D (**4**) was obtained as colorless needles, mp 196−197 °C, $[\alpha]^{23}_D$ +2.0° (*c* 0.15, MeOH). A molecular formula of $C_{27}H_{36}O_6$ was determined for **4** from the protonated molecular ion peak at *m*/*z* 457.2599 (calcd for $C_{27}H_{37}O_6$ 457.2590) obtained by HRTOFMS. Similar to those of compounds **1**−**3**, four methyl groups represented by two singlets at δ_H 1.14 (3H, s, CH_3-19) and 1.54 (3H, s, CH_3-21), and two doublets at δ_H 1.21 (3H, d, J = 6.7 Hz, CH_3-27) and 0.93 (3H, d, J = 6.7 Hz, CH_3-28), were apparent in the ^1H NMR spectrum (Table 3) of **4**. The ^{13}C NMR (Table 4) and other ^1H NMR data of **4** were also closely comparable to those of **1**−**3**, but one difference evident for **4** compared to **1**−**3** was a signal for a nonconjugated ketone (δ_C 209.8) group in the ^{13}C NMR spectrum of **4** instead of the ketal group present in **1**−**3**. This nonconjugated ketone was placed at C-12 on the basis of the observed HMBC correlations from H-9, H_2-11, H-13, H-14, and H-17 to the carbon signal of this ketone. In addition, the chemical shift of the lactone carbonyl carbon (δ_C 175.7) of **4** showed an upfield shift compared to those of **1**−**3**, which suggested that a six-membered lactone ring rather than a five-membered lactone ring was present.[14b] The presence of both this lactone ring and the same A and B rings as those in **1** was confirmed by the 2D NMR correlations observed for **4**. The ^{13}C NMR spectrum of **4** in pyridine-d_5 displayed only 26 carbons, with the signal corresponding to C-17 being absent. However, when $CDCl_3$ was used as the solvent, a very weak signal of C-17 at δ_C 47.4 was apparent, which was assigned on the basis of the correlations from H-17 and CH_3-21 to this weak carbon signal in the HMQC and HMBC spectra.

Subtrifloralactones E (**5**) and D (**4**) differed only in their A rings, analogous to the structural difference between compounds **1** and **2**, by comparison of their ^1H (Table 3) and ^{13}C (Table 4) NMR data. The HRTOFMS of **5** gave a molecular formula of $C_{27}H_{37}O_6$, the same as that for **4**. The observed 2D NMR correlations further supported the positions of two double bonds as being located at C-3, C-4 and C-5, C-6 in **5**.

The HRTOFMS data of **6** and **7** gave the same molecular formula of $C_{27}H_{37}O_7$ for each compound, representing one oxygen atom more than the elemental formula of both compounds **4** and **5**. The ^1H (Table 3) and ^{13}C (Table 4) NMR spectra of compounds **6** and **7** indicated that they are a further pair of isomers with an enone and a nonconjugated ketone in their A ring, respectively. Compounds **6** and **4**, and **7** and **5**, were assigned with the same substituent patterns in rings A−D, as concluded by comparison of their 1D and 2D NMR spectral data. The chemical shifts of the lactone carbonyl carbons (Table 4) of **6** and **7** showed downfield shifts comparable to those of **4** and **5**, and suggested the presence of a five-membered lactone ring rather than six-membered lactone ring in both the molecules of **6** and **7**.[14b] Furthermore, consistent with the HRTOFMS data, the ^{13}C NMR spectra of **6** and **7** displayed one more oxygenated methine than observed in the ^{13}C NMR spectra of **4** and **5**. All of this evidence indicated that compounds **6** and **7** varied from **4** and **5** only in their respective side chains. The side chains of compounds **6** and **7** were both assigned as the same as that present in the known compound ixocarpalactone A[14b] as a result of detailed analysis of their 2D NMR spectral data. The structure of ixocarpalactone A was confirmed by X-ray crystallography.[14b]

Structure Elucidation of Subtrifloralactones H−J (8−10). The ^1H (Table 5) and ^{13}C (Table 1) NMR data of subtrifloralactone H (**8**) were very similar to those of **2**, and suggested the occurrence of the same A and B ring functionalities in these two compounds. The HRTOFMS of **8** provided a molecular formula determination of

TABLE 3. ^{1}H NMR Spectral Data of 4−7[a]

position	**4**[b]	**4**[c]	**5**[b]	**5**[c]	**6**[b]	**6**[c]	**7**[c]
2	5.94, dd (10.0, 2.1)	5.90, dd (10.1, 2.2)	3.28, br d (20.0) 2.76, dd (20.0, 4.7)	3.28, br d (20.2) 2.77, dd (20.2, 4.6)	5.95, dd (10.0, 2.0)	5.90, dd (10.0, 2.2)	3.29, br d (20.2) 2.81−2.85, m
3	6.68, ddd (10.0, 5.0, 2.4)	6.81, ddd (10.1, 5.0, 2.5)	5.57, br dt (9.5, 4.2)	5.64−5.66, m	6.70, ddd (10.0, 4.9, 2.0)	6.83, ddd (10.0, 4.9, 2.4)	5.66−5.67, m
4	3.15, br dd (21.2, 2.4) 2.72, dd (21.2, 5.0)	3.31, br dd (21.2, 2.4) 2.91, dd (21.2, 4.9)	6.05, br d (9.6)	6.10, br d (9.2)	3.18, br d (21.3) 2.74, dd (21.3, 4.9)	3.32, br dd (21.4, 2.3) 2.92, dd (21.4, 4.9)	6.10, br d (9.1)
6	5.41, br d (5.9)	5.60, br d (6.0)	5.54, br d (3.2)	5.64−5.66, m	5.42, br d (5.7)	5.61, br d (5.9)	5.66−5.67, m
7	2.02, m 1.49−1.60, m	2.18, m 1.66, m	2.14−2.18, m 1.57−1.61, m	2.34−2.40, m 1.73, m	2.00, m 1.56, m	2.16−2.27, m 1.70−1.74, m	2.37−2.43, m 1.81, m
8	1.65−1.73, m	1.74, m	1.84, ddd (21.4, 10.7, 5.5)	1.84−1.87, m	1.64−1.69, m	1.70−1.74, m	1.81, m
9	2.34, ddd (12.5, 4.5, 4.5)	2.24−2.27, m	2.48−2.59, m	2.34−2.40, m	2.30−2.41, m	2.37−2.44, m	2.37−2.43, m
11	3.60, dd (12.4, 6.7) 2.75, t (12.4)	3.21, dd (12.5, 4.5) 2.35−2.45, m	3.11, dd (11.4, 3.3) 2.48−2.59, m	2.81, dd (11.8, 4.4) 2.22−2.26, m	3.50, dd (12.1, 4.4) 2.56, t (12.3)	3.23, dd (12.9, 4.6) 2.37−2.44, m	2.81−2.85, m 2.17−2.25, m
13	3.57, t (11.8)	3.02, t (11.7)	3.58, t (11.6)	3.02, t (11.4)	3.46, t (11.9)	2.76, t (11.2)	2.76, t (11.2)
14	1.29−1.34, m	1.38−1.44, m	1.34, m	1.36, m	1.22, m	1.36−1.48, m	1.44−1.48, m
15	2.42, m 1.65−1.73, m	2.35−2.45, m 1.38−1.44, m	2.41, m 1.67−1.71, m	2.44, m 1.40, m	2.30−2.41, m 1.64−1.69, m	2.37−2.44, m 1.36−1.48, m	1.44−1.48, m
16	4.62, br q (6.5)	4.46, br q (6.6)	4.62, m	4.47, m	4.83, br q (7.2)	4.53, br q (7.0)	4.54, m
17	2.52, m	2.35−2.45, m	not observed	2.34−2.40, m	2.91, dd (10.4, 8.5)	2.56, dd (10.3, 8.8)	2.57, dd (10.3, 8.7)
19	1.14, s	1.28, s	1.25, s	1.42, s	1.16, s	1.29, s	1.43, s
21	1.54, s	1.13, s	1.54, s	1.12, s	1.62, s	1.32, s	1.32, s
22	5.34, dd (10.6, 3.8)	4.91, dd (11.2, 3.6)	5.34, dd (10.5, 3.8)	4.91, dd (11.2, 3.6)	4.77, d (6.0)	3.59, d (6.3)	3.56, t (5.9)
23	2.28, m 1.49−1.60, m	2.11, m 1.56, dt (14.2, 3.8)	2.29, ddd (14.2, 10.5, 8.4) 1.57−1.61, m	2.13, ddd (14.2, 11.2, 8.7) 1.56, dt (14.2, 3.8)	4.46, dd (8.3, 6.2)	4.20, dd (8.2, 6.7)	4.20, dd (8.5, 6.5)
24	1.65−1.73, m	1.78, m	1.67−1.71, m	1.79, m	2.46, m	2.16−2.27, m	2.17−2.25, m
25	2.16, m	2.24−2.27, m	2.14−2.18, m	2.22−2.26, m	2.26, m	2.16−2.27, m	2.17−2.25, m
27	1.21, d (6.7)	1.24, d (6.7)	1.20, d (6.7)	1.23, d (6.7)	1.19, d (7.0)	1.24, d (6.4)	1.24, d (6.5)
28	0.93, d (6.7)	1.16, d (6.8)	0.93, d (6.7)	1.15, d (6.8)	1.36, d (6.5)	1.26, d (5.8)	1.26, d (5.8)

[a] Spectra taken at 500 MHz, with chemical shift values assigned on the basis of observed 2D NMR correlations and presented in parts per million. *J* values are given in hertz in parentheses. [b] In pyridine-d_5. [c] In $CDCl_3$.

TABLE 4. ^{13}C NMR Spectral Data of Compounds 4−7[a]

position	4[b]	4[c]	5[b]	5[c]	6[b]	6[c]	7[c]
1	202.9 s	202.8 s	209.2 s	209.1 s	203.0 s	202.9 s	209.2 s
2	127.6 d	127.8 d	39.8 t	39.4 t	127.7 d	127.7 d	39.4 t
3	146.2 d	145.4 d	122.7 d	122.1 d	146.3 d	145.6 d	122.1 d
4	33.3 t	33.3 t	129.2 d	129.0 d	33.3 t	33.3 t	128.9 d
5	135.9 s	135.6 s	140.9 s	140.5 s	135.9 s	135.6 s	135.6 s
6	124.2 d	123.9 d	126.5 d	125.9 d	124.3 d	123.9 d	140.4 d
7	30.4 t	30.3 t[d]	31.0 t[d]	30.4 t[d]	30.5 t	30.2 t	30.4 t
8	39.3 d	39.2 d	38.0 d	37.9 d	39.3 d	38.7 d	37.5 d
9	46.3 d	45.7 d	44.5 d	43.8 d	46.5 d	45.7 d	43.8 d
10	51.0 s	50.7 s	52.6 s	52.3 s	51.0 s	50.7 s	53.9 s
11	44.7 t	44.2 t	43.5 t	42.9 t	44.9 t	43.9 t	42.7 t
12	209.8 s	211.3 s	209.2 s	210.8 s	212.1 s	213.2 s	212.6 s
13	57.3 d	56.7 d	57.6 d	56.9 d	57.3 d	57.3 d	57.6 d
14	51.4 d	50.4 d	51.5 d	50.4 d	51.0 d	49.9 d	49.9 d
15	41.3 t	40.4 t	41.1 t	40.3 t	41.7 t	39.9 t	39.8 t
16	72.5 d	73.5 d	72.4 d	73.5 d	73.2 d	73.5 d	73.6 d
17	NO[e]	47.4 d[f]	NO[e]	47.3 d[f]	47.7 d[f]	45.5 d[f]	45.4 d[f]
19	18.7 q	18.8 q	19.9 q	20.0 q	18.8 q	18.8 q	20.0 q
20	74.9 s	75.7 s	74.8 s	75.6 s	77.0 s	76.7 s	76.7 s
21	22.9 q	22.4 q	22.9 q	22.4 q	22.5 q	21.2 q	21.1 q
22	80.4 d	80.4 d	80.5 d	80.4 d	77.4 d	77.3 d	77.3 d
23	31.1 t	30.2 t[d]	30.6 t[d]	30.3 t[d]	84.7 d	82.3 d	82.8 d
24	31.4 d	31.1 d	31.4 d	31.1 d	41.7 d	41.6 d	41.6 d
25	40.8 d	40.5 d	40.8 d	40.5 d	42.9 d	42.6 d	42.6 d
26	175.7 s	176.0 s	175.7 s	176.0 s	179.2 s	179.2 s	179.2 s
27	14.6 q	14.1 q	14.6 q	14.1 q	13.8 q	13.4 q	13.3 q
28	20.9 q	21.0 q	20.9 q	21.1 q	17.9 q	17.5 q	17.5 q

[a] Spectra taken at 125 MHz, with chemical shift values assigned on the basis of observed 2D NMR correlations and presented in parts per million. [b] In pyridine-d_5. [c] In $CDCl_3$. [d] Data in the same column are interchangeable. [e] Signals were not observed. [f] Very weak signal observed.

$C_{30}H_{40}O_7$ (*m/z* 535.2692 $[M + Na]^+$, calcd for $C_{30}H_{40}O_7$-Na 535.2672), indicating 11 degrees of unsaturation. Consistent with this molecular formula, the ^{13}C NMR spectrum of **8** displayed 30 carbon signals, including a ketal group at δ_C 110.0 (C-12) similar to those in **1**−**3** and a six-membered lactone carbonyl carbon at δ_C 175.6 (C-26), like in **4** and **5**. Compared to those of **2**, the differences evident in the 1D NMR spectra of **8** were the signals of an ethoxyl group at δ_H 1.37 (3H, t J = 7.0 Hz, OCH_2CH_3) and 4.18 and 3.90 (each 1H, m, OCH_2CH_3), and δ_C 56.7 (t, OCH_2CH_3) and 16.1 (q, OCH_2CH_3). In addition, the ^{1}H and ^{13}C NMR spectra of **8** exhibited two downfield signals at δ_H 8.46 and δ_C 161.5. These two signals correlated with each other in the HMQC spectrum, and the DEPT spectrum indicated this carbon was a methine. From a combination of this evidence, a formate ester group[20] was apparent in the molecule of **8**. The observed HMBC correlations from δ_H 4.18 and 3.90 (OCH_2CH_3) to δ_C 110.0 (C-12), from δ_H 8.46 (1H, s, OCOH) to δ_C 76.9 (C-16), and from δ_H 5.53−5.57 (H-16) to δ_C 161.5 (OCOH) indicated that the ethoxyl and formate ester groups were attached to C-12 and C-16, respectively. Six oxygen atoms could be readily assigned, one for the nonconjugated ketone of the A ring, one for the ethoxyl group at C-12, two for the formate ester group at C-16, and two for the δ-lactone ring in the side chain. As a consequence of the molecular formula and the chemical shifts of C-12 (ketal group at δ_C 110.0) and C-20 (δ_C 69.3) of **8**, the seventh oxygen atom had to be attached to both C-12 and C-20 by an oxygen ether bridge. The ^{1}H (Table 5) and ^{13}C (Table 1) NMR data of **8** were assigned by the observed ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY correlations in both pyridine-d_5 and $CDCl_3$.

The molecular formula of subtrifloralactone I (**9**) was determined as $C_{30}H_{40}O_7$, the same as that of **8**. Comparison of the 1D and 2D NMR data of compounds **8** and **9** resulted in the conclusion that **9** is isomeric with **8** in their A rings, in the same manner as described for withanolide isomers **1** and **2**, and **4** and **5**.

Compared to those of **9**, the only difference in the ^{1}H and ^{13}C NMR spectra of subtrifloralactone J (**10**) were the signals of a methoxyl group (δ_H 3.52 and δ_C 48.9) instead of the signals of the ethoxyl group. The HRTOFMS of **10** led to the assignment of a molecular formula of $C_{29}H_{38}O_7$, one methylene less than that of **9**. In the HMBC spectrum of **10**, the methoxy signal at δ_H 3.52 correlated with the ketal at δ_C 110.3, and confirmed that a methoxy group in **10** replaced the ethoxyl group in **9**.

Determination of the Configuration of Subtrifloralactones A−J (1−10). The relative configurations of compounds **1**−**10** were established primarily by analysis of the coupling constants of their ^{1}H NMR signals, and by solvent-induced chemical shift differences in pyridine-d_5 and $CDCl_3$,[14b,21] in addition to the observed correlations from their NOESY spectra. In the same manner as for previously isolated structurally related withanolides,[14,18,19] the angular methyl group (CH_3-19) in compounds **1**−**10** adopted a β-orientation, and rings B and C were *trans*-fused with the 8β and 9α protons. These configurations were assigned on the basis of the observed correlations from CH_3-19 to H-8 and from H-9 to H-11α in the NOESY spectra of **1**−**10**.

In the NOESY spectra of **1**−**3**, clear correlations from H-17 to H-16 and H-13 and from H-13 to H-14 and H-17 were observed, indicating that H-13, H-14, H-16, and H-17 are on the same face of each molecule. Further inspection of their NOESY spectra disclosed correlations from H-15α to H-16 and H-17, and from H-8 to H-15β. These observations permitted the assignment of H-13, H-14, H-16, and H-17 to be in an α orientation; therefore, rings C and D were *cis*-fused in the molecules of **1**−**3**. The H-17 signal of compounds **1**−**3** was observed in each case as a broad singlet (Table 2), indicating the dihedral angles between both H-17 and H-16 and H-17 and H-13 were close to 90°. The CH_3-21 signal of **1**−**3** displayed significant NOESY correlations with H-16, H-17, and H-22, and these observations allowed the relative configurations of C-20 and C-22 to be determined. The assignment was consistent with the *R* absolute configuration of C-20 of previously determined withanolides where the order of priority is OH-20, C-22, and C-17.[5,14b,18,22] The evident chemical shift differences ($\Delta = \delta_{CDCl_3} - \delta_{C_5D_5N}$) of H-23 of compounds **1**−**3** (Δ = −0.43, −0.43, and −0.37 for **1**, **2**, and **3**, respectively) (Table 2) indicated H-23 was 1,3-diaxial to OH-20.[14b,21] The cou-

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TABLE 5. ^{1}H NMR Spectral Data of 8−10[a]

position	**8**[b]	**8**[c]	$\delta_{CDCl_3} - \delta_{C_5D_5N}$	**9**[c]	**10**[c]
2	3.33, br d (20.3)	3.29, br d (20.6)	−0.04	5.89, dd (10.1, 2.2)	5.89, dd (10.0, 2.1)
	2.78, dd (20.3, 4.5)	2.78, dd (20.5, 4.4)	0		
3	5.53−5.57, m	5.60−5.64, m		6.80, ddd (10.1, 5.0, 2.4)	6.80, ddd (10.0, 5.0, 2.1)
4	6.06, br d (9.7)	6.06, br d (7.9)	0	3.26, br dd (21.4, 3.0)	3.26, br dd (21.4, 3.0)
				2.86, dd (21.4, 5.0)	2.86, dd (21.4, 5.0)
6	5.53−5.57, m	5.60−5.64, m		5.53, br d (5.8)	5.53, br d (5.8)
7	2.26−2.32, m; 1.54−1.62, m	2.38, m; 1.72, m		2.06−2.20, m; 1.70, m	2.13−2.19, m; 1.70, m
8	1.54−1.62, m	1.46−1.50, m		1.35, m	1.35, m
9	2.26−2.32, m	2.11, m		1.90−1.97, m	1.89, m
11	2.63, br d (12.6)	2.45, br d (13.4)	−0.18	2.94, br d (13.0)	2.95, br d (11.4)
	1.73, t (12.7)	1.42, t (12.9)	−0.31	1.49−1.53, m	1.27, m
13	3.27, t (10.6)	3.18, t (10.5)	−0.09	3.14, t (10.4)	3.12, t (10.1)
14	2.07−2.16, m	2.27, m		2.06−2.20, m	2.13−2.19, m
15	2.07−2.16, m	2.05−2.08, m		2.06−2.20, m	2.13−2.19, m
	1.98, br d (13.9)	1.92−1.99, m		1.90−1.97, m	1.95, m
16	5.53−5.57, br s	5.38, br s		5.35, br s	5.34, br s
17	2.55, dd (10.2, 4.2)	2.57, dd (9.9, 4.4)	+0.02	2.57, dd (9.8, 4.6)	2.58, dd (10.0, 5.0)
19	1.27, s	1.24, s	−0.03	1.16, s	1.17, s
21	1.59, s	1.47, s	−0.12	1.46, s	1.46, s
22	4.96, dd (11.7, 2.7)	4.67, dd (11.4, 2.8)	−0.29	4.70, dd (11.2, 2.8)	4.70, dd (11.0, 2.8)
23	1.89, ddd (14.1, 11.7, 7.8)	1.92−1.99, m		1.90−1.97, m	1.91−1.97, m
	1.51, dt (14.1, 2.8)	1.49−1.53, m			
24	1.54−1.62, m	1.74−1.78, m		1.78, m	1.78, m
25	2.07−2.16, m	2.05−2.08, m		2.06−2.20, m	2.07, m
27	1.18, d (6.6)	1.20, d (7.1)	+0.02	1.22, d (6.7)	1.22, d (6.7)
28	1.02, d (6.8)	1.09, d (6.8)	+0.07	1.09, d (6.7)	1.09, d (6.8)
−OCOH	8.46, s	8.09, s	−0.37	8.09, s	8.09 s
OCH_2CH_3	4.18, m; 3.90, m	3.87, m; 3.69, m	−0.31; 0.21	4.03, m; 3.75, m	
OCH_2CH_3	1.37, t (7.0)	1.25, t (7.0)	−0.12	1.25, t (7.0)	
OMe					3.52, s

[a] Spectra taken at 500 MHz, with chemical shift values assigned on the basis of observed 2D NMR correlations and presented in parts per million. *J* values are given in hertz in parentheses. [b] In pyridine-d_5. [c] In $CDCl_3$.

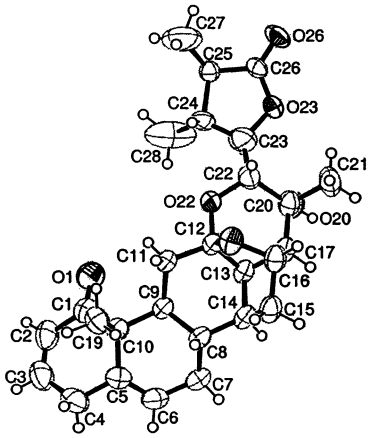

FIGURE 1. ORTEP diagram of subtrifloralactone A (**1**).

pling constants (∼8 Hz, Table 2) between H-22 and H-23 were suggestive of these two protons being in a *trans* relationship in **1**−**3**. The configurations of CH_3-27 and CH_3-28 could be assigned on the basis of the observed NOESY correlations from H-23 to CH_3-28, and from H-24 to CH_3-27, respectively. Although H-22 and H-23 adopted a more stable *trans* relationship in **1**−**3**, clear correlations between these two protons were observed in their NOESY spectra, due to the rotation of the γ-lactone rings. The structure and relative stereochemistry of **1** were verified by a single-crystal X-ray analysis (Figure 1; see below).

In compounds **4**−**7**, the splitting patterns of H-13 and the coupling constants between H-13 and H-14, and between H-13 and H-17, were different from those of compounds **1**−**3**. The H-13 resonance of **4**−**7** was displayed in each case as a triplet with the coupling constant in the range of 11.2−11.8 Hz (Table 3), indicating that the coupling constants between H-13 and H-14, and between H-13 and H-17, were almost the same, with both H-13 to H-14 and H-13 to H-17 being either axially−axially *trans*-coupled or axially−axially *cis*-coupled. On further inspection of their NOESY spectra, correlations from H-13 to H-14 and H-17 were not observed. This led to the assignment of axially−axially *trans*-coupled relationships for H-13 and H-17 and for H-13 and H-14 in **4**−**7**. The H-17 signal of compounds **4** and **5** was overlapped with other proton signals, and this resonance was not observed for **5** in pyridine-d_5. However, in the ^{1}H NMR spectra of compounds **6** and **7** (Table 3), the H-17 signal was exhibited clearly as a double doublet, which was different from the H-17 signal of **1**−**3** (broad singlet). The coupling constants of H-17 and H-13 (∼10.3 Hz) were consistent with their *trans* orientation, while the coupling constants of H-17 and H-16 (∼8.7 Hz) suggested these two protons were axially−axially *cis*-coupled in **6** and **7**. In the NOESY spectra of **4**−**7**, correlations from H-13 to H-8 and H-15β and from H-17 to H-16 and CH_3-21 were observed, suggesting a β-orientation for H-13, with accordingly H-14, H-16, and H-17 all being in an α-orientation. The side chain configurations of **4**−**7** were also established by interpretation of the observed coupling constants, NOESY correlations, and solvent-induced chemical shifts, as well as by comparison of these data with those of published values for the related withanolides,[14b] in a manner similar to those described for **1**−**3**. Finally, the relative stereochemistry of **4** was confirmed unambiguously by a single-crystal X-ray diffraction analysis (Figure 2; see below).

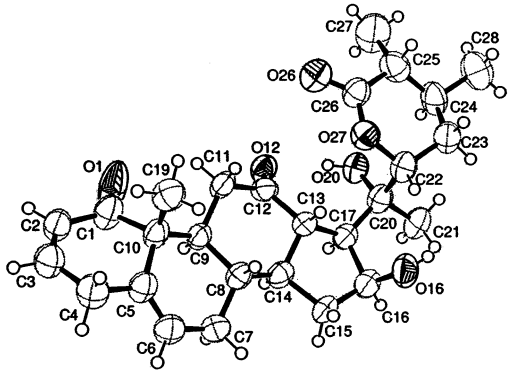

FIGURE 2. ORTEP diagram of subtrifloralactone D (**4**).

The ^{1}H NMR signals of H-13 and H-17 of compounds **8**−**10** were displayed as a triplet and double doublet, respectively, and the splitting patterns and coupling constants of these signals were similar to those of compounds **4**−**7**. However, significant NOESY correlations from H-13 to both H-14 and H-17 were obtained for compounds **8**−**10**. This permitted the determination of *cis* relationships for H-13, H-14, and H-17 in **8**−**10**. The small dihedral angles between both H-13 and H-14 and H-13 and H-17 could be inferred from their large *cis*-coupling constants ($J_{H-13/H-14} \cong J_{H-13/H-17} \cong 10$ Hz) (Table 5). The oxygenated methylene protons of the ethoxyl groups of **8** and **9** and the methoxyl group of **10** showed NOESY correlations with H-11α, indicating that these substituents could be assigned with an α-orientation. The NOESY spectra of **8**−**10** also revealed correlations from H-17 to H-16, CH_3-21, and H-22, from H-22 to CH_3-28, and from H-24 to CH_3-27. These observations, together with biogenetic considerations, suggested that the relative configurations of the C-20 side chains of **8**−**10** were consistent with those of **1**−**7**.

Single-Crystal X-ray Diffraction Analysis of Compounds 1 and 4 and Absolute Stereochemistry of Compounds 1 and 6. As postulated above for **1**−**10** by spectroscopic and chemical methods, compounds **1**−**3** and **8**−**10** possess a ketal group at C-12 and the C and D rings were *cis*-fused in their molecules, while a ketone rather than a ketal functionality was present in compounds **4**−**7**, and the C and D rings were *trans*-fused. A suitable crystal of **1** was obtained from hexanes−EtOAc (1:1), and two X-ray data sets were collected on this compound. The first data set was collected at room temperature with CuKα radiation, and the result determined the absolute configuration of this compound as shown in Figure 1 on the basis of the Flack absolute structure parameter 0.08 (16). However, one of the rings was disordered in the data set collected at room temperature, splitting atoms from C-23 to C-28, and each was set at 50% occupancy. Another set of data was collected at low temperature (150 K) with MoKα radiation. There were two molecules in the asymmetric unit, and no disorder, which further confirmed the structure and stereochemistry of **1** proposed. A small crystal of compound **4** was obtained from hexanes−EtOAc (1:1), 10 × 40 × 40 μm, and was selected for X-ray data collection. It was immersed in glycerol and cooled to 100 K to minimize crystal degradation and X-ray radiation damage. This permitted the generation of an X-ray diffraction analysis structure (Figure 2) and confirmed the structure and relative stereochemistry proposed for **4**.

TABLE 6. QR-Inducing Activity of Compounds 1−10[a]

compd	CD[b] (μM)	IC_{50}[c] (μM)	CI[d]
1	0.55	35.5	64.5
2	>11.1	>11.0	ND[e]
3	0.72	4.9	6.8
4	0.18	1.3	7.2
5	>11.0	>11.0	ND[e]
6	0.42	29.9	71.2
7	>21.2	>42.4	ND[e]
8	>9.8	>9.8	ND[e]
9	3.5	>9.8	>2.8
10	0.62	3.6	5.7
sulforaphane[f]	0.49	11.7	23.9

[a] Test compounds were considered as inactive when the CD value[b] was >5 μg/mL. [b] Concentration required to double QR induction activity (μM). [c] Concentration required to inhibit cell growth by 50% (μM). [d] Chemopreventive index, IC_{50}/CD. [e] ND = not determined. [f] Sulforaphane[14a,15] was used as a positive control substance.

Among the present isolates, the most significant activity in terms of chemopreventive index was demonstrated by compounds **1** and **6** in the quinone reductase assay (see Biological Activity below). Therefore, the absolute configuration of the representative isolate **6** was determined using the Mosher ester procedure. Compound **6** was treated with (*S*)-(+)-α- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride in anhydrous pyridine at room temperature overnight, to afford the (*R*)- and (*S*)-MTPA ester derivatives **6r** and **6s**, respectively. The ^{1}H NMR spectral data of **6r** and **6s** (δ_{S-R}) indicated that the absolute configuration of C-16 was *S*.[23] This result was consistent not only with the absolute stereochemistry of compound **1** determined by X-ray analysis in the present study but also with the previously determined absolute configurations for natural withanolides.[14b,24]

Biological Activity. The potential of **1**−**10** to induce quinone reductase[2b,4,15] was evaluated, and the data obtained are summarized in Table 6. We have previously proposed that 4*β*-hydroxy-2-en-1-one and 5*β*,6*β*-epoxy units of withanolides are necessary for their activities in cell-based quinone reductase induction assay, on the basis of the structural characteristics and the bioassay evaluation results of some withanolides isolated from several plants in the family Solanaceae.[14,15] The present results further verified that an α,*β*-unsaturated ketone structure unit appears to be necessary for the quinone reductase induction activity of withanolides, since all withanolides isolated with such a functionalitiy in their A rings (**1**, **3**, **4**, **6**, **9**, and **10**) exhibited significant quinone reductase-inducing activity (Table 6), with CD values in the range of 0.18−3.5 μM. Compounds lacking this

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structural characteristic (**2**, **5**, **7**, and **8**) were found to be inactive (Table 6) in this assay (CD > 5 μg/mL). Among these novel withanolides, the most potent quinone reductase-inducing activity was demonstrated by compound **4** (CD 0.18 μM), while compounds **1** and **6** showed the highest chemoprevention index values of 64.5 and 71.2, respectively (Table 6).

Experimental Section

General Procedures. Melting points are uncorrected. NMR spectra were obtained on a 500 MHz spectrometer with tetramethylsilane (TMS) as an internal standard. Standard pulse sequences were employed to obtain ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY NMR spectra. The room temperature (298 K) X-ray crystallographic analysis data of compound **1** were collected on an instrument described in ref 25, while low-temperature (150 K) data for this compound were collected on an instrument described in ref 26. Diffraction data of compound **4** were collected at the Advanced Photon Source on a CCD area detector. The SIR-92 direct methods package[27] was used to locate the non-hydrogen atoms, and the WinGX package[28] was used for completing the structure determination, with PLUTON[29] and ORTEP[30] used for the figures. Column chromatography was carried out with Si gel G (230−400 mesh). Analytical thin-layer chromatography (TLC) was performed on precoated 250 μm thick Si gel 60 F_{254} aluminum plates, while preparative thin-layer chromatography was performed on precoated 500 or 1000 μm thick Si gel 60 F_{254} glass plates.

Material. The whole plant of *D. subtriflora* (Ruiz & Pavon) D'arcy (Solanaceae) was collected (number 230) on a river bank at 250 m elevation, 10° 04′ S and 71° 06′ W, in Peru in October 1997. The collection was performed under full sun, and a voucher specimen (P2858) has been deposited at the University of Illinois Pharmacognosy Field Station, Downers Grove, IL.

Evaluation of the QR-Inducing Ability of Isolates. The evaluation was done as described previously.[2b,4,15] Enzyme activity was expressed as CD (the concentration required to double the specific activity of QR), IC_{50} (the half-maximal inhibitory concentration of cell viability), and CI (chemoprevention index), IC_{50}/CD. Test extracts, chromatographic fractions, and pure compounds were considered active when their CD value was <5 μg/mL.

Isolation. The dried and milled whole plant (1.1 kg) was extracted by maceration with MeOH three times (3 × 4 L) at room temperature, for 2 days each. After filtration and evaporation of the solvent under reduced pressure, the combined crude methanolic extract was suspended in H_2O (600 mL) to afford an aqueous MeOH solution (~90%) and then partitioned in turn with petroleum ether (3 × 800 mL) and $CHCl_3$ (3 × 800 mL) to afford dried petroleum ether-soluble (8.7 g), $CHCl_3$-soluble (19.2 g), and H_2O-soluble (26.5 g) residues. The $CHCl_3$-soluble extract showed significant inducing activity (CD < 2.5 μg/mL and IC_{50} > 20 μg/mL) in the cell-based QR assay.

Accordingly, the $CHCl_3$-soluble extract was chromatographed over a Si gel column (7 × 68 cm, 230−400 mesh, 1 kg) and eluted with gradient mixtures of $CHCl_3$–MeOH (from 100:1 to 4:1) to afford eight fractions (F01−F08). The eight fractions obtained were again evaluated in the QR assay, and the CD (μg/mL) and IC_{50} (μg/mL) values of F01−F08 were >10, <2.5, <2.5, 3.7, 4.6, >10, 17.1, and >10 and >20, >20, >20, >20, 12.9, >20, >20, and >20, respectively. Thus, the most active fractions F02 and F03 were selected for further detailed purification.

Fraction F02, eluted with $CHCl_3$–MeOH (60:1 and 40:1), was subjected to purification over a Si gel column (3.2 × 60 cm), using gradient mixtures of petroleum ether−acetone (from 5:1 to 1:1), to give five subfractions (F0201−F0205). Subfraction F0202, eluted with petroleum ether−acetone (4:1), was purified over a Si gel column (2.8 × 55 cm) using hexanes−EtOAc−MeOH (80:40:1) for elution, and afforded compounds **1** (65 mg) and **2** (37 mg), in order of polarity. Subfraction F0203, obtained with petroleum ether−acetone (3:1), was further chromatographed over a Si gel column (3.2 × 55 cm) using $CHCl_3$–acetone (10:1 to 2:1) as the solvent system, to give four further subfractions (F020301−F020304). The second subfraction, F020302, eluted with $CHCl_3$–acetone (8:1), was purified by preparative TLC (60 Å Si gel, 20 × 20 cm, 1000 μm) twice, developed sequentially with hexanes−EtOAc−MeOH (40:40:1; R_f = 0.32) and $CHCl_3$–acetone (9:1; R_f = 0.45), leading to the purification of compound **8** (4.0 mg). Subfraction F020304, eluted with $CHCl_3$–acetone (4:1 and 2:1), was further chromatographed over a Si gel column (2.0 × 25 cm) and eluted with $CHCl_3$−acetone (14:1), and afforded the semipure compounds **9** and **10**, in order of polarity. These were finally purified by preparative TLC (60 Å Si gel, 20 × 20 cm, 500 μm), developed with hexanes−EtOAc−MeOH (60:60:1 and 100:80:1, respectively), and yielded pure compounds **9** (3.0 mg, R_f = 0.35) and **10** (3.5 mg, R_f = 0.33). Subfraction F0204, eluted with petroleum ether−acetone (2:1), was further chromatographed over a Si gel column (2.0 × 25 cm) and eluted with $CHCl_3$−acetone (6:1), to give pure compound **10** (2.4 mg) and a mixture. This mixture was then purified by preparative TLC (60 Å Si gel, 20 × 20 cm, 500 μm), developed with hexanes−EtOAc−MeOH (30:30:1), to afford compounds **4** (7.0 mg, R_f = 0.45) and **5** (5.0 mg, R_f = 0.48).

The second active fraction in the QR-inducing assay, F03, which eluted with $CHCl_3$–MeOH (30:1), was chromatographed over a Si gel column (3.2 × 60 cm), eluted with hexanes−EtOAc (3:1 to 1:1 and then pure EtOAc), to give five subfractions (F0301−F0305). Subfractions F0302 (eluted with hexanes−EtOAc, 2:1), F0303 (eluted with hexanes−EtOAc, 2:1), and F0304 (eluted with hexanes−EtOAc, 1:1), were separately purified by preparative TLC (60 Å Si gel, 20 × 20 cm, 1000 μm), developed with hexanes−EtOAc−MeOH (15:15:1), to afford compounds **3** (5.0 mg, R_f = 0.40), **7** (8.0 mg, R_f = 0.36), and **6** (28.0 mg, R_f = 0.34), respectively.

Data for Subtrifloralactone A (1): colorless needles (hexanes−EtOAc, 1:1); mp 221−222 °C; $[\alpha]^{23}_D$ −13.3° (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.44), 312 (2.87) nm; IR (film) ν_{max} 3507, 1765, 1713, 1576, 1311, 1178, 1079 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 1, respectively; HRTOFMS *m*/*z* 455.2425 $[M + H]^+$ (calcd for $C_{27}H_{35}O_6$ 455.2434); MS−MS (25 eV) *m*/*z* 455.2481 (18), 437.2305 (100), 419.2256 (80), 393.2075 (54), 391.2263 (38), 363.2138 (15), 345.1877 (15), 295.1548 (8), 285.1505 (12), 279.1563 (15), 267.1386 (15), 171.0845 (8), 135.0813 (72), 113.0633 (33).

Monocarbamate of 1 (1a). One drop of trichloroacetyl isocyanate was added to a $CDCl_3$ solution (0.5 mL) of **1** (in a NMR tube), and the monocarbamate of **1** (**1a**) was found to be afforded by checking its ^{1}H NMR spectrum after 10 min: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (1H, s, NH), 6.80 (1H, ddd, *J* = 9.9, 4.9, 2.5 Hz, H-3), 5.89 (1H, ddd, *J* = 9.9, 1.9, 0.9 Hz, H-2), 5.54 (1H, br d, *J* = 5.6 Hz, H-6), 4.46 (1H, t, *J* = 8.7 Hz, H-23), 4.33 (1H, s, H-16), 3.81 (1H, d, *J* = 8.4 Hz, H-22), 3.28 (1H, br d, *J* = 20.3 Hz, H-4a), 3.22 (1H, br s, H-17), 2.86 (1H,

(25) The instrument used is an Enraf-Nonius CAD4 diffractometer and is located at the X-ray Laboratory, 638 Choppin Hall, Chemistry Department, Louisiana State University, Baton Rouge, LA 70803-1804, and operated by Dr. Frank Fronczek (frank.fronczek@chem.lsu.edu).

(26) The instrument used is an Enraf-Nonius Kappa CCD area detector equipped with a rotating anode X-ray generator and Mo Kα radiation and is located at the X-ray Laboratory, 234 Wetherall Hall, Chemistry Department, Purdue University, West Lafayette, IN 47907-1393, and operated by Dr. Phillip E. Fanwick (fanwick@xray.chem.purdue.edu).

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dd, J = 21.5, 4.9 Hz, H-4b), 2.59 (1H, dd, J = 14.7, 6.2 Hz, H-11a), 2.49 (1H, br d, J = 5.0 Hz, H-13), 1.81 (3H, s, H-21), 1.31 (3H, d, J = 6.4 Hz, H-28), 1.25 (3H, d, J = 7.0 Hz, H-27), 1.20 (3H, s, H-19); HRTOFMS m/z 664.1232 $[M + Na]^+$ (calcd for $C_{30}H_{34}O_8NaCl_3$ 664.1248).

X-ray Crystallography of 1. A colorless crystal was obtained from hexanes−EtOAc (1:1), and two data sets were collected on this sample. The first data set was collected at room temperature (298 K) with Cu Kα radiation. Cell parameters: a = 5.6597(2) Å, b = 12.7725(11) Å, β = 98.001(4)°, c = 16.6784(9) Å, V = 1193.92(13) Å^3, space group $P2_1$, Z = 2, D_{calcd} = 1.264 g/cm^3, λ = 1.54180 Å, μ(CuKα) = 0.716 mm^{-1}, F(000) = 488, T = 298(2) K. Data collection yielded 4783 reflections, all of which were considered unique. Full-matrix least-squares refinement led to final R, R (all), and GOF values of 0.0389, 0.0391, and 1.070. The second data set was collected at low temperature (150 K) with MoKα radiation. Cell parameters: a = 11.1278(3) Å, b = 12.7822(3) Å, β = 101.1760(10)°, c = 16.7223(4) Å, V = 2333.44(10) Å^3, space group $P2_1$, Z = 4, D_{calcd} = 1.294 g/cm^3, λ = 0.71073 Å, μ(MoKα) = 0.090 mm^{-1}, F(000) = 976, T = 150 K. Data collection yielded 9060 reflections, all of which were considered unique. Full-matrix least-squares refinement led to final R, R (all), and GOF values of 0.0509, 0.0619, and 1.041. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 184408 (room temperature data set) and CCDC 184409 (low-temperature data set). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Rd., Cambridge CB2 1EZ, U.K. [fax +44 (0)1223 336033 or e-mail deposit@ccdc.cam.ac.uk].

Data for Subtrifloralactone B (2): colorless oil; $[\alpha]^{23}_D$ +14.8° (c 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (3.77), 226 (3.76), 295 (3.00) nm; IR (film) ν_{max} 3454, 1765, 1664, 1539, 1452, 1379, 1315, 1242, 1184, 1099, 1006 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 1, respectively; HRTOFMS m/z 455.2439 $[M + H]^+$ (calcd for $C_{27}H_{35}O_6$ 455.2434); MS−MS (25 eV) m/z 455.2481 (16), 437.2305 (100), 419.2256 (75), 393.2068 (50), 391.2253 (35), 363.2145 (20), 345.1782 (15), 295.1548 (8), 285.1505 (10), 279.1611 (15), 267.1423 (15), 171.0687 (10), 135.0813 (75), 113.0723 (30).

Data for Subtrifloralactone C (3): colorless oil; $[\alpha]^{23}_D$ −30.5° (c 0.21, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.42), 221 (3.42), 312 (2.69) nm; IR (film) ν_{max} 3455, 1773, 1663, 1542, 1379, 1313, 1242, 1184, 1103, 1013 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 1, respectively; HRTOFMS m/z 471.2363 $[M + H]^+$ (calcd for $C_{27}H_{35}O_7$ 471.2383); MS−MS (20 eV) m/z 471.2484 (7), 453.2429 (55), 435.2304 (100), 417.2247 (30), 389.2246 (20), 361.2035 (5), 323.1817 (12), 267.1419 (10), 247.1250 (20), 197.1056 (18), 113.0734 (15).

Data for Subtrifloralactone D (4): colorless needles (hexanes−EtOAc, 1:1); mp 221−222 °C; $[\alpha]^{23}_D$ +2.0° (c 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (3.30), 303 (2.48) nm; IR (film) ν_{max} 3407, 1715, 1574, 1380, 1260, 1095, 1057 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 4, respectively; HRTOFMS m/z 457.2599 $[M + H]^+$ (calcd for $C_{27}H_{37}O_6$ 457.2590); MS−MS (25 eV) m/z 457.2603 (2), 439.3380 (40), 421.3265 (100), 403.3149 (45), 395.3044 (25), 365.2901 (28), 347.2766 (42), 279.2316 (35), 267.1950 (50), 135.1084 (50), 115.0980 (18).

X-ray Crystallography of 4. A small crystal, 10 × 40 × 40 μm, obtained from hexanes−EtOAc (1:1), was selected for data collection. It was immersed in glycerol and cooled to 100 K. Cell parameters: a = 7.797(8) Å, b = 12.954(13) Å, c = 46.880(47) Å, V = 4734(8) Å^3, space group $P2_12_12_1$, Z = 8, D_{calcd} = 1.281 g/cm^3, λ = 0.900 Å, μ = 0.089 mm^{-1}, F(000) = 1968, T = 100(2) K. Data collection at the APS beamline 14-BM-C yielded 21555 reflections, 2837 of which were considered unique. Full-matrix least-squares refinement led to final R, R (all), and GOF values of 0.0510, 0.0580, and 1.075. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 181313. Copies of the data can be obtained as mentioned for compound **1**.

Data for Subtrifloralactone E (5): colorless oil; $[\alpha]^{23}_D$ +12.9° (c 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 232 (3.41), 294 (2.47) nm; IR (film) ν_{max} 3416, 1710, 1575, 1459, 1377, 1264, 1190, 1096, 1054 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 4, respectively; HRTOFMS m/z 457.2607 $[M + H]^+$ (calcd for $C_{27}H_{37}O_6$ 457.2590); MS−MS (20 eV) m/z 457.2603 (2), 439.3380 (38), 421.3265 (100), 403.3149 (48), 395.3044 (25), 365.2901 (28), 347.2766 (45), 279.2316 (20), 267.1950 (55), 229.1712 (18), 143.1196 (20), 115.0980 (18).

Data for Subtrifloralactone F (6): colorless oil; $[\alpha]^{23}_D$ −3.8° (c 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (3.60), 223 (3.63), 309 (2.72) nm; IR (film) ν_{max} 3445, 1767, 1592, 1380, 1180 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 4, respectively; HRTOFMS m/z 473.2560 $[M + H]^+$ (calcd for $C_{27}H_{37}O_7$ 473.2539); MS−MS (20 eV) m/z 473.2508 (2), 455.2523 (8), 437.2422 (100), 419.2362 (50), 393.2205 (90), 363.2075 (8), 325.1879 (8), 267.1485 (6), 135.0873 (5), 113.0691 (4).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of Compound 6. Two portions (each 1.3 mg) of compound **6** were treated with (*S*)-(+)-α- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5 μL) in anhydrous pyridine (0.5 mL) at room temperature overnight. The reaction mixtures were purified over small Si gel columns with $CHCl_3$–MeOH (100:1) as the elution solvent, to afford the (*R*)- and (*S*)-MTPA ester derivatives **6r** and **6s** of **6**, respectively: ^{1}H NMR (**6r**) ($CDCl_3$, 500 MHz) δ 6.83 (1H, ddd, J = 10.1, 5.0, 2.4 Hz, H-3), 5.91 (1H, dd, J = 10.1, 2.4 Hz, H-2), 5.61 (1H, br d, J = 5.8 Hz, H-6), 5.31 (1H, br q, J = 6.3 Hz, H-16), 3.82 (1H, dd, J = 8.1, 6.4 Hz, H-23), 3.32 (1H, br d, J = 21.5 Hz, H-4a), 3.27 (1H, dd, J = 12.5, 4.7 Hz, H-11a), 2.93 (1H, dd, J = 21.5, 4.9 Hz, H-4b), 2.40 (1H, t, J = 12.9 Hz, H-11b), 2.27 (1H, m, H-9), 1.28 (3H, s, CH_3-19), 1.21 (3H, d, J = 6.9 Hz, CH_3-28), 1.17 (3H, d, J = 6.5 Hz, CH_3-27), 1.00 (3H, s, CH_3-21); ^{1}H NMR (**6s**) ($CDCl_3$, 500 MHz) δ 6.82 (1H, ddd, J = 10.0, 5.0, 2.4 Hz, H-3), 5.90 (1H, dd, J = 10.0, 2.3 Hz, H-2), 5.60 (1H, br d, J = 5.9 Hz, H-6), 5.34 (1H, br q, J = 6.4 Hz, H-16), 3.97 (1H, dd, J = 8.2, 6.3 Hz, H-23), 3.32 (1H, br d, J = 21.3 Hz, H-4a), 3.24 (1H, dd, J = 12.4, 4.6 Hz, H-11a), 2.93 (1H, dd, J = 21.4, 4.9 Hz, H-4b), 2.32 (1H, br t, J = 12.8 Hz, H-11b), 2.23 (1H, m, H-9), 1.25 (3H, s, CH_3-19), 1.22 (3H, d, J = 7.0 Hz, CH_3-28), 1.21 (3H, d, J = 6.4 Hz, CH_3-27), 1.13 (3H, s, CH_3-21).

Data for Subtrifloralactone G (7): colorless oil; $[\alpha]^{23}_D$ +28.5° (c 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (3.28), 333 (3.44) nm; IR (film) ν_{max} 3420, 1765, 1712, 1655, 1541, 1378, 1178 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 4, respectively; HRTOFMS m/z 473.2537 $[M + H]^+$ (calcd for $C_{27}H_{37}O_7$ 473.2539); MS−MS (20 eV) m/z 473.2516 (2), 455.2610 (10), 437.2507 (100), 419.2362 (50), 393.2205 (90), 363.2152 (7), 325.1952 (7), 267.1485 (7), 155.0922 (4), 135.0873 (5), 113.0734 (4).

Data for Subtrifloralactone H (8): colorless oil; $[\alpha]^{23}_D$ +49.5° (c 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 233 (3.31), 300 (2.26) nm; IR (film) ν_{max} 3461, 1718, 1713, 1540, 1450, 1356, 1204, 1156, 1092, 1055 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 5 and 1, respectively; HRTOFMS m/z 535.2692 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_7Na$ 535.2672); MS−MS (20 eV) m/z 467.2351 (18), 449.2207 (90), 421.2366 (100), 403.2226 (65), 375.2223 (30), 347.1945 (75), 229.1205 (45), 155.0845 (10), 135.0784 (13).

Data for Subtrifloralactone I (9): colorless oil; $[\alpha]^{23}_D$ −17.3° (c 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 225 (3.70), 311 (2.67) nm; IR (film) ν_{max} 3453, 1720, 1664, 1523, 1450, 1378, 1165, 1092 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 5 and 1, respectively; HRTOFMS m/z 535.2670 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_7Na$ 535.2672); MS−MS (20 eV) m/z 467.2415 (21), 449.2328 (80), 421.2380 (100), 403.2226 (65), 381.1713 (20), 375.2223 (30), 347.1945 (72), 229.1205 (40), 155.0845 (10), 135.0784 (15).

Data for Subtrifloralactone J (10): colorless oil; $[\alpha]^{23}_D$ +10.8° (c 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.43), 220

(3.41), 313 (2.82) nm; IR (film) ν_{max} 3412, 1719, 1663, 1452, 1377, 1165, 1093 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 5 and 1, respectively; HRTOFMS *m*/*z* 521.2519 $[M + Na]^+$ (calcd for $C_{29}H_{38}O_7Na$ 521.2515); MS−MS (20 eV) *m*/*z* 521.2391 (80), 467.2263 (100), 449.2207 (38), 421.2282 (15), 403.2226 (10), 381.1592 (10).

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Supporting Information Available: ^1H and ^{13}C NMR spectra for compounds **1** and **8** in both $CDCl_3$ and pyridine-d_5 and for **4** in $CDCl_3$, ^1H NMR spectra of the monocarbamate of **1** (**1a**) and compounds **2**, **7**, **9**, and **10** in $CDCl_3$ and for **3**, **5**, and **6** in both pyridine-d_5 and $CDCl_3$, and $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra of compound **8** in pyridine-d_5. This material is available free of charge via the Internet at http://pubs.acs.org.

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