Chemical Modification of the Insecticidal Briantheins X and Y[||]

John M. Cronan,[†,§] Angela Lee,[‡] Jun Liang,[‡] Jon Clardy,[‡,⊥] and John H. Cardellina II*[,†]

Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 East Pratt Street, Baltimore, Maryland 21202, and Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, New York 14853-1301

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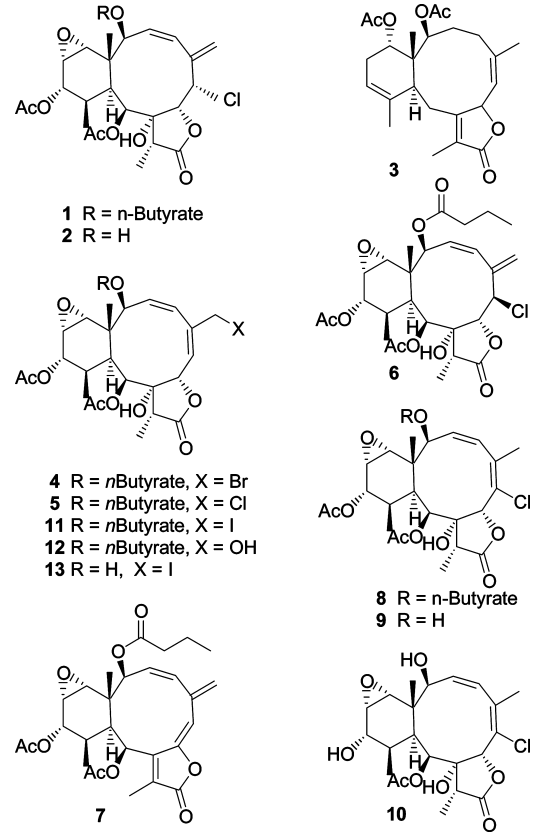
Manipulation of the allylic chloride functionality in briantheins X and Y provided 10 new analogues of these gorgonian diterpenes as part of a continuing study of structure−activity relationships in this family of insecticidal compounds. Modified Finkelstein reaction conditions led not only to halogen substitution products but also to rearrangement, dehydrohalogenation, and dehydration products. None of the new compounds showed superior insecticidal activity to brianthein X or Y, although most did result in lower weight gains versus controls.

Previous work by our group[1−4] and others[5] has explored the insecticidal activity of the briarane diterpenes (e.g., **1** and **2**). Comparative testing of briarane diterpenes[2,4] from the gorgonian *Briareum polyanthes* and the sea pen *Ptilosarcus gurneyi* provided some information about the impact of A-ring (cyclohexane) functionality on the insecticidal activity and also revealed that the less functionalized, non-chlorinated members of this class, such as brianthein W (**3**),[6] lacked potency.

The availability of substantial quantities of briantheins Y and X (**1** and **2**)[1] from a large-scale re-collection of *B. polyanthes* in Bermuda afforded us the material resources to conduct additional structure−activity studies. Herein we report chemical modifications aimed at the allylic chloride functionality in the cyclodecene ring of **1** and **2** and the insecticidal activity screening of the products obtained.



Results and Discussion

The Finkelstein reaction[7] has often been used to prepare bromides or iodides from the corresponding alkyl chlorides as synthetically more useful reagents. In this study, we employed a few modifications of typical Finkelstein conditions. We used acetonitrile as solvent, rather than acetone, because the reaction rate has been reported to be higher in dipolar aprotic solvents.[8,9] We also utilized the catalyst 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane), known to complex cations of inorganic salts in polar aprotic solvents and reported to shift the equilibrium toward the desired products in nucleophilic displacement reactions.[9,10]

Thus, reaction of brianthein Y (**1**) with NaBr provided **4**, after normal-phase HPLC on a cyano-bonded column with *i*PrOH−hexane; HRFABMS showed that this product differed in composition from **1** only by the exchange of bromine for chlorine. Comparison of the ^{1}H NMR spectra of **1** and **4** revealed differences primarily in the region between δ 4.0 and 6.5, which contained signals for H-6, H-7, H-9, and H-16. The most distinctive change in the spectrum of **4** was the disappearance of the H-16 exomethylene proton signals (δ 5.99, 6.32) and the appearance of a new AB pair (δ 4.37, 4.66, J = 11.6). H-6 was shifted downfield (from δ 5.25 to 5.90) and $J_{6,7}$ had increased from 3.3 to 8.9 Hz. ^{13}C NMR and HMQC spectra confirmed the migration of the $\Delta^{5,16}$ double bond to $\Delta^{5,6}$ and placement of the bromine substituent at C-16 (δ 33.0). Detailed analysis of its 1D and 2D NMR spectra indicated that no other changes had taken place relative to **1**. The yield of **4** was increased from 16% to 85% by extending the reaction time from two to nine days; in neither case was there any indication of production of the 6-bromo analogue by direct displacement of the chlorine in **1**.

When KBr was used in place of NaBr, a 1:1 mixture of **4** and a second compound (**5**), isomeric with **1**, was obtained, as confirmed by HRFABMS. The NMR spectra were quite similar to those of **4**, except that the signals for C-16 (δ 44.4) and H-16 (δ 4.45, 4.75, J = 14.3) had shifted slightly downfield. No other changes were obvious, and the structure was therefore assigned as **5**. Thus, **5** was the product of chloride migration (allylic rearrangement) in the presence of excess bromide.

[||] Dedicated to the late Dr. Richard E. Moore of the University of Hawaii−Manoa for his pioneering work on bioactive natural products.

* To whom correspondence should be addressed: ReevesGroup, 9374 Highlander Blvd., Walkersville, MD 21793. E-mail: jhcardellina@aol.com.

[†] University of Maryland Biotechnology Institute.

[‡] Cornell University.

[§] Current address: Magellan Bioscience Group, Inc., 6101 Johns Rd., Suite 8, Tampa, FL 33634.

[⊥] Current address: Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Ave., C-643, Boston, MA 02115.

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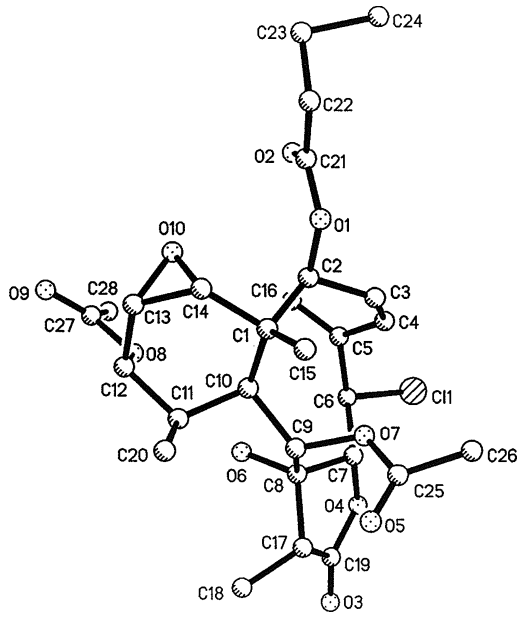

Figure 1. Computer-generated structure of 6-epi-briantheín Y, **6**, derived from X-ray crystallographic data.

Reaction of **1** with NaCl gave a mixture of **5** and a third constitutional isomer of briantheín Y, which were readily separated by HPLC. The ^{1}H NMR spectrum of this new compound was considerably different from that of **1**. H-4 was shifted 0.35 ppm downfield; the H-16 protons were both shifted upfield 0.2 and 0.4 ppm, respectively, and appeared as singlets, and H-6 and H-7 were both shifted upfield 0.53 and 0.41 ppm, respectively. The H-6/H-7 coupling constant had changed from 3.3 Hz (**1**) to 10 Hz. The ^{13}C NMR spectrum revealed that the $\Delta^{5,16}$ double bond was intact and that C-6 and C-7 had shifted downfield 2.2 and 4.4 ppm, respectively. These small chemical shift changes, together with the changes in chemical shift and coupling constants observed in the ^{1}H NMR, strongly suggested that this new molecule was 6-epi-briantheín Y, **6**, with a different conformation of the cyclodecene ring. The structure and altered ring conformation were confirmed by X-ray diffraction analysis of crystals grown from CH_2Cl_2−MeOH (Figure 1).

Next, briantheín Y was treated with NaF under our standard reaction conditions. TLC monitoring of the reaction indicated that two products had formed with R_f values identical to those formed in the reaction with NaCl. Separation of the products and NMR analysis indicated that they were, in fact, **5** and **6**; the yields were 32% and 22%, respectively, marginally better than the yields from the NaCl reaction. Switching to KF in the reaction gave a very complex mixture containing at least 11 minor components. The only product isolated in sufficient quantity for identification was the tetraene **7**, $C_{28}H_{34}O_9$, by HRFABMS, indicating losses of HCl and H_2O from **1**. The ^{1}H NMR spectrum of **7** displayed significant changes in the downfield region between δ 5.0 and 6.6. However, COSY correlations indicated that the H-9 to H-14 spin system and functional groups were intact, although H-9 had shifted downfield by nearly 0.8 ppm relative to **1**, suggesting that dehydration had taken place at C-8/C-17. This was corroborated by the disappearance of H-17 and a downfield shift of the H-18 methyl protons, together with matching changes in the ^{13}C NMR shifts for C-8, C-17, and C-18. With the C-2 butyrate and the C-3 to C-16 diene still intact, dehydrochlorination could only have occurred at C-6/C-7; indeed, a one-proton singlet at δ 6.54 was assigned to H-6 and was supported by long-range COSY and HMBC correlations.

Efforts to substitute a cyano group for the allylic chloride by treatment of **1** with NaCN were unsuccessful, but did provide an additional minor (18%) rearrangement product, **8**, isomeric with briantheín Y. NMR analysis pointed to changes at C-6 and C-16. H-6 and the H-16 exomethylene protons were absent, replaced by a methyl singlet at δ 2.29; in addition, H-7 appeared as a sharp singlet in **8**. Thus, the $\Delta^{5,16}$ olefin had migrated into the ring ($\Delta^{5,6}$) via a 1,3-shift of hydrogen from C-6 to C-16. All other spectral data supported this assignment.

Because of the poor yields observed in the NaCN reaction, the more abundant briantheín X (**2**) was used in a larger scale experiment. In this case, two compounds were obtained, isobriantheín X, **9**, an analogue of **8**, and 12-desacetylisobriantheín X, **10**, both easily identified by comparison to **8** and to **2** and **9**, respectively.

Reaction of **1** with NaI under our standard conditions gave **11** in 6 h, but a mixture of **11** and **12** after nine days. The identification of **11** paralleled that of **4** and **5**; the only notable difference was that the iodo substituent dramatically shielded the C-16 carbon (δ 6.1). The 16-iodo compound **11** was also quite labile to solvolysis, decomposing upon handling and exposure to water or water vapor. This reactivity is the likely reason for the formation of **12** as reaction times were extended. Compound **12** was identified as the 16-hydroxy analogue of **4**, **5**, and **11**, which was confirmed by HRFABMS. The briantheín X-derived analogue of **11**, compound **13**, was prepared by reaction of **2** with NaI in acetone.[7] While NMR and MS data supported the structure assignment, complete characterization was thwarted by rapid decomposition.

The products we obtained were somewhat surprising at first, but each could be rationalized mechanistically. Compound **6** is the only product resulting from direct S_N2 displacement of the allylic chloride with inversion of stereochemistry. Compounds **4**, **5**, **11**, and **13** more resemble the products of an S_N1 reaction, with attack of the halide ion on the more accessible end of the incipient allylic carbocation. Compound **12** results from solvolysis of **11**. Compound **7** would seem to be a minor product of combined dehydration and dehydrohalogenation, from protracted exposure of the highly functionalized **1** to elevated temperature. Compounds **8**−**10** appear to be the result of a formal, albeit forbidden, 1,3-shift of hydrogen from C-6 to C-16. Perhaps the most puzzling case is the accumulation of **5** upon exposure to excess KBr or NaF. We speculate that this is a 1,3-shift of chloride, perhaps facilitated by the crown ether-alkali halide complex and/or proceeding through an S_N1-type reaction.

The 16-chloro (**5**) and 16-hydroxy (**12**) products obtained in this work have several representatives among the approximately 400 known[11,12] naturally occurring briarane diterpenes. Briarein J,[13] nui-inoalide,[14] an unnamed propionate ester analogue of briarein J,[15] juncins R and S,[16] and frajunolide C[17] are 16-chloro-3,5-endocyclic dienes; labouteine,[18] minabein 10,[19] pachyclavulide B,[20] and juncins Q[21] and T[16] are 16-hydroxy-3,5-endocyclic dienes. Closely related compounds include juncin W,[16] gemmacolide F,[19] pachyclavulide C,[20] and frajunolide D,[17] which are 16-acetoxy analogues, and juncins U and V,[16] which are 16-methoxy analogues. Comparison of the ^{13}C NMR chemical shifts of our products with these natural analogues indicated that the new $\Delta^{5.6}$ olefin had the *E* geometry; this assignment was supported by calculations of the expected chemical shifts (ACDLabs). The chemical shifts of C-16 were the most diagnostic.

While most of the new briantheín derivatives elicited reduced weight gain in tobacco hornworm larvae (*Manduca sexta*), none of them caused any mortality in the dose range tested. Thus, briantheins Y and X (**1**, **2**) caused 30% and 10% mortality, respectively, at 100 ppm, with survivors weighing only 70% that of untreated controls. In contrast, the semisynthetic analogues displayed weight gain reductions of 17−43%, except for **9** and **13**, which were inactive; of these, **5** had the most impact on weight gain. It was somewhat surprising that the changes in activity were relatively slight, given the conformational changes induced in many

Table 1. [1]H NMR Assignments[a] for Brianthein Y (**1**) and Modified Brianthein Derivatives **4**−**7** and Continuation of [1]H NMR Assignments[a] for Modified Brianthein Derivatives **8**−**13**

position	**1**[b]	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1											
2	6.22, d (9.1)	5.19, d (9.6)	5.18, d (10.4)	6.21, d (9.1)	5.91, d (9.4)	5.23, d (9.5)	4.17, d (9.7)	4.12, d (9.7)	5.21, d (9.8)	5.29, d (9.6)	5.21, d (9.8)
3	5.60, dd (11.8, 9.1)	5.61, dd (11.0, 9.6)	5.61, dd (10.4, 11.1)	5.61, (4.1, dd 12.0)	5.52, d (9.4, 11.0)	5.54, dd (9.5, 10.9)	5.7, dd (9.7, 10.7)	5.72, dd (9.7, 10.6)	5.61, dd (9.8, 11.1)	5.59, dd (9.6, 11.1)	5.6, dd (9.8, 11.1)
4	5.91, br d (11.8, 1)	6.36, d (11.0)	6.33, d (11.1)	6.26, d (12.0)	6.04, d (11.0)	6.28, d (10.9)	6.22, d (10.7)	6.22, d (10.6)	6.50, d (11.1)	6.32, d (11.1)	6.50, d (11.1)
5											
6	5.25, ddd (3.3, 2.7, 2.3)	5.90, d (8.9)	5.93, d (8.7)	4.72, d (10.0)	6.54, s				5.85, d (8.8)	5.69, dd (1.7, 8.8)	5.85, d (8.8)
7	4.90, d (3.3)	5.06, d (8.9)	5.09, d (8.7)	4.59, d (10.0)		5.29, s	5.29, s	5.27, d (2.4)	4.99, d (8.8)	5.10, d (8.8)	4.99, d (8.8)
8						2.59, s, OH		2.63, s, OH			
9	5.22, br d (8.5)	5.14, d (7.4)	5.17, d (7.3)	5.27, d (8.0)	5.99, d (10.3)	5.17, d (7.4)	5.14, d (7.3)	5.14, d (7.0)	5.12, d (7.4)	5.18, d (7.6)	5.12, d (7.4)
10	2.15−2.26, m	2.18, m	2.15, dd (2.8, 7.3)	2.43, dd (2.7, 8.0)	2.43, dd (2.9, 10.3)	2.20, dd (7.3, 7.4)	2.06, m (7.3)	2.06, dd (4.1, 7.0)	2.18, dd (3.0, 7.3)	2.21, dd (2.7, 7.6)	2.1, dd (3.0, 7.3)
11	2.15−2.26, m	1.99, m	1.94, ddq (7.0)	1.88, ddq (7.3)	1.41, m	2.26, dd (5.5, 7.3)	2.10, m	2.01, dd (2.3, 7.3)	1.97, m (7.3)	2.08, m	1.97, m (7.3)
12	4.68, dd (5.5, 3.2)	4.72, dd (5.5, 2.4)	4.69, dd (2.5, 5.1)	4.72, dd (2.6, 5.7)	4.65, dd (2.7, 5.3)	4.68, dd (2.2, 5.5)	4.70, dd (2.3, 5.5)	3.91, dd (2.3, 5.9)	4.72, dd (2.3, 5.5)	4.70, dd (2.5, 5.5)	4.72, dd (2.3, 5.5)
13	3.55, dd (5.5, 3.3)	3.50, dd (5.5, 3.3)	3.55, dd (3.3, 5.1)	3.52, dd (3.3, 5.7)	3.58, (dd (3.2, 5.3)	3.53, dd (3.3, 5.5)	3.61, dd (3.4, 5.5)	3.55, dd (3.6, 5.9)	3.48, dd (3.3, 5.5)	3.53, dd (3.3, 5.5)	3.48, dd (3.3, 5.5)
14	2.92, d (3.3)	2.95, d (3.3)	2.95, d (3.3)	2.93, d (3.3)	2.96, d (3.2)	2.94, d (3.3)	3.20, d (3.4)	3.36, d (3.6)	2.94, d (3.3)	2.97, d (3.3)	2.94, d (3.3)
15	1.07, s	1.15, s	1.17, s	1.08, s	1.19, s	1.14, s	1.08, s	1.07, s	1.15, s	1.16, s	1.15, s
16a	5.99, d (2.5)	4.37, d (11.6)	4.55, d (14.3)	5.06, s	5.42, s	2.29, s	2.14, s	2.09, s	4.10, d (9.7)	4.54, dq (6.8, 15.6)	4.10, d (9.7)
16b	6.32, d (2.5)	4.66, d (11.6)	4.75, d (14.3)	6.13, s	5.99, s				4.82, d (9.7)	3.12, t (OH, 6.8)	4.82, d (9.7)
17	2.29, q (7.0)	2.20, m	2.28, q (1.6, 7.4)	2.51, q (7.2)		2.18, q (7.0)	2.21, q (7.0)	2.21, q (7.1)	2.27, q (7.1)	2.25, q (6.5)	2.27, q (7.1)
18	1.15, d (7.0)	1.13, d (7.1)	1.06, d (7.4)	1.15, d (7.2)	2.07, s	1.14, d (7.0)	1.14, d (7.0	1.14, d (7.1)	1.12, d (7.1)	1.13, d (6.5)	1.12, d (7.1)
19											
20	1.04, d (7.2)	1.05, d (7.4)	1.16, d (7.0)	1.06, d (7.3)	1.12, d (7.2)	1.05, d (7.3)	1.03, d (7.3)	0.97, d (7.4)	1.04, d (7.5)	1.07, d (7.4)	1.04, d (7.5)
C-9 acetate C═O											
C-9 acetate CH_3	2.05, s[c]	2.06, s	2.04, s	2.03, s	1.98, s	2.07, s	2.08, s	2.14, s	2.05, s	2.08, s	2.05, s
C-12 acetate C═O											
C-12 acetate CH_3	2.14, s[c]	2.15, s	2.16, s	2.17, s	2.10, s	2.15, s	2.15, (		2.15, s	2.16, s	2.15, s
C-2 butyrate CH_3−	0.92, t (6.7)	0.88, t (7.4)	0.92 (t, J = 7.4)	0.93, t (7.4)	0.94, t (7.4)	0.91, t (7.4)			0.91, t (7.4)	0.92 (t, J = 7.3)	0.91 (t, J = 7.4)
C-2 butyrate CH_3-CH_2	1.64, sextet (6.7)	1.63, sextet (7.4)	1.60, sextet (7.4)	1.65, sextet (7.4)	1.66, sextet (7.4)	1.62, sextet (0.4)			1.63, sextet (7.4)	1.59, sextet (7.3)	1.63, sextet (7.4)
C-2 butyrate Et-CH_2	2.29, t (6.7)	2.26, t (7.4)	2.26, t (7.4)	2.29, t (7.4)	2.31, t (7.4)	2.24, t (7.4)			2.26, t (7.4)	2.27, t (7.3)	2.26, t (7.4)
C-2 butyrate Pr-CO_2−											

[a] Bruker AC(F) 300.13 MHz, $CDCl_3$; reported in δ (multiplicity, J = Hz). [b] [1]H NMR assignments were transcribed from ref 1. [c] Resonances for brianthein Y reassigned from HMQC and HMBC experiments.

Table 2. ^{13}C NMR Assignments[a] for Brianthein Y (**1**) and Modified Brianthein Derivatives (**4**−**13**)

position	**1**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	40.8	40.5	40.6	41.3	40.1	40.5	40.7	40.5	40.5	40.6	40.6
2	75.3	77.7	77.4	75.5	76.0	77.8	76.0	76.1	77.4	79.0	75.9
3	131.1	131.6	132.1	131.3	129.6	131.1	135.1	134.9	131.1	131.1	136.1
4	127.9	128.3	127.9	126.3	132.0	129.6	127.5	127.8	128.9	128.9	126.1
5	136.8	142.3	142.6	140.0	147.3	122.8	139.6	138.9	143.8	147.6	142.9
6	62.4	125.4	123.6	64.6	115.9	140.7	123.1	123.5	124.3	120.6	125.2
7	77.4[b]	78.9	78.5	81.8	137.7	77.2	77.0	77.2	78.9	78.7	79.3
8	84.6	82.0	81.9	83.7	145.1	83.1	83.3	83.3	82.0	85.4	82.3
9	69.0[b]	69.0	69.1	69.3	65.4	68.9	69.2	69.6	69.7	69.1	69.5
10	32.7	32.1	32.1	31.9	35.5	32.1	32.5	31.3	32.1	32.1	32.4
11	36.7	37.9	37.8	37.2	34.4	37.7	37.9	41.0	38.0	37.7	38.2
12	69.4[b]	69.6	69.6	69.5	68.9	69.6	70.0	76.1	69.2	69.6	70.1
13	52.6	52.4	52.5	52.4	52.5	52.6	53.1	55.4	52.3	52.6	52.8
14	61.8	61.8	61.9	61.7	60.0	62.0	62.4	64.2	61.8	62.0	62.3
15	15.3[b]	15.8	15.8	15.5	15.7	16.1	14.8	14.6	15.6	15.8	14.7
16	118.8	33.0	44.4	120.5	123.7	18.6	20.6	21.7	6.1	63.4	6.4
17	44.8	43.0	42.9	45.5	133.8	42.8	42.8	42.9	42.9	43.0	43.0
18	6.2[b]	6.3	6.4	6.2	9.9	6.3	6.3	6.3	6.1	6.4	6.3
19	174.4	175.7	175.4	174.2	172.6	174.9	174.9	175.1	175.5	175.7	175.6
20	12.4	12.9	12.7	12.4	12.8	12.9	12.9	13.1	12.9	12.7	13.0
C-9 acetate C═O	170.1[b]	170.3	170.2	170.3	169.5	170.2	170.1	169.6	170.1	170.2	20.8
C-0 acetate CH_3	20.3	20.7	20.6	20.6	20.4	21.8	20.1	22.7	20.8	20.5	170.2
C-12 acetate C═O	170.0[b]	169.8	169.7	170.3	168.6	169.7	169.6		169.7	169.8	21.6
C-12 acetate CH_3	21.8[b]	21.7	21.7	21.9	21.7	20.6	21.7		21.7	21.7	169.7
C-2 butyrate CH_3−	13.5	13.6	13.6	13.6	13.6	13.6			13.6	13.7	
C-2 butyrate CH_3-$\underline{CH_2}$	18.3	18.4	18.4	18.4	18.5	18.4			18.5	18.4	
C- butyrate Et-$\underline{CH_2}$	36.1	36.4	36.4	36.3	36.4	36.4			36.4	36.5	
C-2 butyrate Pr-$\underline{C}O_2$−	172.5	171.8	171.9	172.5	170.0	171.6			171.7	172.8	

[a] Bruker AC(F) 75.47 MHz, $CDCl_3$; reported in δ (multiplicity, J = Hz). [b] Resonances for brianthein Y[1] reassigned from HMQC and HMBC experiments.

of the test compounds. To gain a more complete understanding of the structural requirements for insecticidal activity, the effects of modification on the lactone ring and ring contractions and/or rearrangements must be examined.

Experimental Section

General Experimental Procedures. NMR spectral data were obtained in both normal and inverse detection modes on Bruker Instruments Inc. AC(F) 300 or AM 500 spectrometers. $CDCl_3$ was purchased from Cambridge Isotopes, Inc. Chemical shifts were reported in ppm (δ units) relative to tetramethylsilane (TMS, $\delta = 0$). Low-resolution mass spectrometric data were recorded on either a Hewlett-Packard model 5890 Series II gas chromatograph with a Supelco SBP-1 column (0.25 mm × 30 m, i.d.), coupled to a Hewlett-Packard 5970 mass selective detector, or a VG MM16F. Accurate mass data were recorded on a VG 7070EHF for high-pressure electron capture (HPEC) mass analyses and on a JEOL SX102 using FAB with NOBA or glycerol matrixes. Optical rotation data were measured on a Perkin-Elmer 241 MC polarimeter or an Optical Activity LTD instrument. UV spectra were obtained with a Hewlett-Packard UV/vis spectrophotometer model 8451A. The IR spectra were recorded on a Perkin-Elmer 1320 infrared spectrometer, a Nicolet 5DX FT-IR spectrometer, or a BioRad 2340 FT-IR spectrometer. Briantheins Y (**1**) and X (**2**) were isolated from the gorgonian *Briareum polyanthes*, collected in Bermuda, as described before.[1,5]

Insecticidal Screen. The insecticidal screen used the tobacco hornworm *Manduca sexta*. Eggs were purchased from Carolina Biological Supply and placed on artificial black cutworm agar diet from Bio-Serv Inc. Two days after hatching, the insects were used for the bioassay. The diet was prepared by the addition of 400 mL of boiling water to 100 g of the black cutworm diet and 13.5 g of agar. This mixture was homogenized in a blender and distributed in 10 g aliquots to beakers for each test compound. Compounds being tested were dissolved in 200 μL of either EtOH or CH_2Cl_2 and added to the warm diet with stirring. Once the solvent had evaporated, the diet was distributed at ~1 g per insect into plastic cups with lids. Ten hornworms were used per test compound. Pure compounds were initially assayed at 100 ppm, and dose−response experiments were conducted at 250, 125, 75, 50, and 25 ppm in an effort to determine LD_{50} values. The assay was conducted on a 12 h diurnal cycle with the temperature maintained between 24 and 26 °C. Insects were checked on a daily basis for feeding activity, and on days 3 and 7 the insects' weight and overall health were recorded. Mortality and relative growth rate statistics were calculated for the test compounds versus the controls (with and without solvent).

General Reaction Conditions. CH_3CN (HPLC grade) was dried over 4 Å molecular sieves prior to use. Reactions used 4 equiv of the halide salt per equivalent of brianthein diterpene. The catalyst 18-crown-6 (1,4,7,10,13,16-hexaoxacyclo-octadecane) was added at 0.2 equiv per equivalent of diterpene. A 2 mL microconical vial fitted with a reflux condenser and a $CaCl_2$ drying tube was dried in a vacuum oven (80 °C) for 30 min prior to use. Brianthein Y, **1**, or brianthein X, **2**, was added to the conical vial, followed by dry CH_3CN (0.5−1.5 mL) and then the salt and catalyst. The reaction mixture was heated at reflux (~82 °C).

The reaction was monitored by thin-layer chromatography using cyano-bonded phase silica plates (Merck). The plates were cleaned with MeOH−CH_2Cl_2 (1:1) and dried in a vacuum oven prior to use. A spot of brianthein X or Y was co-developed with a spot from the reaction solution for comparative R_f values. Hexane−*i*PrOH (1:1) was used to separate the components, and both UV light and vanillin/sulfuric acid were used for detection/visualization. To stop a reaction, the vial was cooled to room temperature and the reaction was quenched by the evaporation of the solvent using a stream of N_2 gas. The residue was further dried under vacuum to remove any CH_3CN prior to the addition

of CH_2Cl_2 and H_2O (0.5 mL each). The water layer was removed and discarded only after it was determined that it contained no end product or starting material. The CH_2Cl_2 layer was washed with H_2O (0.5 mL) to remove any salt and complexed crown ether and then concentrated under reduced pressure. The solid was dissolved in dry CH_2Cl_2 for filtration through a Whatman 0.4 μm nylon membrane filter in preparation for HPLC analysis.

Crude reaction product was subjected to HPLC chromatography on Rainin cyano-bonded silica support using a 1 × 10 cm guard connected directly to a 1 × 25 cm semipreparative column. A gradient of *i*PrOH−hexane at 3 mL/min over 40−45 min was used to separate the reaction mixtures. Separations were monitored at 230 nm with an ISCO V^4 variable-wavelength detector.

Preparation, Isolation, and Characterization of Brianthein Derivatives. 16-Bromo-6-dechloro-5,16-dihydro-Δ-5,6-brianthein Y, 4. A reaction mixture consisting of 21.1 mg of brianthein Y, 15.3 mg of NaBr, and 1.8 mg of 18-crown-6 was refluxed in CH_3CN for nine days. The HPLC chromatogram displayed a single peak at 35 min; preparative separation yielded 19.5 mg (86%) of **4**, $[\alpha]_D = -26.8$ (*c* 0.56, CH_3OH); UV λ_{max} (CH_3OH) 232 nm ($\varepsilon = 3400$); IR (thin film on CaF_2) ν_{max} 3465, 3005, 2960, 2935, 1775, 1730, 1455, 1425, 1365, 1215 cm^{-1}; see Tables 1 and 2 for ^1H and ^{13}C NMR assignments, respectively; HRFABMS (NOBA/PEG/EF) *m/z* 635.1494 $[M + Na]^+$, 24% (calcd for $C_{28}H_{37}{}^{79}BrO_{10}Na$, 635.14577).

16-Chloro-6-dechloro-5,16-dihydro-Δ^{5,6}-brianthein Y, 5. A reaction mixture consisting of 20.3 mg of brianthein Y, 5.9 mg of NaF, and 1.9 mg of 18-crown-6 was refluxed in CH_3CN for nine days. HPLC yielded 7.4 mg (36%) of **5**, $[\alpha]_D = -32.9$ (*c* 1.21, CH_3OH); UV λ_{max} (CH_3OH) 224 nm ($\varepsilon = 2000$); IR (thin film on CaF_2) ν_{max} 3465, 3010, 2960, 2930, 1775, 1730, 1455, 1425, 1370, 1220, 1030 cm^{-1}; see Tables 1 and 2 for ^1H and ^{13}C NMR assignments; HRFABMS (NOBA/PEG/EF) *m/z* 591.1959 $[M + Na]^+$, 12% (cacld for $C_{28}H_{37}{}^{35}ClO_{10}Na$, 591.1962). When NaCl was substituted for NaF, the highest obtainable yield of **5** was 17% (3.3 mg).

6-Epi-(6*R*)-brianthein Y, 6. A reaction mixture consisting of 18.5 mg of brianthein Y, 5.5 mg of NaF, and 1.8 mg of 18-crown-6 was refluxed in CH_3CN for nine days. HPLC provided 4.3 mg (23%) of **6**, $[\alpha]_D = -25.3$ (*c* 1.12, CH_3OH); UV λ_{max} (CH_3OH) 234 nm ($\varepsilon = 3300$); IR (thin film on CaF_2) ν_{max} 3470, 3010, 2955, 1775, 1735, 1455, 1365, 1250 cm^{-1}; see Tables 1 and 2 for ^1H and ^{13}C NMR assignments, respectively; HRFABMS (NOBA/PEG/EF) *m/z* 569.2125 $[M + H]^+$, 28% (calcd for $C_{28}H_{38}{}^{35}ClO_{10}$, 569.2143). When brianthein Y was reacted with NaCl in a similar manner, the highest obtainable yield for **6** was 21% (4.2 mg).

6-Dechloro-Δ$^{6,7-8,17}$-brianthein Y, 7. A reaction mixture consisting of 15.6 mg of brianthein Y, 6.4 mg of KF, and 1.4 mg of 18-crown-6 was refluxed in CH_3CN for nine days. HPLC yielded 1.2 mg (9%) of **7**; see Tables 1 and 2 for ^1H and ^{13}C NMR assignments, respectively; HRFABMS (NOBA *m/z* 515.2252 $[M + H]^+$, 11% (calcd for $C_{28}H_{35}O_9$, 515.2271).

Iso-brianthein Y, 8. A reaction mixture consisting of 44.3 mg of brianthein Y, 15.3 mg of NaCN, and 4.1 mg of 18-crown-6 was refluxed in CH_3CN for three days. HPLC separation yielded 8.0 mg (18%) of **8**, $[\alpha]_D = -38.8$ (*c* 0.39, CH_3OH); UV λ_{max} (CH_3OH) 228 nm ($\varepsilon = 3000$); IR (thin film on CaF_2) ν_{max} 3560, 3440 (br), 2960, 2930 (sh), 2870 (sh), 1780, 1735, 1600, 1450, 1365, 1215 cm^{-1}; see Tables 1 and 2 for ^1H and ^{13}C NMR assignments, respectively; HRFABMS (PEG) *m/z* 591.1959 $[M + Na]^+$, 8% (calcd for $C_{28}H_{37}{}^{35}ClO_{10}Na$, 591.1962).

Iso-brianthein X, 9. A reaction mixture consisting of 51.1 mg of brianthein X, 20.2 mg of NaCN, and 5.5 mg of 18-crown-6 was refluxed in CH_3CN for 24 h. HPLC yielded 5.6 mg (11%) of **9**, $[\alpha]_D = -15.0$ (*c* 0.33, $CHCl_3$); UV λ_{max} (CH_3OH) 226 nm ($\varepsilon = 2700$); IR (thin film on CaF_2) ν_{max} 3430, 3340, 2905, 2835, 1765, 1735, 1365, 1215 cm^{-1}; see Tables 1 and 2 for ^1H and ^{13}C NMR assignments, respectively; HRFABMS (NOBA/PEG/EF) *m/z* 997.3367 $[2M + H]^+$, 100% (calcd for $C_{48}H_{63}{}^{35}Cl_2O_{18}$, 997.3374).

12-Desacetyl-iso-brianthein X, 10. A reaction mixture consisting of 58.1 mg of brianthein X, 22.8 mg of NaCN, and 6.2 mg of 18-crown-6 was refluxed in CH_3CN for 24 h. Repeated HPLC provided 5.4 mg (10%) of **10**, $[\alpha]_D = -32.6$ (*c* 0.30, CH_3OH); UV λ_{max} (CH_3OH) 226 nm ($\varepsilon = 2800$); IR (thin film on CaF_2) ν_{max} 3410, 2905, 2835, 1775, 1730, 1590, 1450, 1365, 1215 cm^{-1}; see Tables 1 and 2 for ^1H and ^{13}C NMR assignments, respectively; HRFABMS (NOBA/PEG/EF) *m/z* 913.3190 $[2M + H]^+$, 100% (calcd for $C_{44}H_{59}{}^{35}Cl_2O_{16}$, 913.3164).

6-Dechloro-5,16-dihydro-16-iodo-Δ^{5,6}-brianthein Y, 11. A reaction mixture consisting of 12.8 mg of brianthein Y, 13.7 mg of NaI, and 1.2 mg of 18-crown-6 was refluxed in CH_3CN for 6 h. HPLC gave 13.6 mg (91%) of **11**; see Tables 1 and 2 for ^1H and ^{13}C NMR assignments, respectively; HRFABMS (NOBA) *m/z* 661.1483 $[M + H]^+$, 16% (calcd for $C_{28}H_{38}{}^{127}IO_{10}$, 661.1528).

6-Dechloro-5,16-dihydro-16-hydroxy-Δ^{5,6}-brianthein Y, 12. A reaction mixture consisting of 18.7 mg of brianthein Y, 20.3 mg of NaI, and 1.7 mg of 18-crown-6 was refluxed in CH_3CN for seven days. HPLC of the reaction mixture gave 4.6 mg (25% yield) of **12**, $[\alpha]_D = -23.4$ (*c* 0.64, CH_3OH); UV λ_{max} (CH_3OH) 234 nm ($\varepsilon = 3100$); IR (thin film on CaF_2) ν_{max} 3470, 3005, 2980, 2960, 2935, 2920, 1770, 1730, 1450, 1370, 1220, 1025 cm^{-1}; see Tables 1 and 2 for ^1H and ^{13}C assignments, respectively; HRFABMS (NOBA/PEG/EF) *m/z* 573.2310 $[M + Na]^+$, 5% (calcd for $C_{28}H_{38}O_{11}Na$, 573.2300).

16-Iodo-6-dechloro-5,16-dihydro-Δ^{5,6}-brianthein X, 13. A reaction mixture consisting of 20 mg of brianthein X and 30 mg of NaI was refluxed in acetone for two days. HPLC yielded 14.2 mg (60%) of **13**. The compound decomposed rapidly, precluding $[\alpha]_D$, UV, IR, and MS measurements; see Tables 1 and 2 for ^1H and ^{13}C NMR assignments, respectively.

X-ray Structure Determination of 6-epi-Brianthein Y (6). Crystal data: empirical formula $C_{28}H_{37}ClO_{10}$; formula weight 570.5 amu; crystal color and habit, colorless cube roughly 0.4 mm on an edge, orthorhombic space group $P2_12_12_1$; $Z = 4$; unit cell dimensions from 25 reflections ($25° < 2\theta < 40°$) $a = 10.381(3)$ Å, $b = 13.830(3)$ Å, $c = 20.132(5)$ Å; volume = 2890(1) $Å^3$; $\rho_{calc} = 1{,}294$ g/cm^3. Data collection: Cu Kα radiation; $2\theta < 114°$; variable speed θ, 2θ scans; 3941 unique reflections; room temperature. Solution and refinement: direct methods (SHELXTL); full-matrix least-squares on F^2; anisotropic nonhydrogens and isotopic riding hydrogens (352 parameters); $R = 0.058$ for all data. Crystallographic data for **6** have been deposited with the Cambridge Crystallographic Data Centre.[23]

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Supporting Information Available: X-ray crystallographic data for **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) CCDC 742896 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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